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CURRENT ASPECTS OF NASAL DRUG DELIVERY

PAUL MERKUS

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CURRENT ASPECTS OF NASAL DRUG DELIVERY.

THESIS UNIVERSITY OF AMSTERDAM

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CURRENT ASPECTS OF NASAL DRUG DELIVERY

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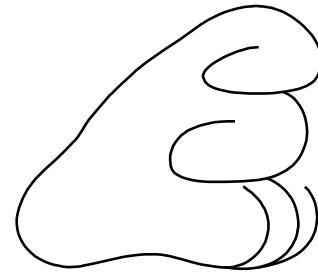
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Voor Rebecca
Voor mijn ouders

Section I General introduction & scope

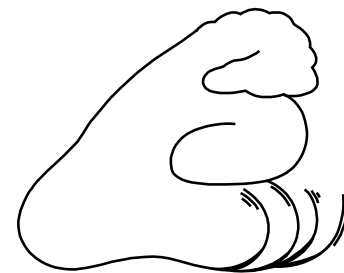
Chapter 1	General introduction: current aspects of nasal drug delivery	11
Chapter 2	Scope and intent of the thesis	51

Section II Nasal drug administration to the middle meatus



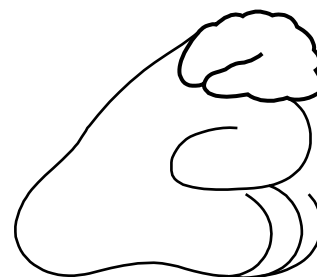
Chapter 3	The 'best method' of topical nasal drug delivery: comparison of seven methods <i>Rhinology, in press</i>	57
Chapter 4	Influence of anatomy and head position on nasal drug deposition <i>submitted</i>	71

Section III Effects of nasal drugs and nasal drug formulations on the nasal ciliary activity



Chapter 5	Classification of cilio-inhibiting effects of nasal drugs <i>Laryngoscope 2001;111:595-602.</i>	89
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Section IV Nasal drug delivery and transport to the CSF and brain



Chapter 6	Method development: Quantitative determination of melatonin in human plasma and cerebrospinal fluid with high-performance liquid chromatography and fluorescence detection. <i>Biomedical Chromatography 2000;14:306-310.</i>	109
Chapter 7	Direct access of drugs to the human brain after intranasal drug administration? <i>Neurology 2003;60:1669-1671.</i>	121
Chapter 8	Hydroxocobalamin uptake into the cerebrospinal fluid after nasal and intravenous delivery in rats and humans. <i>Journal of Drug Targeting 2003;11:325-331.</i>	129
Chapter 9	Uptake of melatonin into the cerebrospinal fluid after nasal and intravenous delivery: Studies in rats and comparison with a human study. <i>Pharmaceutical Research 2004;21:799-802.</i>	143

Section V General discussion and summary

Chapter 10	Discussion and conclusions	159
Chapter 11	Summary	181
	Samenvatting	187

Appendix	Dankwoord	194
	Sponsors	199
	Curriculum Vitae	

Abbreviations

$AUC_{CSF, in}$	Area Under the concentration-time Curve in CSF after nasal delivery
$AUC_{CSF, iv}$	Area Under the concentration-time Curve in CSF after intravenous administration
$AUC_{plasma, in}$	Area Under the concentration-time Curve in plasma after nasal delivery
$AUC_{plasma, iv}$	Area Under the concentration-time Curve in plasma after intravenous administration
BAC	Benzalkonium Chloride
BBB	Blood-brain barrier
C_{max}	Maximal concentration
CBF	Ciliary beat frequency
CNS	Central Nervous System
CSF	Cerebrospinal fluid
EDTA	Sodium Edetate
HB	Head back
HDF	Head down and forward
HPLC	High-performance liquid chromatography
HUR	Head upright
IN	Intranasal
IV	Intravenous
LHB	Lying head back
LHL	Lateral head low
LR	Locke Ringer (solution)
mM	MicroMol
min	Minutes
PK	Pharmacokinetic(s)
PD	Pharmacodynamic(s)
RAMEB	Randomly methylated β -cyclodextrin
RIA	Radio immuno assay
SD	Standard Deviation
T_{max}	Time to reach the maximum concentration
v/v	Volume per volume
w/v	Weight per volume

Chapter 1

General Introduction Current aspects of nasal drug delivery

1.1	Introduction	12
1.2	Nasal anatomy and physiology	12
1.2.1	Nasal anatomy	12
1.2.2	Nasal physiology	15
1.3	Local pathology	16
1.4	Nasal drug delivery	18
1.4.1	Aims & requirements of nasal drug delivery	18
1.4.2	Formulation	20
1.4.3	Devices	21
1.4.4	Techniques of administration	22
1.4.5	Side effects of nasal drugs	24
1.5	Topical treatment	28
1.5.1	Nasal drugs for topical treatment	28
1.5.2	Topical nasal drug deposition	30
1.6	Systemic treatment	31
1.6.1	Nasal drugs for systemic treatment	31
1.6.2	Nasal absorption	32
1.6.3	Nose to brain hypothesis	36
1.7	Current questions in nasal drug delivery	39
	References	40

Introduction

The nasal application of cocaine and psychotropic agents has been known for centuries especially in South American Indian traditional medicine. Surprisingly, the nose as drug administration site for drug uptake in the blood circulation has only received real interest from scientists and the pharmaceutical industry in the last two decades.

Intranasal administration of locally active drugs is much older. Improving irrigation of the nasal sinuses was described in a scientific publication in 1926 about intranasal drug administration for local treatment^{144,145}. Intranasal steroid treatment followed in the 1950s^{52, 55, 171}. Later new formulations were developed to reduce the systemic side effects of the used intranasal steroids^{118, 128}.

The nasal route of administration for systemic drug delivery became popular in the 1980s because the first-pass metabolism via the hepatic circulation can be avoided, the absorption improved and good patient compliance achieved³². Especially drugs that are ineffective orally and/or must be administered by injection received great interest. At this moment about 5 nasal products for systemic use are on the Dutch market and more than 10 in the United States. The number of systemic nasal drugs is growing, not only the amount of prescription drugs but also the number of 'OTC' (over the counter) drugs. In table 1, 2 and 3 a list of respectively prescription, OTC and investigational drugs is given. In this chapter a number of key issues concerning nasal drug delivery will be explained and an introduction is presented to current scientific questions influencing the future development in nasal drug delivery.

1.2 Nasal anatomy and physiology

To understand nasal drug delivery some basic knowledge about the nasal anatomy, physiology and pathology is mandatory.

1.2.1 Nasal anatomy^{143, 122, 170}

General anatomy. In general we can divide the nose in two compartments containing similar structures. Only one-third of the nose and nasal cavity is externally visible, the rest is well hidden centrally in the frontal skull. The nose is 5cm high and 9cm long and has a frontal part, the vestibule, a middle part, containing three turbinates and just before the nasopharynx a posterior part, the choanae.

The *nasal vestibule*, is covered with skin and hairs (vibrissae) and narrows down towards the middle part of the nasal cavity. The narrowest point is called the *nasal valve or internal ostium*, which is located approximately 1.5cm from the nasal tip. The cross-sectional area of the valve is only 30mm² (about 5 by 6 mm) on each side and accounts for 50% of the total resistance of the respiratory airflow from nostril to lung aveoli.

The middle part of the nose, right after passing the nasal valve, has on the medial side the nasal septum and on the lateral side, from top to bottom, three turbinates, a *superior, a middle and an inferior turbinate*. They are important in the regulation of airflow, humidity and temperature of the inspired air, controlled by the slit-like passages (meatus) lateral and under the turbinates. The middle meatus is in local disease and drug delivery an important area, called the *osteomeatal complex*. Most of the sinuses have their opening and drainage in this area underneath the middle turbinate and patency of this region is essential in the cause and treatment of disease. The osteomeatal complex is difficult to reach by an ordinary intranasal spray¹²².

The *nasal septum* is dividing the nasal cavity in two halves and the frontal third is richly vascularized. The region around the superior turbinate is a sort of narrow 'roof' and contains the *area of olfaction*. This roof of the nasal cavity is a fenestrated bone, the lamina cribrosa or cribriform plate, which allows the olfactory nerve cranially to enter right underneath the nasal mucosa, caudally.

Epithelial layers and cells. The nose has a large surface area, especially compared to the relative small cavity. The total surface area of both nasal cavities is about 150cm² and the total volume is about 15ml. The surface epithelium contains three epithelial layers, squamous epithelium, respiratory epithelium and olfactory epithelium.

The vestibule is covered with keratinized *squamous epithelium*, posteriorly changing in transitional and promptly to *respiratory epithelium*. Most of the septum, middle and inferior turbinates, just like rest of the airway, is lined with respiratory epithelium.

This epithelium layer, as shown in figure 1, contains columnar cells next to goblet cells. Each *columnar cell* has about 300 microvilli, which are short finger-like cytoplasmic expansions, increasing the surface area of the epithelium. The microvilli promote exchange processes and prevent the the surface from drying by retaining moisture. Columnar cells are either ciliated or non-ciliated. Cilia are motile hairlike appendages extending from the surface of epithelial cells. The number of cilia per cell is about 200, and they are beating in the direction of the nasopharynx with a frequency of 15Hz in vivo and about

10Hz as measured in in vitro test systems¹⁰⁹. Non-ciliated columnar cells are found in the first one-third part of the nose and ciliated cells are seen in the whole posterior part (including all sinuses) starting at the inferior turbinate head. Less cilia are seen in the areas with increased airflow, low humidity and low temperature¹⁴³.

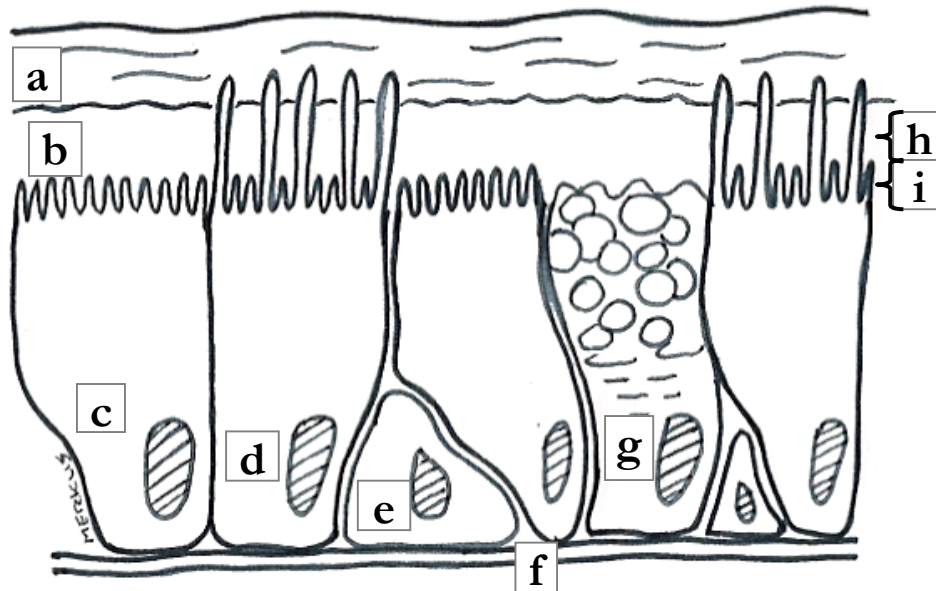


Figure 1. Nasal mucosa: ciliated, non-ciliated and goblet cells under a blanket of mucus. **a.**Mucus gel/top layer; **b.**Mucus sol layer; **c.**Non-ciliated columnar cell; **d.**Ciliated columnar cell; **e.**Supporting cell; **f.**Basal membrane; **g.**Goblet cell ; **h.**Cilia ; **i.**Microvilli .

Goblet cells, characteristic for airway epithelium, are mucus producing cells, increasingly located posteriorly in the nasal cavity. Their volume of secretion is probably small compared to that of submucosal glands. Goblet cells are, in contrast to the tight-junctions between columnar cells, less connected because of discontinuity of tight junctions²⁹. Tight-junctions opening or discontinuity could play a role in nasal drug absorption¹⁰⁹ as will be explained further on (paragraph 1.6.2).

Olfactory epithelium. Only the top part of the nose is covered with olfactory epithelium and comprises about 10- 20cm² (8%) of the nasal surface epithelium in humans. In contrast to animals this is a small area; in rats the olfactory area is about 50% of the nasal cavity⁷⁴. The olfactory epithelium has columnar cells with microvilli as supporting cells next to olfactory receptor neurons (ORN). These ORN extend from the nasal mucosa through the

cribriform plate into the olfactory bulb (figure 6). The ORN endings, the fila olfactoria, can be found in the top part of the nose, sometimes as far as the front of the middle turbinate¹⁰⁰. The potential role of this area as a transport route of certain drugs to the brain will be described in 1.6.3.

Glands. In the nose there are two types of glands, more anteriorly about 300 serous glands and more posteriorly about 100 000 seromucous glands. They produce the major part of nasal secretions, more watery anteriorly and a higher viscoelastic secretion posteriorly. The other secretions are from goblet cells and from plasma exudation, especially in an inflammatory state. The serous and seromucous glands are innervated parasympathetic (cholinoceptors).

Blood vessels. Several types of bloodvessels are located in the nose and differ from the rest of the airway vasculature in three ways. First, there are *venous sinusoids* in the nose, mainly located in the inferior turbinates. They are normally found in a semi-contracted state but can swell in certain conditions. Second, nasal vasculature shows *cyclical changes of congestion* (see 1.2.2 Nasal cycle and congestion). Third, there are *arterio-venous anastomoses*, probably related to temperature and water control and creating a shunted blood flow of at least 50% of the total nasal blood flow. Therefore, total blood flow through the nose per cm³ is greater than in muscle, brain or liver⁴⁷.

1.2.2 Nasal physiology

Nasal cycle, congestion and airflow. The width of the nasal passage depends on the congestion state it is in. Nasal cavity congestion and decongestion alternates from left to right and visa versa in a 2-4h interval. This is called “the nasal cycle” and is actively regulated via sympathetic innervation and tone of the venous sinusoids in the turbinates. The nasal airflow is influenced by this cycle and the primary respiratory airflow is under the inferior turbinate of the decongested site. Discussion in literature is ongoing about individual differences of airflow and how frequent the nasal cycle is present^{49, 61, 86, 103}.

Mucus and mucociliary transport. Nasal mucus is 95% water, 2% mucus glycoproteins and several other proteins, salts and lipids. The mucous glycoproteins are formed by the goblet cells and submucosal glands providing the viscoelastic properties of the mucus. The mucus layer that is formed can

be divided in a superficial blanket of gel, on top of the cilia, and the layer between the cilia called (an aqueous) sol layer.

The direction of the mucus layer is towards the throat and moves in approximately 3- 25mm/min (average 6mm/min). This nasal mucociliary clearance limits the residence time of particles or a drug formulation in the nose to only about 15 min^{94, 108}. The mucociliary clearance removes bacteria, viruses, allergens and dust from the respiratory tract, which makes it an important cleaning mechanism and 'first line of defense' against respiratory infection.

In research the effect of certain drugs on the mucociliary clearance is measured by the mucus transport time (MTT) or the ciliary beat frequency (CBF)⁴². In MTT the time of a stained saccharin drop from the head of the inferior turbinate to the pharyngeal cavity (dye visible or drop tasted) is measured in certain conditions. CBF is an in vitro photoelectric measurement of the ciliary beat frequency.

1.3 Local pathology

In nasal drug delivery there are two ways to look at nasal pathology. First, pathology treated with nasal drugs (paragraph 1.4.1) and second, pathology influencing nasal drug delivery (paragraph 1.6.2). In this paragraph some basic knowledge is given.

Nasal congestion. The reason for congestion of the nasal turbinates can be various (e.g. allergy, common cold, irritants, physiological). The venous sinusoids of mainly the inferior turbinate can swell and block the airway lumen in part (physiological) or complete (in disease). Blockage of airflow is annoying and tiring, which causes a desire for instant relief.

Allergic rhinitis. Exposure to an aeroallergen in allergic patients triggers an inflammatory reaction. At first, histamine, the most important mediator in an allergic reaction, causes itching, sneezing and also hypersecretion and vasodilatation of the nose. Secondly, cell influx of histamine-releasing-cells (mast cells and basophils) in the nasal mucosa is increased. Plasma exudation from postcapillary venules (a 'runny nose') is characteristic for inflammation in allergic rhinitis. Treatment of allergic rhinitis can be done by allergen avoidance, pharmacotherapy (oral antihistamines, nasal antihistamines, cromoglycate and steroids) and in some cases immunotherapy (desensibilization).

Infectious rhinitis and sinusitis. Rhinosinusitis is an infection of the nasal cavity and the adjacent sinuses, with as most important region the middle meatus. Patency of this region (osteomeatal complex) is crucial in the cause and treatment of this disease⁸⁷. Like the inflammatory reaction in allergy, a mediator reaction and cell influx set symptoms and appoint severity.

Sinusitis can be classified in three main groups: Acute, Recurrent and Chronic sinusitis¹⁰². In an acute infection the treatment comprises a combination of systemic antibiotics, local decongestants and/ or a nasal douche with saline. In chronic or recurrent infections the topical nasal treatment is done by corticosteroids (locally sometimes systemically) to maintain middle meatus and sinus patency^{50, 67}. If changes are seen on CT scan surgery is optional.

Nonallergic noninfectious rhinitis. Many causes are in this cluster of diagnoses. Some examples: Rhinitis medicamentosa, an overuse of topical vasoconstrictors. Drug induced nonallergic rhinitis, a reaction of the nasal mucosa to systemic drugs. Rhinitis senilic or rhinitis of the elderly, a persistent watery rhinorrhea without other nasal symptoms in elderly patients. Rhinitis sicca/atrophica, non functional and dry mucosa, of unknown origin. As last cause of nonallergic noninfectious rhinitis, if all known causes are excluded: Idiopathic rhinitis or rhinitis ‘e causa ignota’¹⁵¹.

Nasal polyposis. These blue-gray protuberances originate in the area of the ethmoid bone, the middle meatus and middle turbinate. This location is very specific since nasal polyps do not originate from the mucous membrane of the inferior turbinate^{95, 162}. The reason for this as well as the pathofysiology of nasal polyposis are still unknown. Like in infectious rhinitis the number of infectious cells can be increased in nasal polyposis. Polyps react well on treatment with local (and also systemic) corticosteroids. This treatment is considered as “golden standard” and if obstructive polyposis is not reacting to medication polyp, extraction is indicated.

Septal deviation. The nasal septum is seldomly positioned exactly in the midline and as a reaction to the deviation compensatory inferior turbinate hypertrophy is often encountered^{66, 75}. Only little known about the influence of a septal deviation on nasal drug absorption and on drug deposition. Future research is needed to increase knowledge about the influence of septal deviations on nasal drug delivery.

Impaired mucociliary function. Theoretically, impaired mucociliary function, change in mucus composition or secretion, and destruction of the nasal epithelial layer due to pathological conditions will most likely alter drug deposition and/or absorption, but scientific evidence is missing. Conditions like chronic rhinosinusitis, Sjögren syndrome, cystic fibrosis and Kartagener's syndrome will cause mucociliary dysfunction^{34, 76, 156, 175} and change the quality or quantity in periciliary fluid or mucus ('pathologic secretion')^{34, 156}. Also bacteria, low relative humidity, smoking, preservatives in nasal formulations and even insulin-dependent diabetes have been shown to destroy ciliated epithelium or cause ciliostasis^{48, 148, 155}.

1.4 Nasal drug delivery

Nasal drug delivery is an increasingly important route to administer drugs to patients. To create a basic understanding of the used terms, methods and current aspects in nasal drug delivery, we will go over this matter in five paragraphs. First we look at the aims of nasal drug delivery, before we touch upon the requirements for these aims. Second and thirdly, aspects of the formulation and the devices will be discussed. In the fourth paragraph the several techniques of administration are closely looked at, before some disadvantages and possible side effects are reviewed.

1.4.1 Aims & requirements of nasal drug delivery

Aims in topical treatment.

Topical nasal drug treatment we can allocate in five main goals: decongestion, anti-inflammatory, rinsing & cleaning, and 'other' goals.

Decongestion. Aim: To diminish the swelling of the nasal mucosa and especially the swollen middle and inferior turbinate. How: Either a vasoconstrictor action or a sympathetic signal are likely to establish this effect. Where: The inferior turbinate is the main site of swelling it is likely that a decongestive drug has to be deposited here.

Anti-inflammatory (allergic and non-allergic). Aims can be: desensibilisation (preventing an inflammatory reaction/ immunotherapy), decrease of inflammatory reaction (drug use before reaction), or symptom relief (reaction took place). How: treatment can focus on a down regulation of the inflammatory response, decreasing cell influx or cell activation, or counteract with the mediator (effects). Where: In anti-allergic drug deposition there is no scientific evidence of an optimum location, in inflammatory rhinosinusitis the osteomeatal complex area will be more beneficial.

Rinsing and cleaning. Aim: helping the normal cleaning and filtering function of the nose. How: mechanically increasing the rinsing and cleaning fluid, avoiding obstruction. Where: There is no scientific evidence of an optimum location for rinsing solutions, but easily obstructed locations or important mucus clearance routes will probably benefit most.

Other goals of topical nasal drugs: *Local anesthesia*, as used in an ENT practice, will be successful when efferent nerves fibers are effectively 'numbed'. High concentration of anesthetic on the nerve endings, without harmful interference with normal physiology will achieve this goal. *Softening or humidifying* the nasal cavity can be helpful in rhinitis sicca or after nasal (sinus) surgery. Restoring or covering the nasal mucosa or mucus layer will help to achieve this goal.

Aims in nasal systemic treatment.

For some drugs used as injection, for instance in pain and migraine, the nasal route of application is an interesting alternative. Also for some oral drugs the nasal route may have specific advantages. Some examples of nasal drugs and their target organ/ disease are shown in table 1 and 3.

In general the aim of all nasal drugs for systemic treatment is good bioavailability and no local side effects. In fact good nasal systemic drug delivery is a balance between the various factors influencing nasal absorption (paragraph 1.6) and the nasal bioenvironment. One of the most important advantages is that nasally absorbed drugs avoid the liver as first station in the blood stream, like after oral administration (first-pass effect) and as a consequence bypass drug degradation by liver metabolism. Good distribution in the nasal cavity and a long residence time may improve absorption.

New aims in nasal drug delivery

Nose to brain. When the target organ is the central nervous system (CNS) and especially the brain, some researcher claim a new route of drug delivery: direct transport of drugs from the nose to the brain/CNS. Clearly deposition in the olfactory region and a good absorption are essential. The possibility and basis for this new aim will be highlighted in paragraph 1.6.3.

Nasal vaccination. To create mass and rapid immunization, a nasally applied aerosol vaccine has a great potential. Development of nasal immunity and generalized immunization in a whole population has been proven successfully in several pilot studies in Russia and South America¹⁵³. Roth et al. gives a good overview of the potential of aerosol immunization as

it seems promising in cost –effectiveness, side effects and technical requirements¹⁵³.

1.4.2 Nasal Drug Formulation ^{15, 22}

A nasal formulation can be applied in various dosage forms (as solution, powder or gel) and will contain the drug and several pharmaceutical excipients.

Various dosage forms. The one most used is an aqueous *solution*. It is perhaps the most simple and most convenient form of formulation and practical in different types of administration devices (sprays and drops). When the environment (like temperature, light etc.) is more demanding a *powder* could be more suitable, on account of the more physical stability. Advantages are the absence of preservative and superior stability of the formulation. A disadvantage would be the nasal irritancy and gritty feel in the nose. A *nasal gel*, a high-viscosity thickened solution or suspension is a rather new dosage form in nasal drug delivery. It has some advantages, because it reduces post nasal drip and anterior leakage out of the nostril after application and may give little irritation to the nasal mucosa. Disadvantage of a gel is the difficulty in delivering an exact dose. Other dosage forms are *emulsions and ointments* of which too little is known whether they are really useful in nasal drug delivery.

Drug and formulation properties and their influence on drug absorption will be mentioned in *paragraph 1.6.2*.

Excipients. *Preservatives* are usually added to a nasal formulation. Several preservatives are used nowadays. Preservatives are still a current aspect in the discussions about safety. During the development of new nasal drugs the choice of an effective (sterile) preservative-free device or the use of preservatives in the nasal formulation is a key issue.

Other excipients added to a nasal drug formulation are: *Humectants*, like glycerin, used as a moisturizer, *Buffer systems*, to maintain the desired pH of the nasal formulation, *Antioxidants*, to prevent drug degradation, and *Absorption enhancers*, which may improve the nasal absorption.

1.4.3 Nasal drug delivery devices

Drop delivery devices. Drops can be delivered by several types of devices: a drop bottle, an one-unit dose container (*nasule*) or a *rhinyle*. Because of an awkward position of applying and an ‘open’ *dropcontainer*, which makes preservatives necessary, the bottle is more and more replaced by a spray or *nasules*. A *nasule* is a small plastic container mostly for one time use after removing the top part (e.g. Flixonase/ Flonase *nasules*®). Advantage of *nasules* is that the formulation can be preservative-free. Disadvantages could be the ‘squeeze force’-dependent volume (\sim dosing accuracy) and the head position dependent application²². A *rhinyle*, a calibrated plastic catheter from mouth into the nasal vestibule will blow the nasal drops/ powder in the nasal cavity and depending on the force of blowing the distribution is more posterior than with a nose spray^{41,63}. Compliance and reliability are debatable, low costs and the use of preservative-free device attracts pharmaceutical industry.

Sprays. There are three spray types known: the squeeze nebuliser, the propellant driven sprays and the mechanical dispensing pump sprays.

In a *plastic bottle ‘squeeze’ nebuliser* (e.g. Otrivin®, Nasivin®) the distribution and dosage given depends on the pressure of the squeezing hand¹¹⁹, making this device less suitable for potent drugs where a constant dose and distribution is preferred. Furthermore the open squeeze-bottle allows bacteria to enter the system, which will contaminate the fluid inside the container²².

Propellant driven or pressurized aerosol sprays deliver the drug as in an aerosol and are well known in the inhalation therapy. The use of CFCs in these devices is banned, consequently other propellants are used and investigated. Disadvantages are the cold sensation and the impact force.

Mechanical dispensing pump sprays are the most frequently used type of nasal sprays and can be divided in unit-dose and multi-dose systems. Unit-dose is preferred for a infrequent-used application, whereas the multi-dose or container spray will be more suitable for the frequent user.

Due to the availability of metered dose pumps and actuators, a nasal spray can deliver an exact dose from 25 to 200 μ L. The particle size and morphology (for suspensions) of the drug and viscosity of the formulation determine the choice of pump and actuator assembly. Spray developments can be expected in different modifications of the tip, the swirl chamber, counting mechanism, ergonomics, design and even chip-controlled sprays, but the clinical relevance of these modifications has to be seen²². In addition, different spray

performances *in vitro* do not necessarily translate into deposition differences in the nose *in vivo*¹⁶³.

Powder is delivered to the nose by mechanical pump spray, a nasal inhaler or a rhinyle^{41, 77}. In principle any pulmonary powder inhaler can be adapted for nasal applications³⁸. Powder can be delivered accurately, repeatably and easily just as solution sprays.

Gel delivery has been difficult because exact dosage delivery was not able until a few years ago. Now metered dosage is possible.

1.4.4 Techniques of administration

A scala of factors play a role in the technique of administration of a nasal formulation as a spray or as drops. Head position, volume and frequency of administration, angle of spraying, inhaling or sniffing and compliance have all been investigated by many research groups. We have to emphasize that all studies were done with healthy volunteers and therefore the outcome might differ from the actual therapeutic outcome in patients.

Head position. Nose sprays for nasal drugs are generally multidose container *sprays* and used in the upright position. The administration of nose *drops* is different. Four positions to instill nose drops have been described, all shown in figure 2:

The most simple (but unsuccessful) technique to use a nose drop is the Head Back (HB) position. This technique will give the drop the opportunity to go down the inferior meatus with a quick slide to the throat^{105, 31}.

The Lying Head Back (LHB) position is “Lying down in supine position with the head just off the bed in hyperextension, so that the chin is the highest point of the head”. It is recommended by some manufactures and it is actually the first position published (1926)^{144, 145} When republished in 1979 this position was the first of a sequence of steps and since then this position is often named after Mygind¹²⁰. The sequence of 6 steps is probably too difficult for patients in their daily routine, but the initial position is comfortable and easy to use.

Head down and forward (HDF) is often referred as “Praying to Mecca”; “Kneeling down and with the top of the head on the ground. The face is upside down, the forehead close to the knees and the nostrils are facing upward”³¹.

Lateral head-low position (LHL)^{134, 135} later described as the “new” Ragan position¹⁴⁷ is the fourth known head position: “Lying on the side with the

parietal eminence resting on the bed (pillow under the shoulders or no pillow). Nasal drops are instilled into the lower nostril².

These techniques of nasal drug administration to the middle meatus have been an ongoing topic for study and debate. Consensus about a superior administration method is lacking and remains a very interesting subject for further research.

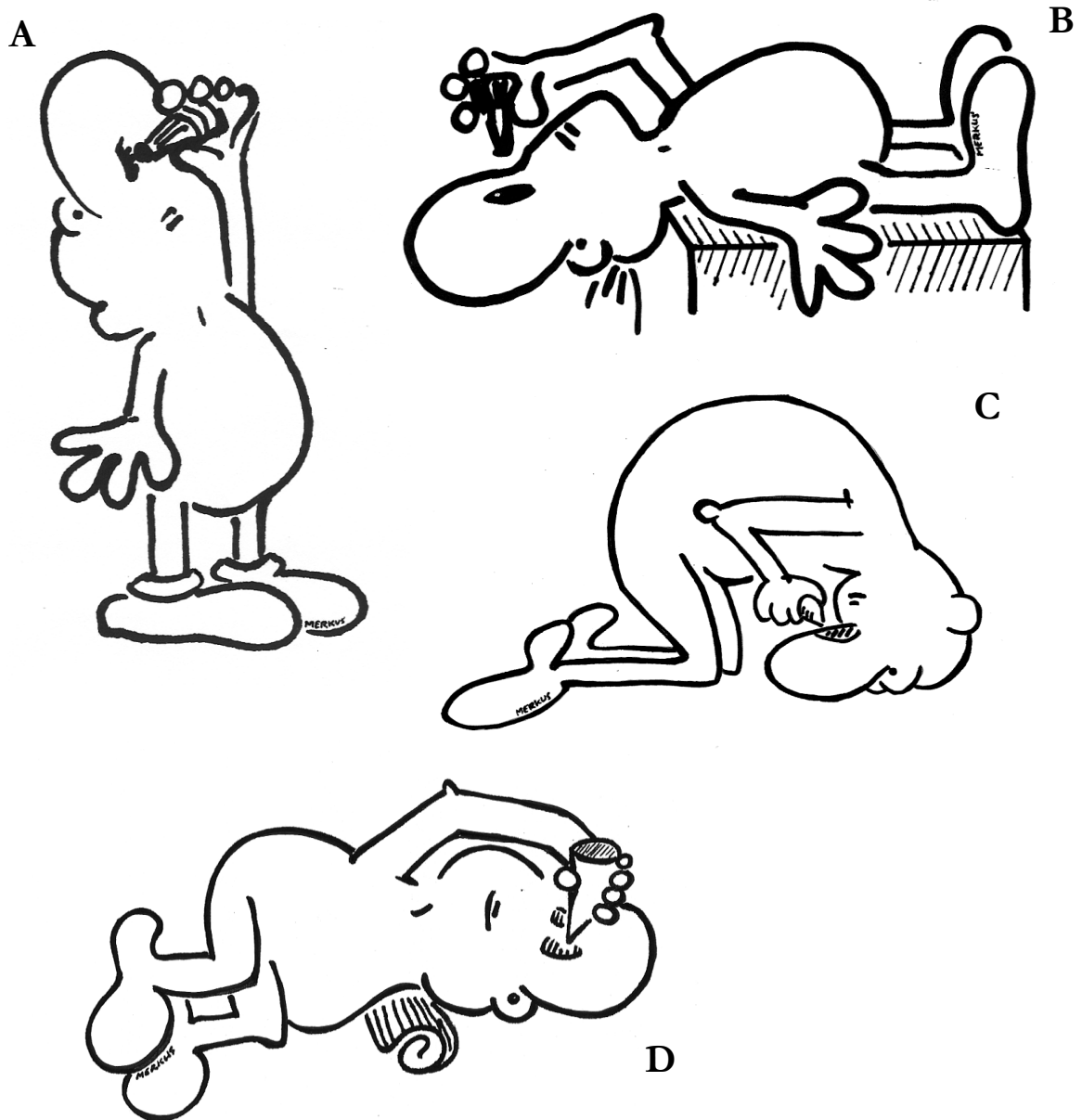


Figure 2. Four head positions to instill nasal drops.

A. Head Back (HB), **B.** Lying Head Back (LHB) also called Mygind position, **C.** Head Down and Forward (HDF), also called 'praying to Mecca' position, **D.** Lying Head Lateral

Head position affecting compliance. Some head positions may be uncomfortable, affecting compliance. HDF was the most uncomfortable position followed by LHB and HB^{82, 83, 91, 92}. The LHL position was suggested to be the most favorable position for patients to adopt^{82, 147}. Training a spray technique improves the compliance, but whether this is true for different head positions remains to be seen⁵³.

Volume and Frequency. The optimum volume and frequency has not been extensively studied and a multi-factorial evaluation (incl. intraindividual differences, compliance, efficacy) is still needed.

Nasal aerosol pump sprays with a larger volume (100- 160 μ l) have a significant greater nasal distribution area compared to smaller volumes (50- 80 μ l)^{125, 127}. Even when the total volume is the same, *local* distribution is improved when the administered volume is given all at once (100 μ l) rather than twice half the volume (50 μ l)¹²⁵. This seems to be in accordance with the clinical effect of a topical nasal steroid, seeing that once a day seems to be frequent enough²⁵.

In contrast to local treatment, in systemic treatment done via a nasal spray, two doses of each 50 μ l, is more efficient than a single dose of 100 μ l as the bioavailability of desmopressin increased (figure 5)^{63, 64}. Nasal clearance of twice a doses of 50 μ l was only slightly slower than 100 μ l at once, which again is in favor of the uptake in systemic treatment.

Angle of spraying. Consensus about the influence of the cone angle of a nose spray is not available, although there is a slight tendency towards a 35-45 degree angle^{13, 23, 119, 124, 125, 173}. The difference in research methods used prevents us from drawing conclusions.

Inhaling or sniffing. The effect of vigorously inhaling whilst spraying had no significant effect on the distribution of an aqueous spray^{60, 68, 119, 127}. In contrast to these studies, in a nasal model cast an increased inspiratory flow rate will give an increased deposition⁸⁹ and some researchers found that the clearance rate increased when sniffing during aerosol spray delivery¹¹¹. When a 'sniff-like' inhalation takes place right after spraying some already deposited droplets will move posteriorly¹¹⁹.

1.4.5 Local side effects of nasal drugs

The effect of nasal drugs and excipients on ciliary activity

It is obvious that during chronic intranasal drug application, the drug itself and the formulation excipients should not disturb the nasal mucociliary clearance, because it is a very important defense mechanism of the respiratory tract. Frequent nasal drug use can cause degenerative changes and impairment of mucociliary transport, which may be responsible for nasal obstruction and posterior nasal drip⁹⁸.

The influence of drug formulations on the ciliary beat frequency (CBF), measured in 'in vitro' experiments, is an ongoing issue to establish the safety of nasally administered drugs. Various formulation excipients such as preservatives^{14, 18, 33, 152} and absorption enhancing compounds^{115, 152} have been tested. Remarkably the cilio-inhibiting effects of some daily used nasal corticosteroids, have not been investigated.

CBF Research method. Some tests to assess the influence of drugs and drug compounds on the ciliary activity in vitro have been using human adenoid tissue. Already in 1982 Van de Donk et al. proved that in CBF measurements chicken embryonal tracheal tissue is a good substitute for human adenoid tissue⁴³ and in 1999 Boek et al. confirmed these findings^{19, 20}.

Other local side effects of nasal drugs

Next to cilio-inhibiting effects of nasally applied drugs, there are several other side effects known from the literature. Still most of them are linked with the use of a topical drug, which we will explain in the next paragraph. One side effect which could be applicable to all nasal sprays is a septal lesion caused by the nasal applicator^{17, 173}. This can be due to frequent improper use of the device, which makes good instruction on 'how to use' essential.

Table 1. Prescription nasal drugs

Drug	Examples of products	Indication
Azelastine	Astelin®, Allergodil®	Allergic rhinitis
Beclomethasone dipropionate	Beconase®, Vancenase® Beclometason	Management of seasonal and perennial (allergic) rhinitis
Budesonide	Rhinocort® Budesonide	Management of seasonal and perennial (allergic) rhinitis
Buserelin (acetate)	Suprecur®, Profact®	Prostate carcinoma, endometriosis
Butorphanol tartrate	Stadol NS®	Management of pain/ Migraine
Calcitonin	Miacalcic®	Postmenopausal osteoporosis
Desmopressin acetate	Minrin®, Octostim®	Nocturnal enuresis, Management of diabetes insipidus, Hemophilia A, von Willebrand's disease (type 1)
Dexamethasone	Decadron®	Treatment of inflammatory nasal conditions or nasal poliposis
Dihydroergotamine mesylate	Migranal®	Management of migraine
Estradiol	Aerodiol®	Management of menopause symptoms
Flunisolide	Syntaris®	Management of seasonal and perennial (allergic) rhinitis
Fluticasone propionate spray and drops	Flixonase®	Management of seasonal and perennial (allergic) rhinitis
Ipratropium bromide	Atronase®	Treatment of bronchospasm
Levocabastine	Livocab®	Allergic rhinitis
Mometasone furoate	Nasonex®	Management of seasonal and perennial (allergic) rhinitis
Nafarelin acetate	Synarel®	Treatment of symptoms (dysmenorrhea, dyspareunia and pelvic pain) associated with endometriosis.
Nicotine	Nicotrol®	Smoking cessation
Oxytocine	Syntocinon®	Stimulates milk ejection in breast feeding mothers
Sumatriptan	Imigran®	Management of migraine
Triamcinolone acetonide	Nasacort®	Management of seasonal and perennial (allergic) rhinitis

Zolmitriptan	Zomig®	Management of migraine
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Table 2. Examples of non-prescription nasal drugs, OTC ('over the counter') drugs.

Drug	Example(s) of product	Indication
Cromolyn sodium	Allergocrom®, Lomusol®, Vividrin®	Allergic rhinitis
Naphazoline	Rhinex®	Decongestion
Oxymetazoline	Nasivin®	Temporary relief of nasal congestion
Phenylephrine	Sinex®	Temporary relief of nasal congestion
Tramazoline	Bisolnasal®	Decongestion
Xylometazoline	Otrivin®	Temporary relief of nasal congestion

Table 3. Examples of investigational nasal drugs

Drug/ disease	Examples
Antibiotics	gentamicin
Benzodazepines	lorazepam, midazolam, diazepam
Hormones	insulin, human growth hormone, steroid hormones
Pain medication	morphine, fentanyl
Vit B ₁₂ deficiency substitute	hydroxocobalamin
Parkinson medication	apomorphine
Vaccines	influenza vaccine

1.5 Topical treatment

1.5.1 Nasal drugs for topical treatment

Nasal decongestants. Imidazoles (like oxymetazoline and xylometazoline) or sympathomimetic amines (like phenylephrine) are the main components used as decongestant (table 2, OTC drugs). They are used in the treatment of an inflammatory or idiopathic rhinitis (infectious, allergic or a common cold). Although these drugs are very potent, only a symptomatic relief is provided due to the short duration of their effect. Whether decongestants (sometimes in combination with other drugs) shorten the duration of an acute or chronic sinusitis, is still debatable^{9, 132, 164}.

Nasal decongestants may have serious *side effects*, reason to limit their use to a maximum of 5 to 7 days. The most well known side effect is rhinitis medicamentosa. Rijntjes and others, showed in rhinitis medicamentosa patients, those with an abnormal (addictive) period of frequent imidazoles use, that several mucosal changes are seen^{98, 150, 168}. Hyperplastic epithelium including goblet cells, an increased number of gland openings and a chronic inflammatory and hypersecretory state of the mucosal layer were noted.

Another important side effect of decongestants is the rebound effect: when quitting daily use, after use for several days, the congestion will return prominently (rebound) and can even cause drug addiction⁵⁶. Finally by frequent decongestant use, the drug itself and the additives and/or preservatives can be harmful to the ciliary activity (paragraph 1.4.5). It seems clear that safety of these 'over the counter' drugs remains an important topic for further research.

Nasal anti-histamines. Antihistamines, (histamine-1 receptor antagonists), are an effective treatment for allergic rhinitis, but not first choice in the treatment of chronic (allergic) rhinosinusitis. Only in mild or incidental symptoms nasal antihistamines are advised in allergic rhinitis. This is due to the minimal effect of antihistamines on mucosal swelling, especially compared to corticosteroids¹⁷⁴.

Side effect of (older) nasal anti-histamines is drowsiness because of the good systemic absorption^{136, 172}.

Nasal corticosteroids. Several corticosteroid nasal drops and sprays are on the market nowadays, as shown in table 1. The clinical efficacy of the corticosteroid sprays (like triamcinolone acetonide, fluticasone propionate, budesonide and mometasone furoate) exhibits no major differences^{30, 35, 101}.

They are very potent inflammatory drugs, by avoiding cell influx and cell activation, used in chronic rhinosinusitis and polyposis^{10, 50, 67, 121} and are preferred drugs in the World Health Organization consensus statement on treatment of allergic rhinitis^{26, 30}.

Long use of nasal corticosteroids is proven to be safe¹⁶, without suppression of the hypothalamic-pituitary-adrenal axis. This resulted in the approval of intranasal corticosteroids in young children (from 4 years old) in recent years^{51, 90, 158}. Although the (low) systemic uptake, still caution should be taken when increasing the licensed doses⁹⁹.

Another current issue is the use of corticosteroid *drops* (as compared to ‘the usual spray’) as effective treatment of nasal polyposis^{8, 85, 137}. Whether drops are more effective than a spray or powder in polyposis treatment remains to be seen and could be strongly depending on the difference in drug deposition between drops and spray.

Side effects of nasal corticosteroids are epistaxis, pharyngitis, nasal crusting and drying, and possible atrophic rhinitis or even a septal perforation. Discussion about odor and taste¹¹, reduction of the recovery time after an acute rhinosinusitis¹¹³ and the ‘best’ technique of spraying¹⁷ are ongoing aspects of nasal corticosteroids.

Nasal ipratropium bromide. This anticholinergic drug is used mainly in the treatment of asthma, but can be effective on the nasal glands in the treatment of constant rhinorrhea (as in rhinitis of the elderly)^{54, 106, 166}. Strangely enough ipratropium bromide as a nasal spray is available in several European countries, but not on the Dutch market anymore.

Saline solutions. Nasal 0.9% saline douches are used in several nasal problems as a moisturizing and cleansing liquid. Especially when patency is important and removal of crustae or debris are necessary, nasal douches can be helpful. The positive effect of nasal irrigation with isotonic salt solution (saline 0.9%) on patients with sinonasal symptoms has been proven^{12, 65, 167}. Changing this solution to a more salty, hypertonic solution has a negative effect on the mucosa⁶⁹ and changing to Ringers lactate solution could improve mucosal ciliary function²¹. Clinical consequences of these solution changes are unknown.

Nasal anesthetics. For fast local anesthesia used by physicians, some sprays, gels or drops are on the market. The main components of these drugs are cocaine derivatives, like lidocaine and tetracaine (1-10%). Although these

products act fast (1-5 minutes) they may cause a stinging and burning sensation. As explained further on, this could be due to the physical properties of the drug or the drug additives. Another local side effect could be the absent swallow reflex, causing potential aspiration. Serious systemic events are seen when overdosing leads to cardiovascular or nervous system side effects.

Antibacterial nasal drops/ointment. Nasal ointments, like mupirocine (Bactroban®) or Terra-Cortril® with polymyxin B, are effective in the treatment or prevention of a local bacterial infection or nasal carriage of (resistant) bacteria^{138, 160}. A side effect of these ointments could be myospherulosis, especially post-surgery using lipid-based packing material¹⁶⁰. Also local irritation and burning are possible.

Capsaicin. Although this drug is still investigational, recent work by van Rijswijk¹⁵¹ and earlier studies^{107, 149, 176} have clearly proven the potential role of capsaicin as treatment of idiopathic rhinitis.

However the exact working mechanism is unknown, repeated applications of capsaicin will lead to desensitization of the ‘pain receptors’ of the nasal sensory neurons. Side effects of intranasal capsaicin are nasal burning and lacrimation, but no serious or systemic side effects have been noticed.

Other nasal ointments/ solutions. In rhinitis sicca/atrofica, or non functional and dry mucosa, several drugs and treatments are suggested, like bromhexine^{123, 165}, dexpanthenol⁸⁴ and propylene glycol nasal gels.

1.5.2 Topical nasal drug deposition

Based on a review of the literature, the American Academy of Otolaryngology-Head and Neck Surgery Foundation has tried to define the best method of administering intranasal corticosteroids but interestingly, they could not draw definitive conclusions¹⁷. This is remarkable, since large groups of patients are put on daily corticosteroids for the treatment of their nasal polyposis or (chronic) rhinosinusitis in the absence of a widely accepted advice how to use the prescribed drug.

Multiple factors play a role in the pathway of drugs towards the middle meatus when treating both nasal polyposis and (chronic) rhinosinusitis. First of all the type of drug formulation, drug volume, particle size and various delivery devices will have influence^{22, 60, 93, 116}. Secondly the great variety of used research methods and small investigational groups of volunteers and

patients impede clear conclusions^{2, 6, 17, 68, 173}. Thirdly, individual anatomical differences will probably alter the nasal drug delivery⁴⁶, but the performed studies have not been taken these differences into account and draw their conclusions based on healthy volunteers investigations. Finally, the effect of pathological conditions, like nasal polyposis, is not tested in relation to topical nasal drug delivery, even though these conditions are the main reason for this type of treatment.

1.6 Systemic treatment

1.6.1 Nasal drugs for systemic treatment: a wide variety of drugs

Intranasal administration of systemic drugs has the advantage of a relatively large surface area⁷, a rich vascular network and access to the nonhepatic systemic circulation³². Due to these facts bioavailability of some drugs given intranasally, is even similar to intravenous administration. For instance, some drugs poorly absorbed orally can be well absorbed intranasally.

Nasal drugs for systemic treatment are easy to administer, without pain or gastro-intestinal discomfort, improving compliance. Not surprisingly there is an increasing number of nasal drugs available for systemic treatment on the market (table 1), or in clinical trails (table 3)^{81, 15, 114}. The number and variety of indications is still growing (e.g. hormones, central nervous system drugs, cardiovascular drugs, antibiotics).

Nasal drug delivery as way of delivering drugs to the human body has also disadvantages and restrictions. It is only suitable for drugs active in low doses and for drugs that are soluble in a watery solution and able to pass the mucosal layer. Nasal drugs should not cause local irritation or interfere too much with normal physiology. Drugs designed for slow absorption or a constant blood concentration are not optimal for nasal drug delivery, because the absorption of nasal drugs show a fast “pulsatile” absorption profile.

1.6.2 Nasal absorption

There are four known pathways across the epithelium, three types of transcellular transport and one paracellular pathway. These ways of absorption, as briefly explained below, are a more experimental model in basic (animal) research and are still discussed in pharmacokinetic and pharmacodynamic literature. Absorption in general is influenced by: formulation-, nasal-, and delivery factors.

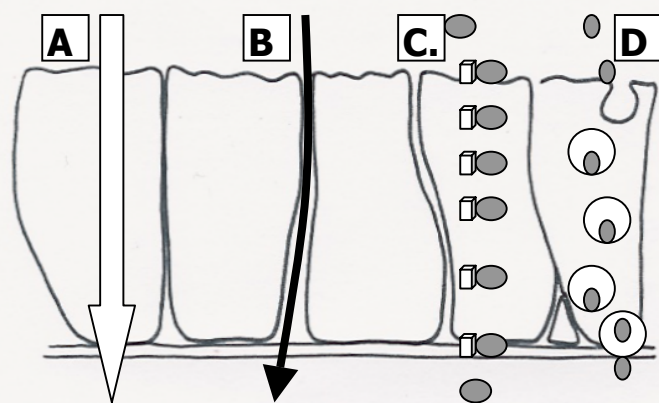


Figure 3.
Routes of absorption
A. Passive intracellular /transcellular transport,
B. Paracellular/ tight junction transport,
C. Carrier-mediated transcellular transport,
D. Transcellular transcytosis

Routes of nasal absorption. (Figure 3)^{71,112,170}.

A. *Passive intracellular/transcellular transport*, the drug is transferred by passive diffusion through the cytoplasm of the cell, **B.** *Paracellular/ tight junction transport*, that is, through the cell-cell junctions and the spaces between cells, **C.** *Carrier-mediated transcellular transport*, a specific carrier takes the drug through the cell, **D.** *Transcellular transcytosis*, which is drug uptake into vesicles which cross the cell.

Formulation factors. Absorption of intranasal drugs is affected by a number of formulation and drug-specific characteristics, like molecular weight and size, solubility, lipophilicity, ionization, pH, osmolarity and viscosity^{7, 71, 81, 146, 170}.

When *molecular weight* is below 300 daltons (Da) most drugs may permeate through the membranes¹¹², between 300 and 1000 Da absorption is influenced by molecular size, and when molecular weight exceeds 1000 Da the absorption decreases rapidly^{1, 32}.

Drug *solubility* is important in determining absorption, but insufficient data are available to define clear standards. On increasing *lipophilicity* the permeation of a compound increases through nasal mucosa. But a too high degree of lipophilicity diminishes water solubility and the drug could be swept away by mucociliary clearance⁹⁹. *Ionization* of the drug in the nasal formulation and the *pH* of the formulation, together with the physico-chemical properties of the drug molecule, are key factors in the absorption process of some drugs. For each drug these factors can be very complicated and may lead to extensive pharmaceutical-chemical and animal studies, in order to elucidate the nasal absorption mechanism. When looking at *osmolarity*, an isotonic solution is preferably the best nasal solution, because hypertonicity will lead to shrinkage of the nasal mucosa^{129,130}. *Viscosity* has controversial effects: higher viscosity increases contact time with the nasal mucosa (increasing permeation time),

probably causing a better absorption. However, in some cases a highly viscous formulation may delay the permeation of the drug molecule through the mucus layer on top of the nasal epithelial cells, disturbing the nasal absorption process. Also a viscous formulation may disturb the mucociliary clearance.

Formulation factors improving absorption. To improve systemic absorption several changes to the properties of the formulation can be altered. Improving dosage forms, like changing to a powder^{77, 142}, a gel dosage¹¹⁷, using bioadhesives or absorption enhancing agents^{72, 109, 112}, or change viscosity¹³⁹ could increase the systemic uptake. Noteworthy is that use of enhancers, preservatives and additives, in order to improve the efficiency of the drug, have to be chosen carefully because of the potential harmful influence on the mucosal epithelium or the ciliary activity.

Nasal factors in drug absorption. The nose can be divided in different regions with microscopic and macroscopic differences having their impact on permeability⁷. The *nasal vestibule and nasal valve* area have due to the nasal hairs, the narrow region and stratified keratinized squamous epithelium, the least permeable surface. More posteriorly, the *respiratory region* (area of the middle and inferior turbinate and meatus) is the most permeable region due to the large surface area (micro- and macroscopically), rich vasculature and maximum nasal secretions. It has the highest concentration goblet cells (with discontinuity of tight junctions) that could be very important in the absorption of drugs deposited here^{7, 169}. The *olfactory region* (area of superior meatus and turbinate) has specialized ciliated olfactory nerve cells, less vascularization and is hard to reach by nasal drugs, which makes it less suitable for drug absorption.

Although several studies describe the role of *nasal enzymes* in drug degradation^{36, 63, 154}, or ways to avoid this degradation⁷, it seems a theoretical problem. The absorption of drugs in the nose is so fast (within 15-30 minutes) that never any role of enzymatic degradation of the drug in the nose has been found in all the nasal drugs that are on the market.

The *mucus*, of which 1.5-2.1 L is produced a day, may influence the permeability. A too thick or too thin layer of mucus will inhibit the mucociliary clearance and the time of contact between drug and mucosa. Also changes in mucociliary clearance (paragraph 1.3 Local pathology) can change drug absorption^{34, 109, 159}.

Nasal pathological conditions. It is hard to give the exact influence of pathological conditions, because they have not been studied in sufficient detail. Paragraph

1.3 describe briefly the most common nasal pathologies and some consequences of these conditions, but it would be a sheer guess to what extent they could alter the drug absorption. The only confirmed outcome is that systemic absorption of several different drugs, is *not* decreased by a *common cold or rhinitis*: buserelin⁹⁶, desmopressin¹³¹, dihydroergotamin⁷⁰, nicotine¹⁰⁴ and estradiol⁴⁵.

Other single study remarks, about nasal pathological conditions and their influence on drug absorption, are cited below.

On the 'hollow', concaved, side of a *septal deviation* mucociliary transport time is increased, total of cilia is decreased⁷⁹ and drug distribution is decreased on the prominent convex side¹⁷³. *Nasal polyposis* can reduce drug absorption¹⁴³, decrease clearance rates but leave the deposition pattern unchanged⁹⁷. Seasonal *allergic rhinitis* will diminish the nasal absorption compared to absorption outside the pollen season and absorption in healthy subjects⁵⁷. Contrary, perennial house dust mite allergy has *no* effect on the nasal absorption⁵⁸. A congress report shows that a 'runny nose' contributes to a fast clearance and that a congested nose can block the passage of the applied formulation²³. Theoretically, *impaired mucociliary function*, change in mucus composition or secretion, and destruction of the nasal epithelial layer due to pathological conditions will most likely alter drug deposition and absorption, but there is no scientific proof^{23, 59}.

The real influence on nasal drug absorption in all these pathologic conditions remains largely unrevealed and undoubtedly a challenging field for future research.

Delivery factors in nasal absorption. As mentioned before systemic uptake may be increased by longer residence time and a wide spread over the mucosa. These factors are tested in spray or drop delivery device studies and only a few studies have covered this topic.

Longer residence time: Clearance of a spray is much slower than drops, since most of the spray is deposited on the non-ciliated regions. Although distribution and clearance of drops is less predictable than after spray administration²⁸, a shorter residence time is seen because especially the drop solution spread more extensively over the ciliated area (figure 4)^{62, 126} using pump sprays, 111 using an aerosol spray

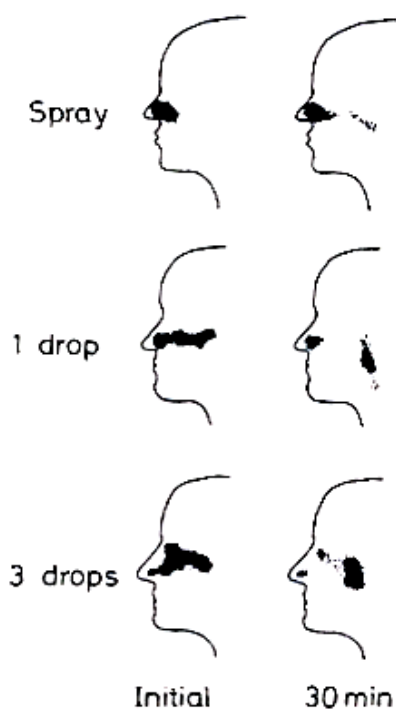


Figure 4. Distribution of a nasal spray compared to drops.

From Hardy 1985, with permission of Journal of Pharmacy and Pharmacology, Pharmaceutical Press, London, UK

Area of distribution. A larger distributed area will improve systemic uptake, as confirmed by deposition of an ointment in two nostrils compared to one nostril³⁹. The best site of deposition in the nose is debatable and depending on the properties of the drug. For instance, for a well absorbed compound like nicotine, the nasal site of deposition appeared *not* to influence the nasal bioavailability⁸⁰.

Volume. The spread of volumes seems to improve nasal absorption of a drug with low intranasal absorption, as two doses of each 50 μ l, seems more efficient than a single dose of 100 μ l of desmopressin⁶⁴. Nasal clearance of twice a dose of 50 μ l was slightly slower than 100 μ l at once, which was also in favor of the uptake⁶⁴. Increasing the volume above 100 μ l did not increase the uptake (figure 5)⁶³. These experiments are interesting but should be repeated with other drug properties, because desmopressin is a hydrophilic drug with a relatively high molecular weight and a low intranasal absorption.

Device. When comparing systemic uptake after drop or spray administration, better uptake after spray administration was seen in two studies^{62, 63}.

Given the studies mentioned above, the advice in systemic treatment seems more in favor of drug delivery by spray when compared to drops. Still confirmation is needed and comparison with a gel, powder or ointment are not (sufficient) available.

Angle. Either in local therapy as in systemic nasal drug delivery consensus about the influence of the cone angle of a nose spray is not available.

Olfactory delivery. An optimal method for drug delivery to the olfactory area has not (yet) been investigated, but the outcome of the head position studies suggests that drops and gravity together have advantage over a spray^{63, 82, 147}.

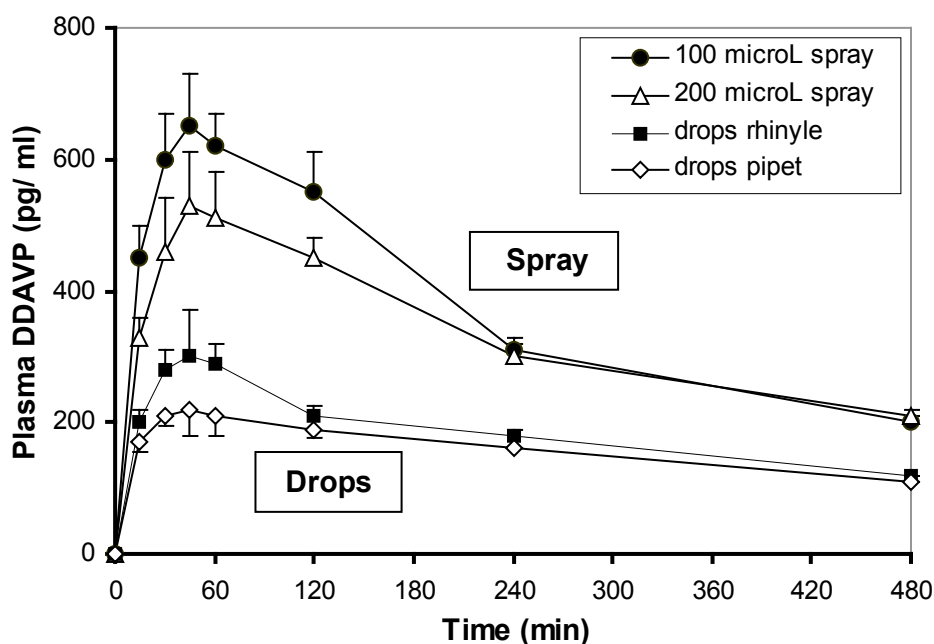


Figure 5. Improved systemic uptake with a 100 μ l spray compared to a 200 μ l spray and drops of desmopressin (DDAVP).

Adapted from Harris 1986 with permission of John Wiley & Son, Inc., Hoboken, USA.

1.6.3 Nose to brain hypothesis

One of the most interesting topics in recent nasal drug delivery research is concerning the question: “Is it possible to circumvent the blood-brain barrier (BBB) and achieve a direct access to the cerebrospinal fluid (CSF) or brain by administering drugs intranasally?”

For more than 30 years a large number of studies, mainly in animals, have described the direct transport of a variety of compounds directly from the nose to the CSF after intranasal administration^{37, 73, 110}. In 2002 a human study suggests that “sniffing neuropeptides” may lead to an accumulation of these peptides in the CSF within 80 minutes²⁴. This new route would be a revolution in drug delivery because nowadays many drugs targetting the human brain have great difficulties in passing the BBB.

Already physiological and histological studies in animals and men have demonstrated that mucosa in the upper part of the nose is connected with the cerebral perivascular spaces and the subarachnoid spaces of the brain olfactory lobes, which would make this pathway for drug transport feasible^{78, 100}. It is suggested that cerebrospinal fluid (CSF) runs directly underneath the olfactory mucosa see figure 6²⁷. According to Pardridge, following intranasal application a drug has to traverse two epithelial barriers in series, i.e. the nasal

olfactory mucosa and the arachnoid membrane, in order to gain access to the CSF compartment¹³³. Diseases of the central nervous system (CNS) like Parkinson's, epilepsy and Alzheimer's are prone to benefit from nasal drug delivery if this 'nose to brain' route is confirmed. The question is whether this new route of drug delivery is a real treatment option or merely a scientific hype.

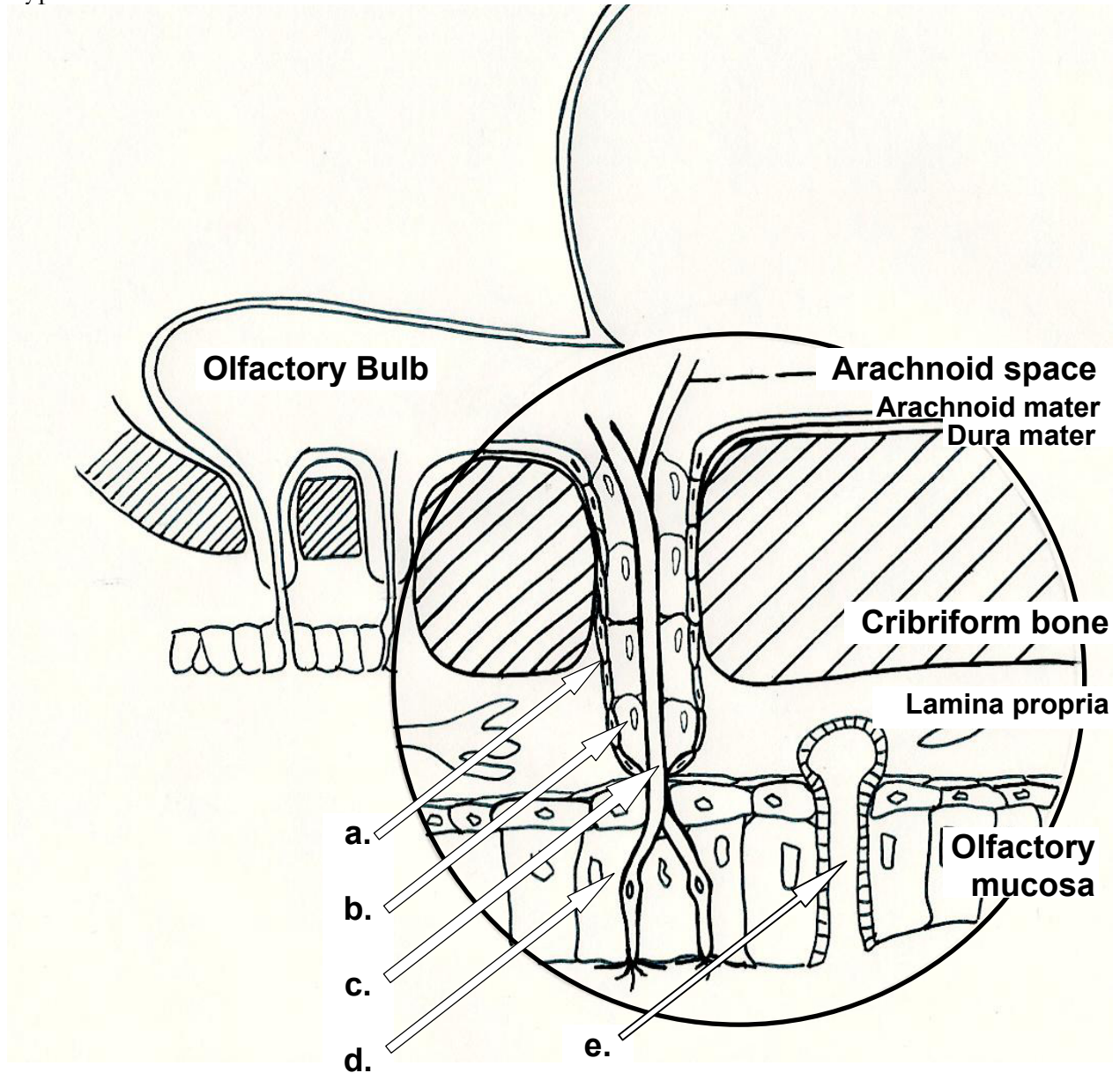


Figure 6. Arachnoid 'slieve' through the cribriform plate.

"Slieves" of arachnoid space surround olfactory nerve endings through the cribriform bony plate into the nose. This anatomical appearance in the nose could be important in 'nose to cerebrospinal fluid' drug delivery. **a.** perineurial cells, **b.** Schwann's cells, **c.** fila olfactoria/olfactory receptor neuron, **d.** olfactory mucosa supporting cell, **e.** Bowman's gland.

Figure is modified from Bradbury 1981 with permission of American Journal of Physiology, Bethesda, MD, USA

Animal studies have shown direct drug transport from the nasal cavity to the CSF or (directly) to the brain. Dyes, viruses, metals, amino acids, proteins, hormones, antibiotics, antiviral agents and genes have subsequently been reported over the past 75 years¹¹⁰. From the results of these studies one may expect that the same route is feasible in humans. In animals however there is a much larger olfactory area while CSF volume and turnover rate differ largely from the human situation^{74, 170}. Also some of the formulations used in the animal studies contained mucosa-damaging permeation enhancers (e.g. organic solvents)^{3,4} and some nasal formulations were used in a relatively aggressive way (continuous perfusion, insufflation with an atomizer)¹⁵⁷. Such a treatment would be unrealistic in the human situation.

Human studies. Up to 2002 some pharmacodynamic human studies are supporting the nose to brain hypothesis but did not provide clear pharmacokinetic evidence. Pietrowsky et al. have proven that brain potentials could be directly influenced by nasal drug administration compared to intravenous injection of cholecystokinin and vasopressin in humans^{140, 141}. Also intranasal angiotensin II has a direct central nervous action compared to intravenous administration⁴⁰. In a comparable setup intranasal administration of ACTH 4-10 and insulin gave direct central nervous effects^{88, 161}. These studies provide pharmacodynamic evidence in advantage of the 'nose to brain' hypothesis.

In 2002 Born et al. published the first pharmacokinetic human data after administering neuropeptides intranasally and detecting a good uptake in the CSF, with low plasma levels. The results suggest that very small amounts of peptide molecules travel to the CSF via the olfactory region, but the authors admit that their data cannot establish that intranasal administration results in greater uptake in the CSF than does intravenous administration²⁴. Moreover, 20 years ago in experiments with other neuropeptides in dogs, no direct or facilitated transport from nose to the CSF could be demonstrated⁵. Obviously the nose-to-brain transport pathway hypothesis is still controversial. Well-controlled studies in humans are missing in which a comparison is made of the CSF/brain levels of drugs after intranasal and intravenous administration of similar doses of the same drug in the same patient.

1.7 Current questions in nasal drug delivery

Nasal drug delivery is a constant process of new developments and changing concepts. The past paragraphs gave an overview and some basic knowledge about nasal drug delivery. It is clear that many questions are still unanswered in local drug delivery, and also in the area of drug absorption of nasally administered systemic drugs.

To our opinion there are many questions and current scientific topics of nasal drug delivery:

- What is the influence of anatomy and pathology on topical nasal therapy?
- What is the best technique of delivery to the middle meatus?
- What is the influence of currently used drugs and excipients on the nasal cilia?
- Is nasal vaccination a realistic option?
- What is the influence of nasal anatomy and pathology on nasal drug absorption for systemical treatment?
- How to improve nasal drug absorption?
- Is direct ‘nose to csf/brain’ drug delivery in humans possible?

To our opinion and within our line of research three questions have been chosen to be further analysed and investigated:

1. How do drugs for topical treatment, reach the middle meatus and what role does anatomical differences play?
2. Are nasal drugs potentially harmful to the cilia and mucociliary clearance, and is it possible to compare ciliostatic effects of drugs, preservatives and other excipients with each other?
3. Do intranasally administered drugs reach the CSF directly via the olfactory region, without being absorbed first into the systemic circulation and without passing the blood-brain barrier, in other words: do nasal drugs have a direct route to the cerebrospinal fluid?

These three topics are the “current aspects of nasal drug delivery” investigated and discussed in this thesis. Three separate sections in this thesis are dealing with the topics related to (1) methods of drug administration, (2) effects of nasal drugs and drug formulation on nasal ciliary activity and (3) nasal drug delivery and drug transport to the CSF.

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Chapter 2

Scope and intent of the thesis

Scope and intent of the thesis

Current intranasal drug delivery is rapidly increasing, accordingly new devices and applications are invented, new formulations are developed and new routes of drug administration investigated as demonstrated in the literature review of **chapter 1**. It looks like the limits of nasal drug delivery are not found yet. Still caution should be taken since some drugs for local use will not reach the site of action, some formulations are not as safe as they seem and a supposed new route of administration still has to be confirmed.

The scope and intent of the investigations in this thesis was to analyze three key issues in nasal drug delivery. We have studied these subjects in detail dividing the core of this thesis in three sections:

Section:

II. Methods of nasal drug administration

[chapter 3 & 4]

III. Effects of nasal drugs and nasal drug formulations on the nasal ciliary activity

[chapter 5]

IV. Nasal drug delivery and drug transport to the CSF and brain

[chapter 6-9]

Section II: Methods of nasal drug administration

Consensus about the most optimal method of administration of nasal corticoid drops and sprays is still lacking. This is striking because millions of people use these nasal drugs on a daily basis.

In this section several aspects of nasal drug deposition will be studied, aiming for the best deposition around the middle meatus. In **chapter 1** we have seen that several techniques can be used to analyze drug deposition around the middle meatus and the use of decongestants or anesthetics is not preferred in the investigation. Furthermore, nasal drug delivery is multifactorial requiring a standardized method and an intra-individual comparison. We created a setting in which volume, formulation, anatomy and delivery methods are consistent. We even introduced a new device in topical nasal drug delivery to have a real comparison between drops and spray, with identical head positions. Hopefully

our setting will give clarity in the current discussion about the best method of topical nasal drug delivery.

In **chapter 3**, in a comparison of seven different methods, an attempt will be made to establish the 'best method' of topical nasal drug delivery. In **chapter 4** we investigated the influence of anatomy and head position on nasal drug deposition.

Section III: Effects of nasal drugs and nasal drug formulations on the nasal ciliary activity

The influence of drugs, excipients and nasal drug formulations on the ciliary activity has been studied in the past two decades by many research groups (chapter 1). Most of these studies have been using in vitro methods, which are extremely sensitive. Whether the results of these investigations are predictive for the in vivo situation is still debatable. Nevertheless it is widely accepted that the in vitro effects of drugs and excipients may be relevant for the design of safe nasal drug formulations. In **chapter 5** we will try to classify the in vitro effects of drugs, excipients and drug products in relative terms, by comparing the negative or even toxic effects on ciliary movement of individual compounds. To create a physiological test the reversibility of the cilio-inhibiting effects is tested after 15 minutes comparable to the normal residence time of drugs in the nasal cavity. The aim was to classify each drug and compound as either ciliofriendly, cilio-inhibiting or ciliostatic via repeated cilia beat frequency measurements in vitro.

Section IV: Nasal drug delivery and drug transport to the CSF and brain

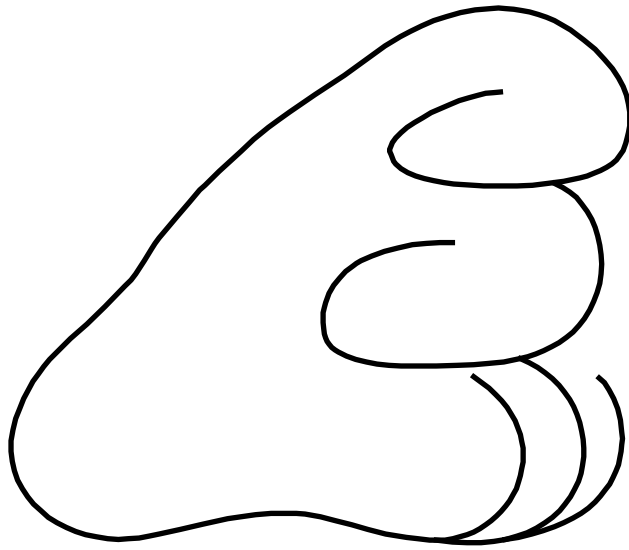
Many diseases of the central nervous system, like dementia, Alzheimer's disease, Parkinson's disease, epilepsy and depression are difficult to treat. The reason is that drugs cannot easily reach the brain in therapeutic quantities, because drugs have to be transported from the blood to the brain via the blood-brain barrier (BBB). A large number of papers have been published in the past two decades claiming that it is possible to circumvent the BBB by nasal drug administration (**chapter 1**). It is suggested by many authors that drugs can be transported via the olfactory area directly to the CSF and brain on the basis of animal experiments, mostly in mice and rats. Recently one research group claimed that they have found evidence for a direct transport of three peptide drugs after nasal administration in human volunteers. Their

research was lacking an intravenous comparison that is necessary to have real evidence of this new route of drug transport.

The purpose of our investigations was to explore the possibility of direct 'nose to brain' transport of drugs in human subjects. After developing a new detection method for one of the chosen model compounds (**chapter 6**), we conducted a controlled comparison of intranasal versus intravenous administered drug in the same individual and compared the levels in plasma and in the CSF after administration (**chapter 7**). To explore the existence of a direct transport of drugs from nose to CSF/brain and in the hope to confirm the human results, animal experiments were carried out under comparable and controlled circumstances (**chapter 8 & 9**).

SECTION II

NASAL DRUG ADMINISTRATION
TO THE MIDDLE MEATUS



Chapter 3

The 'best method' of topical nasal drug delivery: comparison of seven techniques

Accepted Rhinology

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Abstract

Objective: To determine whether there is a ‘best’ technique for delivering drugs to the middle meatus.

Design: Single-blind cross-over study in healthy individuals using endoscopic video-imaging.

Participants: A dyed test formulation was administered intranasally on seven non-sequential days to ten healthy individuals with no ‘nasal’ history. The participants were recruited through advertisement.

Main outcome measures: Comparison of seven different techniques, 20 nostrils and 140 endoscopic videos for the deposition patterns of dyed test formulation. Analysis was possible in 90% of all endoscopic videos. Three head positions were tested for both nasal drops and nasal sprays.

Results: Deposition of dyed test formulation near the middle meatus was observed in 43% of all observations. No significant differences were observed in terms of delivery between any of the seven techniques.

Conclusions: Our study suggests there may not be a single ‘best’ technique for topical nasal drug delivery. A more individual approach to topical nasal drug treatment, taking anatomy and head position into account, would seem to be more appropriate.

Key words: Nasal drug delivery, nasal spray, nasal drops, distribution, nasal polyposis

Introduction

Based on a review of the literature, the American Academy of Otolaryngology-Head and Neck Surgery Foundation has tried to define the best technique of administering intranasal corticosteroids¹. Unfortunately, they failed to provide us with definitive conclusions. This is remarkable, since large groups of patients receive daily corticosteroids for the treatment of nasal polyposis, allergic rhinitis, rhinosinusitis or chronic rhinosinusitis.

Reaching the middle meatus is of importance when treating both nasal polyposis and chronic rhinosinusitis², but individual anatomical and physiological differences challenge nasal drug delivery to this area.. Furthermore, the great variety of used methods and small size of most investigational groups prevents consensus about the best technique for administering topical nasal drugs^{1,3}.

In this study we compared four nasal drug delivery techniques currently in use and tried to define the best technique for administering intranasal corticosteroids. In addition to the four techniques already in use, we investigated three new techniques for topical nasal drug delivery. These new techniques used the single-unit dose nasal spray, a known intranasal drug delivery device, re-designed to overcome the role of gravity and combining the advantage of a spray mechanism with the possibility of delivering drugs in non-upright head positions.

Material and Methods

Healthy volunteers

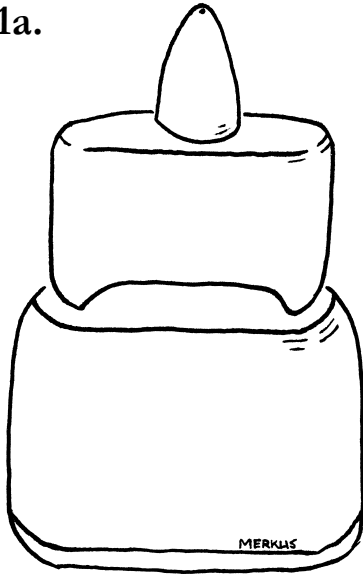
Healthy volunteers were recruited through an advertisement. Volunteers with frequent epistaxis, a history of smoking, an absent middle turbinate or a severe septal deviation (defined as severe enough to prevent visualisation of the anterior end of the middle turbinate without decongestion) were excluded. Volunteers taking medications (corticosteroids, antibiotics) known to interfere with nasal mucosa and volunteers having difficulties in assuming the different head positions for administration were excluded. All included subjects were required to read and sign an informed consent form. The study was approved by the Medical Ethical Committee of the Amsterdam University Medical Center.

Test drug formulation for spray and drop

The same dyed formulation was used in each test. The content of fluticasone nasal drops (Flixonase nasules® (1 mg/mL), GlaxoSmithKline, Zeist,

Netherlands) was used as the test formulation and dyed with 0.1% methylene blue (methylthionin chloride 1 mg/mL of pharmaceutical grade). In order to guarantee comparable volumes of test formulation in all test situations, the usual daily dose of fluticasone in a metered atomizing nasal spray (Flixonase®, GlaxoSmithKline, Zeist, the Netherlands), 2 puffs each nostril, (approximately 0.18mL) was used as the standard test volume. Dose and volume were checked by two physicians prior to delivery.

1a.

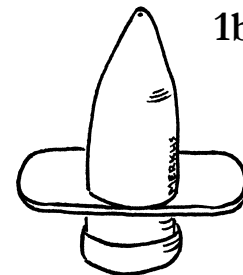


Nasal sprays

Metered atomizing nasal sprays for fluticasone (further referred to as '*container spray*', Figure 1a) were emptied and filled with dyed test formulation. These devices deliver 0,089 mL during each spray. After priming, two puffs per nostril were administered (equals approximately 0.18 mL per nostril) to each volunteer sitting in the Head in Upright position (HUR).

The second spray, the *unit-dose spray* (Figure 1b, Bidose MK3®, Valois, France), was adapted by the manufacturer to deliver 0.18 mL of test formulation per nostril in one spray when filled with 0.20 mL (0.18 mL dose volume, 0.023 mL residual volume). The manufacturer supplied residual volumes and these were checked using pre- and post delivery weight measurements. The single-unit dose spray is, unlike the container spray mentioned above, capable of delivering drugs in different head positions against gravity. Three different head positions were tested (see head positions and Figure 2 & 3).

1b.



1c.



Figure 1a-c.

Three drawings showing the devices used.

a. Container spray, a multidose spray, used in one head position; **b.** Unit-dose spray, an 'one time use' spray functional in different head positions; **c.** Nasule, an 'one time use' plastic container, used in different head positions.

Nasal drops

Nasal drops were administered using nasules (Figure 1c, Flixonase nasules®). Each nasule was filled with test formulation to a total volume of 0.20 mL, delivering 0.18 mL after one firm squeeze (0.18 mL dose volume, 0.02 mL residual volume). This volume also resembles the 'normal' dosage of half a fluticasone nasule (0.2mL). Three different head positions were tested (see head positions and Figure 2 & 3).

Figure 2 *Summary of the seven techniques used.* The head positions are shown in Figure 3.

Device	Sprays				Drops		
	Container Spray	Unit-dose Spray			Nasal Drops		
Head Position	HUR Head UpRight	LHB Lying Head Back	LHL Lateral Head Low	HDF Head Down Forward	LHB Lying Head Back	LHL Lateral Head Low	HDF Head Down Forward

Study design

Single-blind randomized crossover study using seven different nasal drug delivery techniques (Figure 2). Each volunteer was tested on seven non-sequential days.

Head positions

Head upright (HUR): This position is widely used for all multidose container sprays. All other head positions are explained below and drawn in Figure 3.

3a.

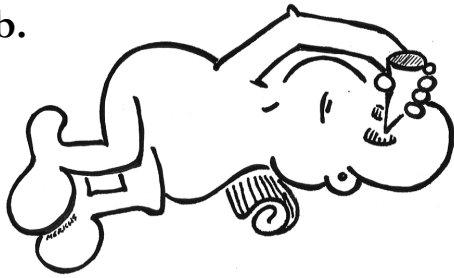


Lying head-back position (LHB): Lying down in supine position with the head just off the bed in hyperextension, so that the chin is the highest point of the head. This head position was first described by Proetz in 1926^{4,5} and modified by Mygind in 1979⁶.

Figure 3a-c. (this and next page)

Three head positions: a. Lying Head Back (LHB, chin as highest point), b. Lateral Head Low (LHL, lying on one side) and c. Head Down and Forward (HDF, 'Praying to Mecca').

3b.



Lateral head-low position (LHL)⁷⁻⁹
Lying on the side with the parietal eminence resting on the bed (no pillow or a pillow under the shoulders). The nasal formulation is administered to the lower nostril.

Head down and forward (HDF), also known as 'Praying to Mecca'¹²:

Kneeling down, placing the top of the head on the ground and the forehead close to the knees with the nostrils facing upwards.



Protocol

All healthy volunteers received instructions during the first visit. Subsequently, and on all other visits, the first ENT physician administered the test formulation using one of the techniques described in the study design (Figure 2). The delivery of dyed test formulation was directed towards the lateral epicanthus of the ipsilateral eye. Volunteers were not allowed to deliver the test formulation themselves. After administration, each volunteer had to remain in the position in which drugs were delivered for 60 seconds. The first ENT physician provided strict supervision of administration. Nose blowing was allowed prior to administration. During the test, vigorous sniffing and nose blowing were not allowed. In an adjacent room, a second ENT physician, who was not informed about the technique used, performed nasal endoscopy within three minutes after the administration of dyed test formulation. The drug delivery technique was revealed just before statistical analysis of the data.

Endoscopic investigation

A 2.7mm 0° Storz rigid nasendoscope was used and the images were recorded using digital video registration (Stroboview® 2000, Alpatron medical & microwave systems BV, Rotterdam, The Netherlands). The endoscope was placed near the anterior end of the middle turbinate and then retracted slowly while recording images. An example of endoscopic photo imaging is shown in Figure 4. This procedure is based on a combination of the photographic analysis described by Weber et al.^{11,12} and the endoscopic evaluation described by Homer et al.¹³. No local anesthetic or decongestant was used.

Video analysis

Three independent ENT specialists analysed all video images. The deposition of dyed test formulation was scored as either 'head of the middle turbinate not sufficiently visible' (not on the video/poor view), 'absence of dye' or 'presence of dye'. Dye scoring was rehearsed to reduce inter-observer variability. The analysis was based on observer consensus, with at least two observers independently agreeing on deposition scoring. This is a statistical valid method often used in histological grading ¹⁵. The videos in which the middle turbinate was not visible were excluded from the analysis results.

Statistical analysis

Statistical analysis was conducted with SPSS (version 12.01, SPSS Inc., Chicago, USA). Data are expressed as median values. Cochran Q non-parametric tests for related samples were performed to check for significant in between-group variability. McNemar non-parametric tests for related samples were performed for between-group comparisons. P values of less than 0.05 were considered statistically significant.

Table 1	Container	Nasal		Nasal		Unit-dose		Total
	spray	Drops	Drops	Drops	Spray	Spray	Spray	
		LHB	LHL	HDF	LHB	LHL	HDF	
Dye: absent	8	11	11	13	8	5	10	66
present	10	7	7	5	10	14	7	60
Not sufficiently visible	2	2	2	2	2	1	3	14
N	20	20	20	20	20	20	20	140

Dye around the head of the middle turbinate per technique.

Absolute figures for the seven techniques tested in twenty nostrils. 'Container spray' is a multi-dose spray and 'unit-dose spray' is a single-unit dose spray, used in different head positions (LHB = lying head back, LHL = lying head lateral and HDF = head down and forward).

Overall the dye was present and absent in an almost equal numbers of observations. In 90% of all endoscopies, clear observation of the middle turbinate was possible; amount of observations around the head of the middle turbinate which were not sufficiently visible are shown on the row 'not sufficiently visible'. The data are presented as percentages in Figure 5.

Results

Ten volunteers were included, 2 males and 8 females with a median age of 23 (19- 28) years. Nostrils were evaluated separately (n=20). Seven different drug delivery techniques were compared and a total of 140 recorded endoscopies were analyzed.

Table 1 and Figure 5 show the overall presence of dye around the middle turbinate is. Values scored as 'head of the middle turbinate not sufficiently visible' were excluded from the analysis results (16% of all observations, mainly observations in 'higher' narrow cephalic regions). Ten per cent of the observations around the head of the middle turbinate were excluded from the analysis results. In general, there was less dye towards the head of the middle turbinate (47% presence, 43% absence, Table 1).

Statistical analysis revealed no significant difference between the amounts of drug delivered near the head of the middle turbinate by the seven investigated techniques (Figure 5, $n = 7$, $p = 0.115$). Although not significant, a clear improvement in deposition near the head of the middle turbinate using the single-unit dose nasal spray was observed for all techniques (HUR, LHB and LHL head position, Figure 5). The single unit-dose nasal spray was superior to nasal drops in all head positions used. This difference attained significance when all observations for both delivery devices were taken together ($n = 3$, $p = 0.039$, Figure 6). Caution should be taken when transposing these figures to the clinical setting (see discussion).

In general, the different techniques for topical nasal drug administration were easily accepted, although most volunteers mentioned some discomfort during the HDF head position, confirming the findings of Kayarkar ¹⁵. The test formulation was tolerated well, but some volunteers noticed some discomfort (sneezing, itching). No adverse effects were observed. In some cases, congestion disturbed the quality of endoscopic video imaging. These images were excluded from the analysis results.

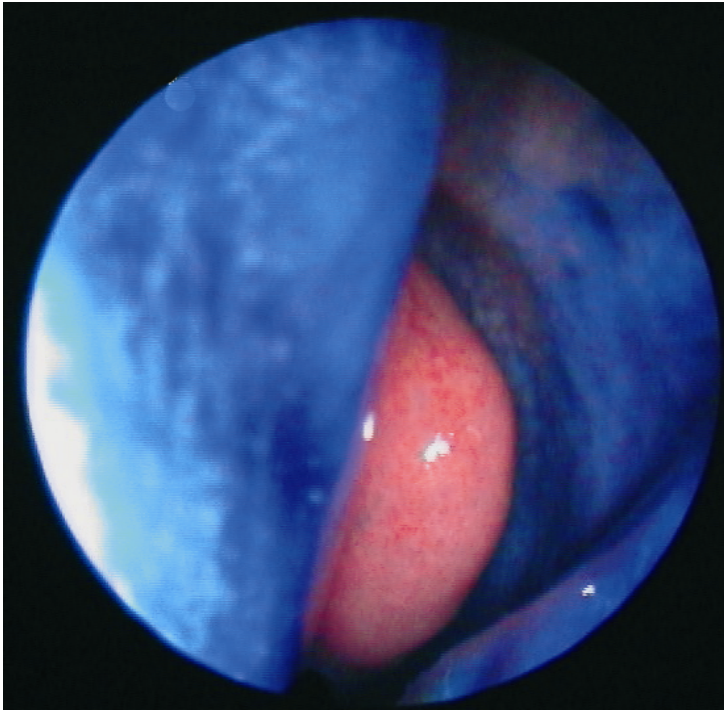


Figure 4. Photograph of an endoscopic view of a left nostril immediately after the administration of the test formulation. Dyed formulation is clearly visible lateral and medial (septum) of the middle turbinate.

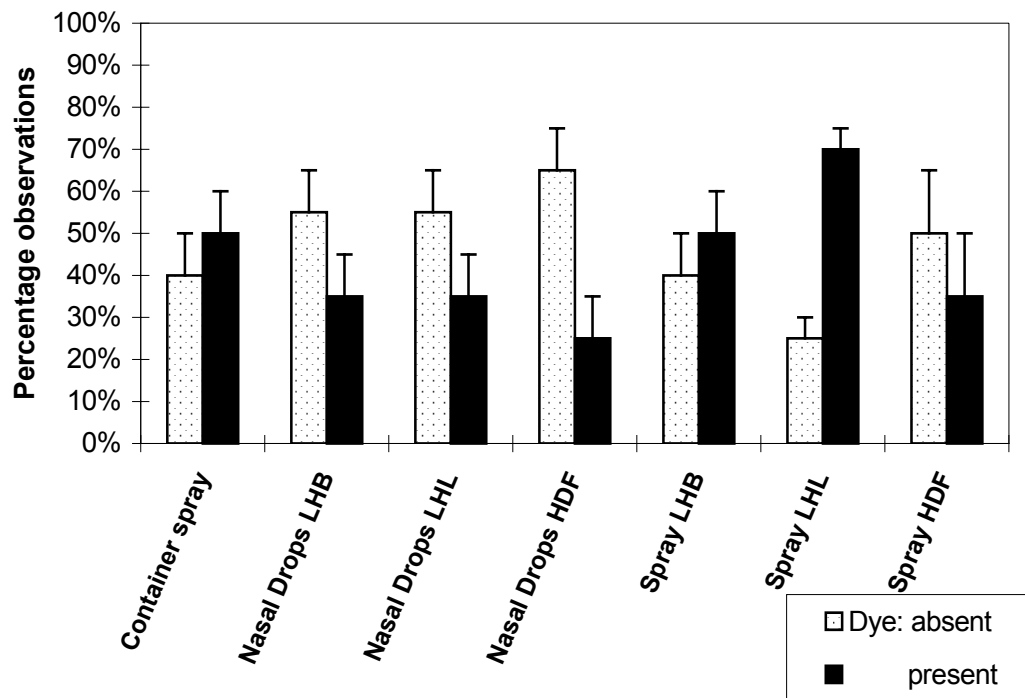


Figure 5
Presence or absence of dye around the head of the middle turbinate after nasal drug delivery using seven different techniques. The black bars (presence of dye) or white dotted bars (absence of dye) represent the percentages of observations with or without dye around the middle turbinate.

Discussion

Nasal drug delivery is a multifactorial process and therefore hard to investigate. Individual anatomical differences, different head position, the type of drug formulation, drug volume and different delivery devices all affect topical nasal drug delivery. Furthermore, since there are numerous investigational methods, comparison between studies is even more difficult³. All of these factors may explain why Benninger *et al*, in their thorough review, failed to arrive at definitive conclusions about the best technique for administering topical nasal drugs¹. In our present study we tried to optimise the investigational method used for the assessment of topical nasal drug delivery by combining photographic analysis^{11,12} with endoscopic evaluation¹³ and by standardising the test formulation, test volume and head position. Our standard volume throughout the experiments was chosen carefully on the basis of the daily volume of a nasal container spray (delivers around 0.18 mL after 2 puffs in one nostril) and was comparable to the volume delivered as nasale drops (half the content of one nasule, approximately 0.2 mL).

Despite the optimisation of the study method, no significant differences were found between the seven topical nasal drug delivery techniques. On the basis of these and other results, it may be realistic to conclude that there is *no* such thing as ‘a best technique’ for topical nasal drug delivery. In a number of healthy volunteers, anatomical variations, although small, seemed to influence topical nasal drug delivery. This may explain the unsuccessful search for the best nasal drug delivery technique for a whole group, in spite of the best technique per individual. This has already been suggested by earlier publications¹⁶.

We observed a trend indicating that the single-unit dose nasal spray was on the whole superior to nasal drops in a comparison of three devices (Figure 6). We believe this spray could be a promising new device for topical nasal drug delivery, but additional testing will be required to establish the true value of this innovative device. The longer tip of this nasal spray (bypassing the nasal valve area and vestibule hairs), the higher velocity of administration (to increase penetration) and the possibility of directing drugs may account for these differences. Again, we believe that further studies are necessary to confirm these results.

Our study reveals that all head positions commonly used for the delivery of drugs in nasal drops are equally effective, although a slight trend in favour of the LHB and LHL head position was observed, confirming the findings of Karagama *et al*¹⁷. A similar trend was seen in drops *and* spray, which may

indicate that head position, like anatomy and delivery device, is an independent factor determining the outcome of topical nasal drug treatment. Drug delivery to the nose via the HDF head position revealed that drugs are delivered at more cranial locations. This head position may, therefore, be useful in the treatment of nasal polyps located superior to the middle meatus or in reaching the olfactory region.

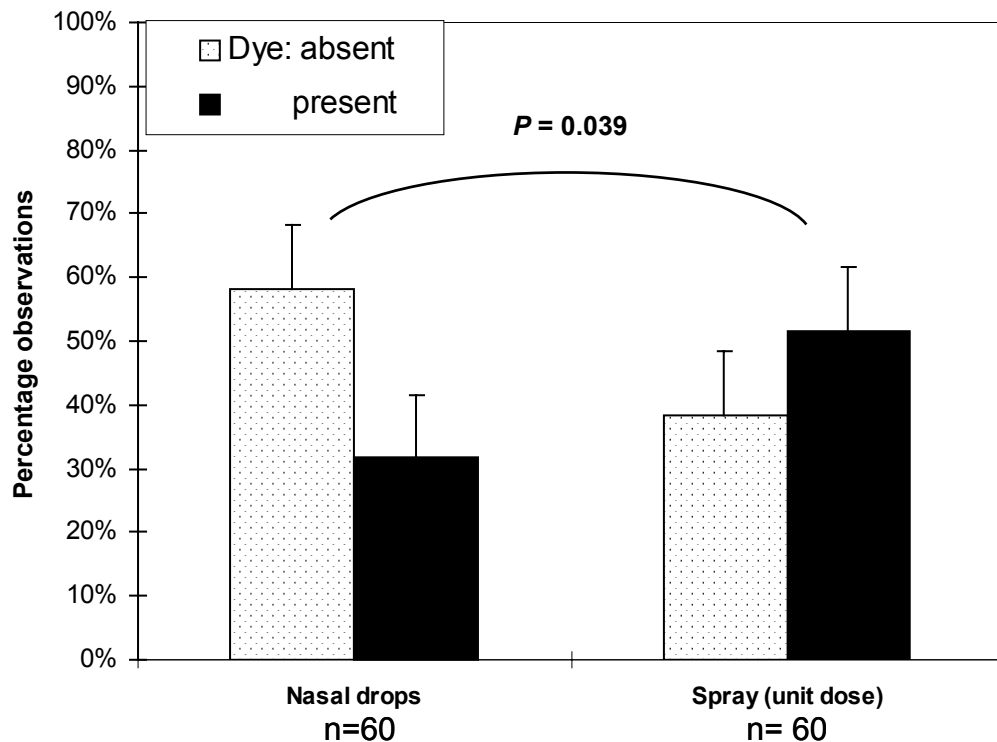


Figure 6
Comparison of nasal drops and the single-unit dose spray without regard to head position. The black bars (presence of dye) or white dotted bars (absence of dye) represent the percentage observations with or without dye around the middle turbinate ($p= 0.021$, $n= 60$). Caution should be taken to convert these figures to the clinical setting.

Although our study provides important additional information about topical nasal drug treatment, we were unable to investigate some other important determinants of nasal drug delivery such as the variability between repeated drug administrations, the effect of time on nasal drug delivery (mucociliary transport) and quantification of the amount of dye reaching the middle meatus. Although an investigational method to quantify topical nasal drug delivery has been described by Aggarwal *et al.*³, we think that this method will not identify a true 'best drug delivery technique' since local anesthetics and

decongestants alter nasal anatomy and physiology significantly.

In general, we wish to stress that results from studies in healthy individuals are difficult to extrapolate to pathological conditions, such as major septal deviations, allergic rhinitis, chronic rhinosinusitis and nasal polyposis, and that additional studies in diseased patients will be necessary before implementing results in clinical practice.

From our study, we conclude that topical nasal drug delivery is multifactorial and hard to investigate, and that the identification of a single ‘best technique’ for topical nasal drug administration is unrealistic. A more individual approach to topical nasal drug treatment, taking anatomy and head position into account, seems more appropriate. We hope that future research will include the single–unit spray and patients instead of healthy volunteers.

Acknowledgement

We like to thank Valois (France) for their support with the single-unit dose device.

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Chapter 4

Influence of anatomy and head position on intranasal drug deposition

Submitted

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Abstract

The objective of this study was to determine the influence of individual anatomical differences on intranasal drug deposition.

The data of a comparison of 7 different administration techniques in 10 healthy volunteers was used in this single-blind crossover pilot study. After intranasal administration of a dyed test formulation endoscopic video imaging was done on seven non-sequential days. The deposition pattern per individual around the head of the middle turbinate was analyzed of each technique and correlated with the individual anatomy.

Decreased deposition of dyed test formulation in the target area around the head of the middle turbinate was observed in the presence of minor septal deviations, narrow nasal valve areas or inferior turbinate hypertrophy; a lateral head position helps to bypass a minor septal deviation.

Although results are preliminary, we conclude that anatomy and head position are important factors in the deposition of topical nasal drugs and may be the key to improving individual local nasal (steroid) treatment.

Key words: Nasal drug delivery, nasal polyposis, rhinosinusitis, anatomy, distribution, head position.

Introduction

A recent thorough review shows that only eight studies have proven the efficacy of topical intranasal corticosteroids in the treatment of patients with chronic rhinosinusitis (5 studies) and nasal polyposis (3 studies) [6]. Although this treatment is often successful, topical corticosteroids sometimes fail to reduce polyp size effectively or are not decreasing rhinosinusitis complaints. Many factors determine the outcome of topical nasal drug treatment: formulation characteristics, delivery device, delivery technique, site of deposition, anatomy, pathophysiology and compliance, for example. This means that there are many uncertainties confronting the ENT surgeon when optimizing treatment for individual patients.

It seems rational to aim for the middle meatus when treating nasal polyposis and chronic rhinosinusitis [22]. Several studies have looked at the best way to reach this area but, remarkably, the American Academy of Otolaryngology-Head and Neck Surgery Foundation has failed, on the basis of a review of these studies, to draw definitive conclusions regarding the best technique of topical nasal treatment [3]. An explanation could be the underestimation of the influence of individual anatomy. If anatomical obstructions reduce the delivery to the middle meatus of topical nasal drugs, it would seem unlikely that there is a single administration technique appropriate for all patients. In a recent publication [14], we confirmed the absence of a 'best technique' for topical nasal drug delivery; in the present pilot study we correlate the drug deposition data with the individual anatomical differences. Ten volunteers and seven techniques of drug delivery were used to determine whether anatomical obstructions influence drug deposition and whether obstructions can be avoided by changing the technique of administration.

Material and Methods

Healthy volunteers

Healthy volunteers without nasal symptoms were recruited through an advertisement. Volunteers with frequent epistaxis, a history of smoking, an absent middle turbinate or a severe septal deviation (defined as severe enough to prevent visualization of the anterior end of the middle turbinate without decongestion) were excluded. All anatomical differences were carefully described and recorded prior to inclusion. Patients with various anatomical differences (except for extreme septal deviations as described above) were included. Volunteers taking medication (prednisone, antibiotics) known to interfere with nasal mucosa and volunteers with difficulties in assuming the

different head positions for administration were excluded. All subjects were required to read and sign an informed consent form. The Medical Ethical Committee of the Amsterdam University Medical Center approved this study.

Test drug formulation for sprays and drops

The same dyed formulation was used in each test. The test formulation selected was fluticasone nasal drops (Flixonase nasules® (1 mg/ml), GlaxoSmithKline, Zeist, Netherlands). It was dyed with 0.1% methylene blue (methylthionin chloride 1 mg/ml of pharmaceutical grade). In order to ensure a comparable volume of test formulation in all test situations, the usual daily dose for fluticasone in a metered atomizing nasal spray (Flixonase®, GlaxoSmithKline, Zeist, Netherlands, 2 puffs each nostril, approximately 0.18ml) was used as the standard test volume.

Nasal sprays

Metered atomizing nasal spray for fluticasone (further referred to as ‘container spray’) was emptied and filled with dyed test formulation. This device delivers 0.089 ml during each spray. After priming, two puffs per nostril were administered (equals approximately 0.18 ml per nostril) to each volunteer sitting in the Head in UpRight position (HUR).

The manufacturer adapted a single-unit dose spray (Bidose MK3®, Valois, France) to deliver 0.18 ml of test formulation per nostril (fill volume 0.203 ml). This single-unit dose spray is, unlike the container spray, capable of delivering drugs in different head positions. Three different head positions were tested (see below, Figure. 1 & 2).

Figure 1. Summary of the seven techniques used.

Figure 2 shows the head positions

Device	Sprays				Drops		
	Multi-Dose Container	Single-Unit Dose			Nasules		
	HUR	LHB	LHL	HDF	LHB	LHL	HDF
Head Position	Head UpRight	Lying Head Back	Lateral Head Low	Head Down Forward	Lying Head Back	Lateral Head Low	Head Down Forward

Nasal drops

Nasal drops were administered using nasules (Flixonase nasules®). Each nasule was filled with test formulation to a total volume of 0.20 mL, delivering 0.18 mL after one firm squeeze (0.18 mL dose volume, 0.02 mL residual volume). This volume also resembles the 'normal' dosage of half a fluticasone nasule (0.2mL). Three different head positions were tested (see below, Figure 1 & 2).

Study design

Single blind randomized crossover study using seven different nasal drug-delivery techniques (Figure 1). Each volunteer was tested on seven non-sequential days. The correlation between dye deposition and individual anatomy was analyzed.

Head positions

Head upright (HUR) This position is widely used for all multidose container sprays. The three other head positions are explained below (Figure 2)

2a.

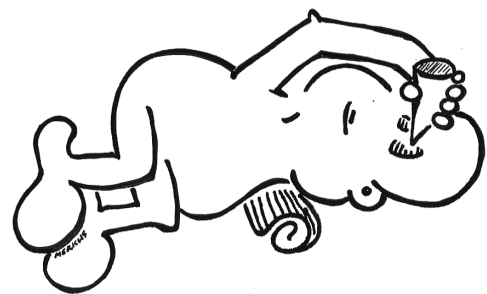


←Lying head back (LHB)

Lying down in supine position with the head just off the bed in hyperextension, so that the chin is the highest point of the head. This head position was described first by Proetz [19,20] in 1926 and modified by Mygind [16] in 1979.

Lateral head low (LHL) [17,18,21] →

Lying on the side with the parietal eminence resting on the bed (no pillow or a pillow under the shoulders). The nasal formulation is administered in the lower nostril.



2b.

2c.



←Head down and forward (HDF)
(Praying to Mecca) [4,13]

Kneeling down with the top of the head on the ground and the forehead close to the knees with the nostrils facing upwards.

Protocol

Three ENT physicians reviewed and graded the anatomical differences between the selected individuals. All healthy volunteers received instructions during the first visit. Subsequently, and at all later visits, an ENT physician administered the test formulation using one of the techniques described in the study design (Figures 1 & 2). After administration, the volunteer remained in the same position for 60 seconds. Vigorous sniffing and nose blowing were not allowed during the test (this was only allowed prior to administration and after endoscopy). In the next room, a second ENT physician, who was not informed of the technique used for drug administration, performed a nasal endoscopy within three minutes after administration. The technique used for drug delivery was revealed after scoring of three independent observers and after closing of the database.

Endoscopic investigation

A 2.7mm 0° Storz rigid nasendoscope was used and images were captured using digital video registration (Stroboscopy® 2000, Alphascope medical & microwave systems BV, Rotterdam, Netherlands). The endoscope was placed near the anterior end of the middle turbinate and subsequently retracted slowly while recording the images. This procedure was based on a combination of the photo analysis described by Weber et al. [25] and the endoscopic evaluation described by Homer et al.[9]. No local anesthetic or decongestant was used.

Video analysis

Three independent ENT specialists analyzed the video images. Deposition of dyed formulation was scored as either ‘head of the middle turbinate insufficiently seen’ (not on the video), ‘absence of dye’ or ‘presence of dye’. Presence of dye was scored at several pre-defined locations (Table 1) and dye scoring was rehearsed to diminish inter-observer variability. Observer consensus – with at least two observers independently agreeing about deposition scoring – was used in analysis. This is a statistically valid method often used in histological grading [23]. “Non-consensus videos” were excluded from analysis. The videos in which the middle turbinate was not visible were also excluded from analysis.

Results

Ten volunteers were included: 2 males and 8 females, median age 23 (19- 28) years. Nostrils were evaluated separately (n=20). Seven different drug-delivery

techniques were compared and a total of 140 videos were analyzed. Anatomical differences were defined as “narrow valve area” (3 volunteers/6 nostrils), “hypertrophic or congested inferior turbinate” (10 nostrils) and “septal deviation/slight septal deviation” (5 volunteers/5 narrow nostrils & 5 contralateral “open” nostrils). Three ENT physicians, proceeding without objective measurements and without selection, independently agreed upon the interpretation of these anatomical differences. The results are presented in Table 1. Values counted as “head of the middle turbinate insufficiently seen” or without consensus (minority) were excluded from analysis (16% of all observations, mainly observations in narrow cephalic regions, only 10% in the head of the middle turbinate region). Positive scores for the overall presence of dye were found in 45% of observations, with 55% of observations resulting in negative scores (median values). On and around the middle turbinate, the number of observations without dye (55-72%) exceeded those with dye (28-45%).

Table 1. *Deposition of dyed test formulation.* Results of 140 independently-reviewed nasal deposition videos. Nine pre-defined locations were assessed. Only “valid” observations (videos in which the location was visible) were assessed and scored as “dye present” or “dye absent”. A decreased amount of dye is observed when going from the vestibulum (97%) to postero-cranial locations (above the middle turbinate, 17%).

Location	Dye Present
Vestibulum	97%
Inferior turbinate head	83%
Inferior turbinate tail	83%
Septum	68%
Lateral wall	36%
Lateral of middle turbinate	28%
Middle turbinate head	45%
Medial of middle turbinate	30%
Superior of middle turbinate	17%
Median	45%

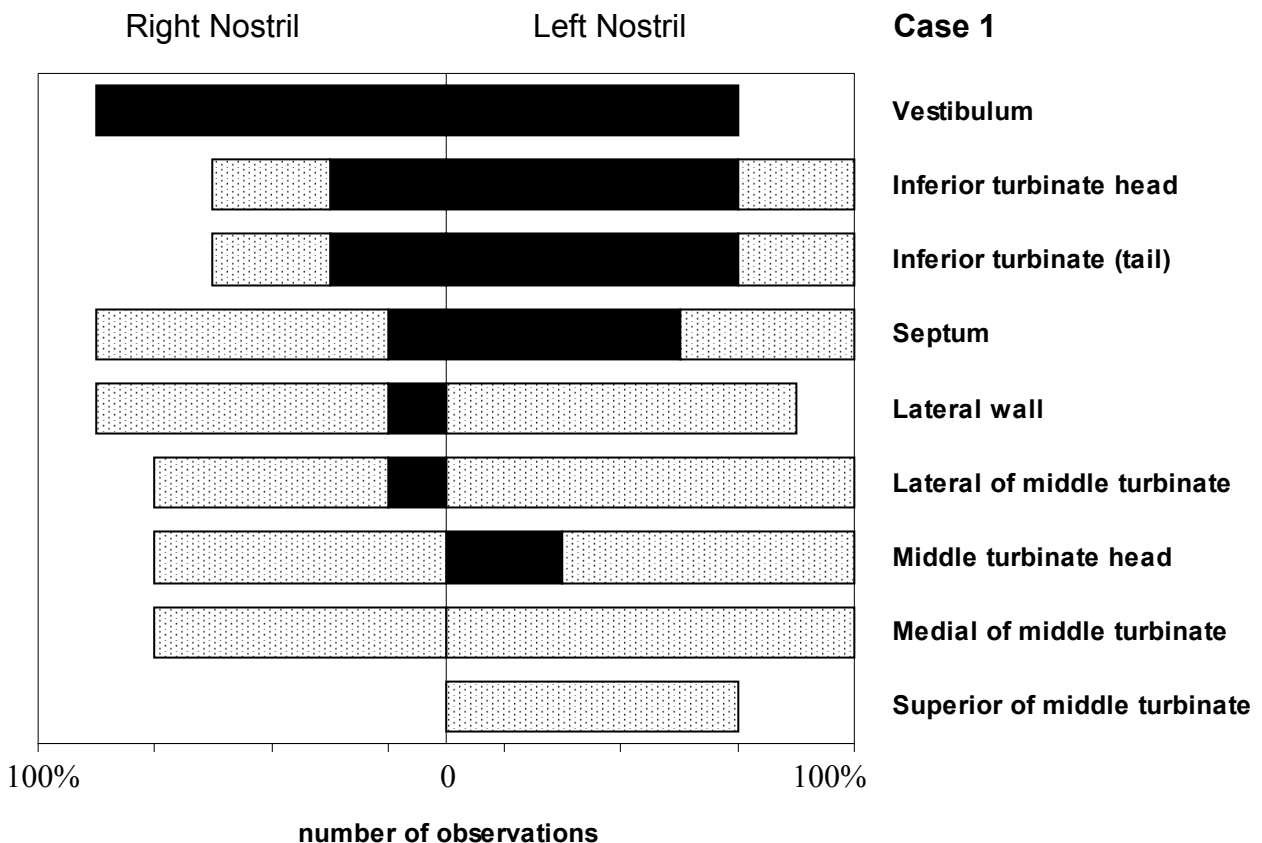
Looking at anatomical differences between individuals, a trend emerges indicating that anatomy affects the site of deposition. Figure 3a-c shows the cumulative deposition pattern in three individuals after testing all seven techniques. Only a few techniques reached the area around the middle turbinate in volunteers with a narrow valve area or hypertrophic inferior turbinate (Figure 3a). Dye deposition was good at all sites and with all techniques in volunteers with an “open” nose (Figure 3b). A mild septal deviation caused a decrease in the amount of dye present in the area around

the middle turbinate on the obstructing convex side and an increase or “normal” amount of dye on the concave side (Figure 3c).

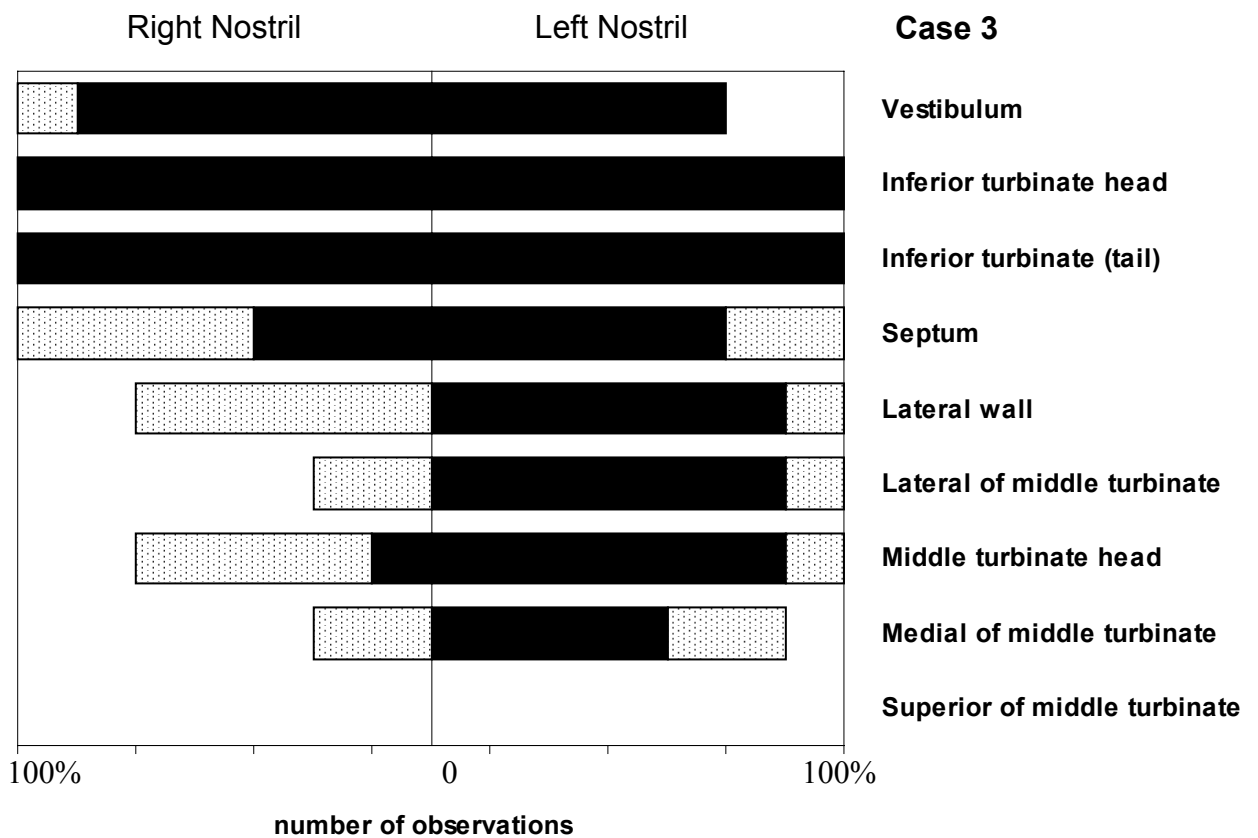
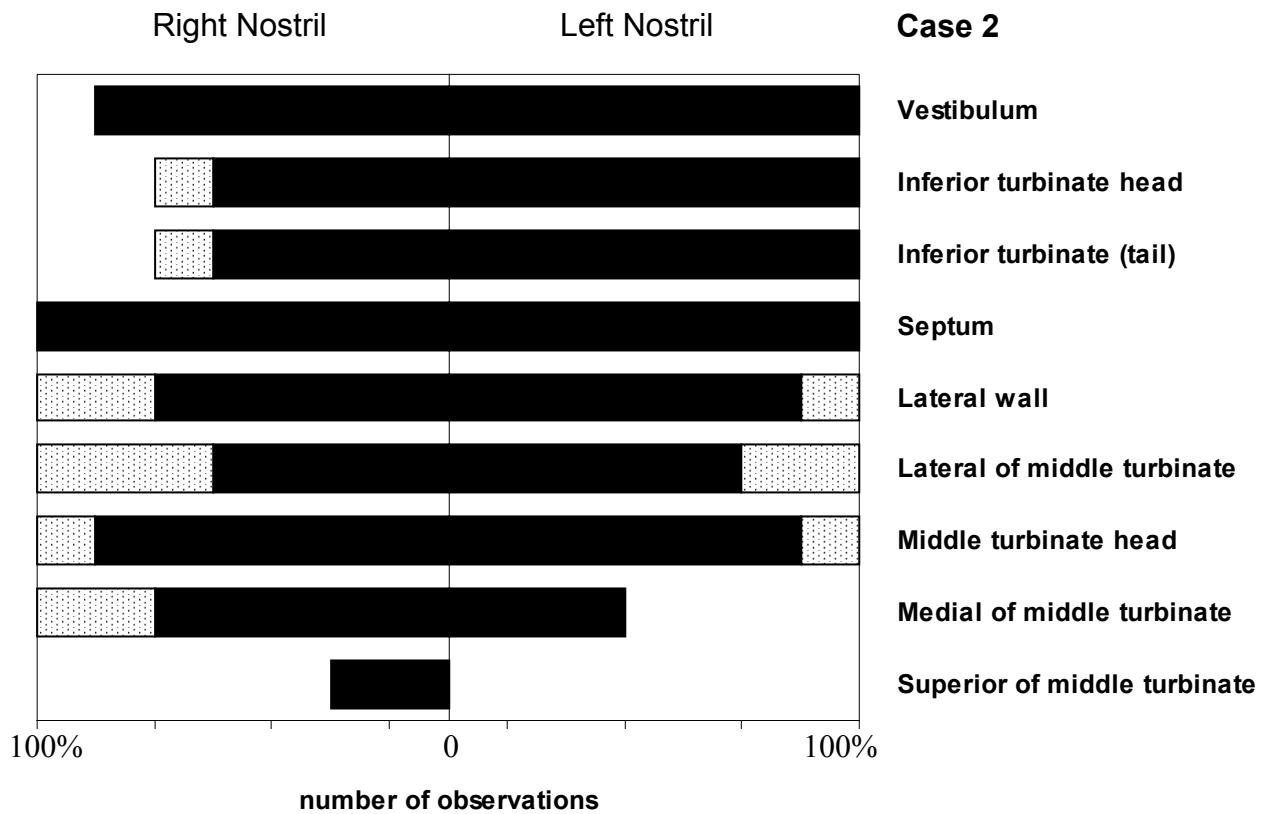
Figure 3a-c. Individual deposition (cases 1-3) and anatomical correlation.

Deposition of dye at various locations shown for both left and right nostrils of three individuals after administration using 7 techniques. The presence or absence of dye per technique is cumulatively represented by a bar on the x-axis (100%=7 techniques). Bar length= amount of videos scored. The white dotted bar shows the number of videos scored as ‘absence of dye’. The black bar shows the number of videos scored as ‘presence of dye’. The anatomical locations are on the y-axis. Each bar represents the percentage of observations. A clear correlation between observed deposition and anatomy can be seen.

- a. Case 1: septal deviation to the right, narrow valve area
- b. Case 2: an “open nose” (next page)
- c. Case 3: septal deviation to the right and an “open” valve region. (next page)



Influence of anatomy and head position on intranasal drug deposition

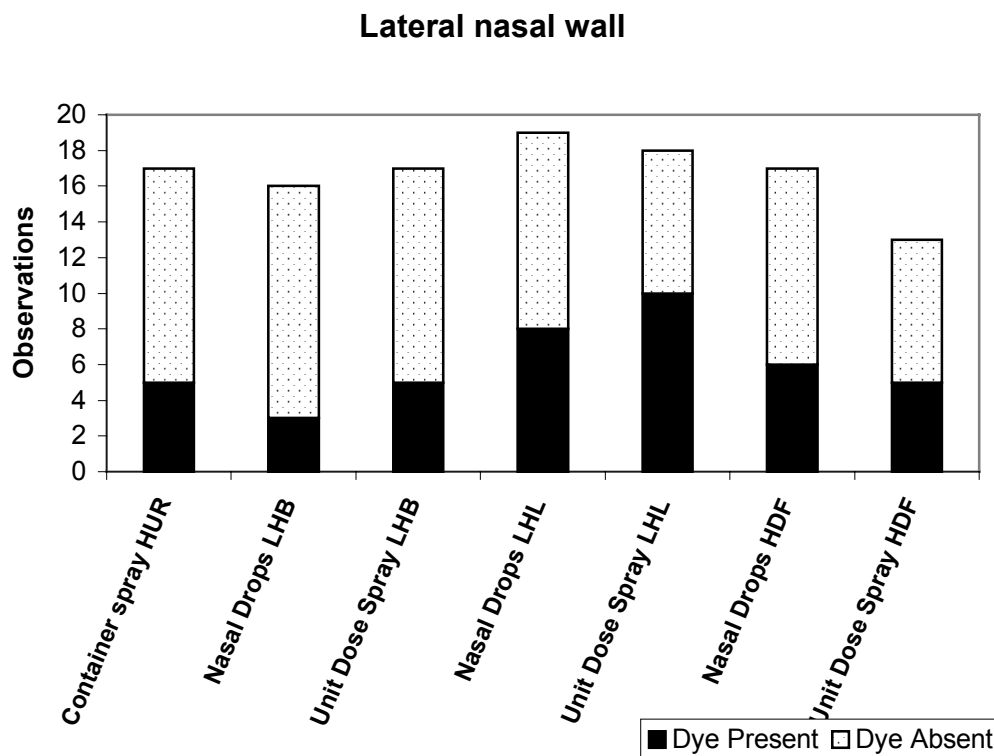


Head position (read: gravity) seems to have a substantial influence on drug delivery to the middle meatus. Increased amounts of dye are present in more lateral locations (this is especially important when challenging septal

deviations) when using the LHL head position (Figure 4) and in the superior region when using the HDF head position (data not shown). These results support the idea that gravity affects drug deposition.

In general, the different techniques of topical nasal drug administration were easily accepted, although most volunteers mentioned some discomfort associated with the HDF head position. This confirms the findings of Kayarkar *et al.*[11] The test formulation was tolerated well, but some volunteers noticed some discomfort (sneezing and itching). No adverse effects were observed.

Figure 4. Deposition lateral nasal wall. The number of valid observations per technique is around 16 /20 (84%). Dye was present on the lateral nasal wall in about 6/20 observations (36%) of these observations. The most favourable head position during administration for reaching the lateral nasal wall is Lateral Head Low (LHL) (10 observations with dye present using the single-unit dose nasal spray and 8 observations with dye present using nasal drops).



Discussion

When the literature fails to provide definitive conclusions about the best technique for administering topical nasal drugs, it is difficult to investigate “a best technique”, even supposing that one exists. In a recent review, Aggarwal *et al.*[1] clearly point out why topical nasal drug deposition is hard to

investigate. Individual anatomical differences, different head positions and the use of sprays or drops all affect topical nasal drug administration. Moreover, the wide variety of research methods used renders comparison between studies difficult. In that perspective, we have gathered data in a standardized manner relating to techniques with drops *and* sprays *and* different head positions. We studied ten volunteers in an intra-individual and inter-individual comparison [14].

This pilot study establishes that individual anatomical differences, even though they seem trivial upon first inspection, explain the impossibility of identifying a single “best technique” for topical nasal corticosteroid administration [14]. The outcome of topical nasal drug treatment is even harder to predict when there are pathological changes. Obstruction by either a hypertrophic inferior turbinate or a narrow nasal valve area confines delivery of topical nasal drugs to the head of the middle turbinate (Figure 3). These findings confirm the results of Dowley *et al.*[5], who showed that congestion of the inferior turbinate significantly reduced drug delivery to the middle meatus. Weber suggested that a septal deviation may affect nasal drug deposition [26], but we are not aware of any other study that investigates this suggestion. In concordance to most drug delivery studies we excluded patients with severe septal deviations in order to ensure adequate observation of the head of the middle turbinate [5,8-10,24]. In spite of this exclusion criterion, we show that even slight septal deviations can have major consequences on nasal drug deposition. Only five volunteers with “minor” septal deviations were included in our study, still we were able to show that their drug deposition patterns (70 observations) are remarkably similar. Furthermore, administering topical nasal drugs in certain head positions (LHL, LHB) bypasses septal deviations, thereby increasing the amount of drug delivered to the head of the middle turbinate. Improving nasal drug deposition to the middle meatus when the individual’s anatomy is unfavorable may therefore be a matter of changing head position.

In a small study (n=5) of Homer *et al.* [8] it is suggested that there is an optimal delivery technique for each individual; some volunteers do better on nasal drops whereas others are best treated with nasal sprays. In our study, we also investigated both techniques, and we conclude from our data that individual anatomical variations are the most important factor in determining the outcome of topical nasal drug treatment. In 1985, Hardy *et al.*[7] concluded that nasal drops are superior to nasal sprays in penetrating the nasal valve area. From our data, we conclude that considerable amounts of dye fail

to penetrate the nasal valve area with all techniques and that nasal sprays are superior, albeit not significantly, to nasal drops for bypassing the valve area. The decrease in deposition towards the cephalic nasal regions (Table 1) supports the idea that the middle meatus area is difficult to reach and that most of the administrated formulation will never reach this area [9,15,26]. It is possible that a narrow valve and vestibule hair area can be bypassed using a longer nasal-spray tip and high-velocity administration, increasing drug delivery to the head of the middle turbinate. This spray advantage is in contrast with the efficacy study of fluticasone *drops* of Aukema *et al.*[2], which seems to be more effective in the treatment of nasal polyposis when comparing the results to treatment with fluticasone *spray* as studied by Lund *et al.* [12] An explanation for this can be the questionable predictive value of healthy volunteers in our study.

Although we were able to investigate several aspects of nasal drug delivery, our study has several limitations: video imaging simplifies the nose to a 2D structure, it is not a quantitative measure, and the rigidity of the endoscope occasionally prevents assessment of every area of the nose. Furthermore, it is not known whether the test solution reaches the area of the middle turbinate later as a result of mucociliary clearance. This is especially important in the case of nasal drops, because droplets do not necessarily reach the target area of the middle turbinate at the same time and in the same way as nasal sprays [7]. By comparison with a recommended, more quantitative, assessment [1,8], we did not alter nasal physiology by using a decongestant and local anesthetic. Since our technique is well tolerated, repeated testing is possible, making the comparison between different techniques in one subject possible.

Although our results reveal differences in topical nasal drug deposition associated with “normal” anatomical variations, they are not statistically significant. Furthermore, in this pilot study, we did not select the patients for their nasal anatomy; we investigated whether there were correlations between anatomy and deposition in the nose. Extrapolation of our data to patients suffering from rhinosinusitis with or without nasal polyposis is difficult, especially since intranasal deposition and distribution patterns are presumed to be different in these diseases. Investigating patients with pathological conditions like nasal polyposis should therefore be the next step in nasal drug delivery studies.

Although these results are still preliminary, we recommend taking even “minor” anatomical differences into account when trying to optimize topical nasal drug treatment for individual patients. Head position during

administration should be adapted to individual anatomical characteristics. The single-unit dose spray seems to present potential advantages for topical nasal drug delivery and it therefore merits additional testing.

Acknowledgement

We wish to thank Valois (France) for their support with the single-unit dose device.

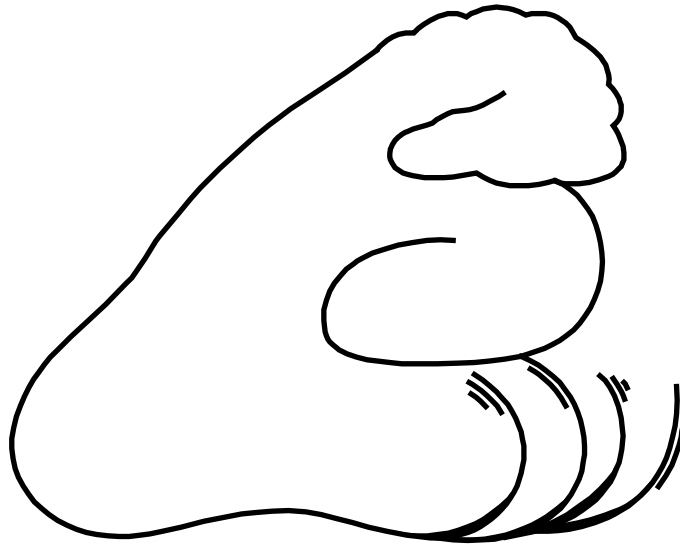
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SECTION III

EFFECTS OF NASAL DRUGS
AND NASAL DRUG FORMULATIONS
ON THE NASAL CILIARY ACTIVITY



Chapter 5

Classification of Cilio-inhibiting Effects of Nasal Drugs

Laryngoscope 2001; 111: 595-602

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Abstract

Objective/ Hypothesis: Nasal drug formulations are widely used for a local therapeutic effect, but also for systemic drug delivery. In the development of new nasal drugs the toxic effect on the mucociliary clearance and therefore on the ciliated tissue is of importance. In this study the effect of nasal drugs and their excipients on the ciliary beat frequency (CBF) is investigated.

Study Design: Experimental, in vitro.

Methods: CBF is measured by a photo-electric registration method. Excised ciliated chicken trachea tissue has been incubated for 15 min in the formulation, followed by a reversibility test. In order to estimate the ciliostatic potential a classification is given of all tested formulations. According to the CBF after 60 min every drug or excipient could be classified as follows: *Ciliofriendly*: after 60 min the CBF has regained 75% or more of its initial frequency. *Cilio-inhibiting*: after 60 min the CBF has regained between 25 and 75% of its initial frequency. *Ciliostatic*: after 60 min the CBF has regained 25% or less of its initial frequency.

Results: Most formulations used are ciliofriendly or cilio-inhibiting. Only some are ciliostatic. Preservatives have a major role in the cilio-inhibiting effect of the drug. Also other additives can contribute to the toxicity profile of nasal drug formulations.

Conclusion: This classification of the cilio-inhibiting potential of nasal drug formulations is a valuable tool in the design of safe nasal drugs. The number of animal studies in vivo can be reduced substantially by using this in vitro screening technique. This study demonstrates that the effect on ciliary movement of most drug formulations is due to the preservatives and/or additives and mostly not to the drug itself.

Key words: Nasal drug, preservatives, ciliary beat frequency, ciliostatic, cilio-inhibiting, ciliofriendly.

Introduction

Nasal drug formulations, containing for instance decongestants and corticosteroids, are widely used for a local therapeutic effect. The nasal mucosa is also a very attractive site for systemic drug absorption. It is an effective alternative for other routes of drug administration (oral, injection) for instance in the case of antimigraine substances^{1,2}, steroids³ and peptide and protein drugs^{4,5}. Nasal drug absorption can be very efficient because the nasal epithelium has a relatively large permeability and the subepithelial layers are highly vascularised.⁶

Nasal drug delivery has a number of clear advantages, including ease of administration, patient acceptability and prevention of first-pass effect.⁷ The relatively small surface area of the nasal cavity and the mucociliary clearance are drawbacks in nasal drug delivery. The residence time of a drug formulation in the nose is limited to only about 15 min, because of the nasal mucociliary clearance.⁸⁻¹⁰ It is obvious that during the acute or chronic nasal drug application, the drug itself and the formulation excipients should not disturb the nasal mucociliary clearance, because it is an extremely important defence mechanism of the respiratory tract. By the mucociliary clearance bacteria, viruses, allergens and dust are removed from the respiratory tract. Since ciliary movement is a major factor in mucociliary clearance, the influence of drug formulations on the ciliary beat frequency (CBF) is an important issue to establish the safety of nasally administered drugs and various formulation excipients such as preservatives¹¹⁻¹³ and absorption enhancing compounds.^{13,14}

The aim of this study was to test the cilio-inhibiting effects of a number of drugs, using ciliated chicken embryo tracheal tissue. Chicken trachea has shown to be a valid substitute for human material in studying ciliary activity *in vitro*.^{15,16} Moreover, the reversibility of the observed effects was established after exposure of the ciliated tissue to the nasal drug formulations during 15 min, comparable to the situation *in vivo*. The evaluation of the influence on ciliary movement may offer a possibility to classify drugs and excipients according to their inhibiting effect.

Materials and Methods

The nasal formulations selected for the present study are widely prescribed drugs for local and systemic effects, some excipients, and investigational drug formulations indicated for systemic nasal drug absorption. Products have been

selected which are available on the market in the US and Europe, although brand names may differ sometimes.

Materials:

Benzalkonium chloride (BAC; U.S.P. quality) was from Brocacef (Maarssen, The Netherlands), chlorobutanol was from Sigma-Chemie (Dreisenhofen, Germany), and sodium edetate (EDTA; P.A. quality) from Merck (Darmstadt, Germany). Randomly methylated β -cyclodextrin (RAMEB; degree of substitution 1.8) was obtained from Wacker (Burghausen, Germany). All other chemical compounds were from Sigma –Chemie (Dreisenhofen, Germany) and the drug substances were from Bufa (Uithoorn, the Netherlands). The species of the chickens used was Hubbard-Golden Comeet (Vossensteijn, Groenekan, The Netherlands).

(Non-) Prescription Nasal Drug Formulations:

All nasal formulations selected for the present study are widely used prescription and non-prescription drugs for local or systemic effects, and were studied for their influence on ciliary beating in undiluted form. The following formulations were investigated:

Estradiol (Aerodiol®; Servier, Paris, France) 0.2% w/v, containing randomly methylated β -cyclodextrin (RAMEB) 2.0% w/v; Fluticasone (Flixonase®; Glaxo Wellcome B.V., Zeist, The Netherlands) 0.05% w/v, containing BAC 0.02% w/v and phenylethylalcohol 0.25% w/v;

Sumatriptan (Imigran®; Glaxo Wellcome B.V., Zeist, The Netherlands) 20% w/v in a phosphate buffer pH 5.4; Salmon calcitonin (Miacalcic®; Novartis Farmaceutica, Barcelona, Spain) 2,200 IU/ml, containing benzalkonium chloride (BAC) 0.01% w/v; Desmopressin (Minrin®; Ferring, Malmö, Sweden) 0.01% w/v, containing chlorobutanol 0.5% w/v; Triamcinolone acetonide (Nasacort®; Rhône Poulenc Rorer B.V., Amstelveen, The Netherlands) 0.05% w/v, containing cellulose, sodium carboxymethylcellulose, polysorbate 80, BAC and EDTA; Oxymetazoline (Nasivin®; Merck, Darmstadt, Germany) 0.05% w/v, containing BAC and EDTA; Oxymetazoline (Nasivin® pur; Merck, Darmstadt, Germany) 0.05% w/v, preservative-free; Mometasone fuorate (Nasonex®; Schering-Plough B.V., Maarssen, The Netherlands) 0.05% w/v, containing BAC, polysorbate 80 and phenylethylalcohol; Xylometazoline (Otriven®; Novartis Consumer Health, Munich, Germany) 0.1% w/v, containing citric acid, sodium citrate and glycerol, preservative-free; Xylometazoline (Otrivin®; Novartis Consumer Health, Breda, The Netherlands) 0.1% w/v, containing BAC and EDTA;

Budesonide (Rhinocort®; Astra Pharmaceutica, Zoetermeer, The Netherlands) 0.1% w/v, containing potassium sorbate and sodium edetate (EDTA); Oxymetazoline (Sinex®; Richardson Vicks B.V., Rotterdam, The Netherlands) 0.05% w/v, containing BAC 0.02% w/v, chlorhexidine digluconate, EDTA 0.01% w/v, and also menthol, camphor, eucalyptol and tyloxapol.

Investigational Nasal Formulations:

The investigational hydroxocobalamin formulation consisted of hydroxocobalamin 1.2% w/v and NaCl 0.7% w/v in 20 mM sodium acetate buffer of pH 4.5. Melatonin nasal preparations contained melatonin 0.2% w/v, NaCl 0.9% w/v and the solubilizer β -cyclodextrin 0.75% w/v in water. The midazolam formulation consisted of midazolam hydrochloride 3.1% w/v, benzylalcohol 1% v/v and propylene glycol 25% v/v in water. Propranolol hydrochloride 1.0% w/v was dissolved in Locke-Ringer.

Excipients:

A number of excipients used in the (non-) prescription and investigational nasal drug formulations were measured for their effect on ciliary beat frequency, after dissolving these substances in Locke-Ringer solution: the solubilizer/absorption enhancer RAMEB in concentrations of 2.0 % w/v, the preservative BAC in concentrations of 0.01% and 0.02% w/v, and the preservatives phenylethylalcohol and chlorobutanol in concentrations of 0.5% w/v. Additionally, combination preparations of the preservative BAC 0.01% and potassium sorbate 0.2% with EDTA 0.1% w/v in Locke-Ringer were tested. Three vehicle solutions were investigated: 120 mM phosphate buffer (adjusted to pH 5.4), 20 mM sodium acetate buffer containing NaCl 0.9% w/v (adjusted to pH 4.5), and benzylalcohol 1% v/v with propylene glycol 25% v/v in water.

Locke-Ringer (Control Solution):

Locke-Ringer (LR) is an isotonic solution of the following composition per liter of water: NaCl, 7.72 g (132 mmol); KCl, 0.42 g (5.63 mmol); CaCl₂·2H₂O, 0.16 g (1.24 mmol); NaHCO₃, 0.15 g (1.79 mmol); glucose, 1.00 g (5.55 mmol). Locke-Ringer solution was prepared using Millipore-deionized water, and the solution was subsequently sterilized for 20 min at 120°C. The pH of the Locke-Ringer solution was established at 7.4.

Ciliary Beat Frequency Measurements:

Ciliary beat frequency (CBF) measurements were performed on the ciliated epithelium of isolated chicken embryo trachea as described previously.^{13,17} Briefly, the chicken embryo trachea was dissected from the embryo and sliced into small rings of about 1 mm thickness. The trachea slices were placed in stainless steel supporting rings, and were allowed to recover for 30 min in Locke-Ringer solution. Thereafter, the tissue samples were put in a well containing 1.0 ml of the test solution, and placed under an Olympus BH-2 light microscope. The microscope table was connected with a thermostat to maintain a temperature of 33°C. The CBF was subsequently monitored using a photo-electric registration device. A light beam was transmitted through the moving cilia, and after magnification by the microscope the flickering light was projected to a photocell. The electrical signal generated by this photocell was visualized with a computer monitor. The frequency of the signal was calculated electronically by Fast Fourier Transform algorithm and displayed as a frequency distribution.

After starting the incubation, the CBF was measured at 5, 10 and 15 min. Thereafter, in order to test the reversibility of CBF, the trachea slices were washed by shaking them vigorously in a tube with 3 ml Locke-Ringer. Then the slices were replaced in pure Locke-Ringer and CBF was measured again every 5-10 min until 60 min after the start of the incubation. Every formulation has been tested using tissue samples of at least 6 different chickens.

CBF data were calculated as the relative frequency of the initial frequency measured in Locke-Ringer solution at the start of the experiment, the latter being expressed as 100%.

Classification of Effects on CBF:

The influence of the studied nasal drug formulations and excipients on CBF was classified into three categories (Fig. 1):

Ciliofriendly: after 60 min the CBF has regained 75% or more of its initial frequency.

Cilio-inhibiting: after 60 min the CBF has regained between 25 and 75% of its initial frequency.

Ciliosstatic: after 60 min the CBF has regained 25% or less of its initial frequency.

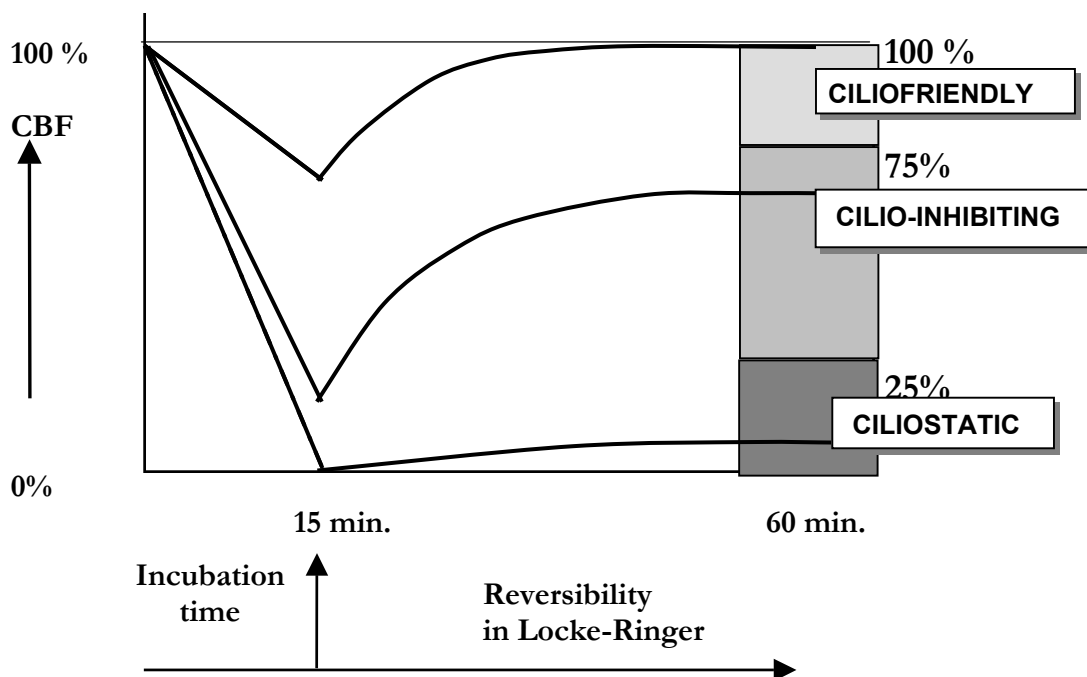


Figure 1. Classification of the effect of nasal formulations on ciliary beat frequency (CBF). CBF is expressed as percentage of the initial frequency (100%). After 15 min incubation of the ciliated tissue in the nasal formulation, the reversibility of the CBF in Locke-Ringer solution is measured. At 60 min after the start of the incubation, the degree of reversibility is classified into 3 categories, i.e. ciliofriendly, cilio-inhibiting or ciliostatic.

Results

A summary of the results is shown in Table I-III. The CBF of the control solution (Locke-Ringer) remained 100% of the initial frequency at least one hour in all experiments (Table I).

Nasal Products:

Imigran®, Rhinocort®, Nasacort® and Aerodiol® reduce CBF, and this effect is reversible. Imigran® arrested the ciliary beating within 5 min, but the mean CBF recovered to 96% of the initial frequency at completion of the reversibility test. Rhinocort® (Fig. 2), Nasacort® and Aerodiol® resulted in mild effects on the CBF after 15 min incubation: the mean CBF decreased to 25, 38 and 42%, respectively. In the subsequent reversibility test CBF increased to 98, 78 and 97% of their initial frequency.

Miacalcic® (Fig. 2) and Flixonase® appeared to have almost identical effects on CBF. Their initial frequency dropped to 12 and 9% after 15 min incubation. After washing and putting the ciliated tissue back into pure Locke-

Ringer, the CBF regained up to 58 and 62% of their initial frequency. Both products contain BAC as a preservative.

Nasivin® pur, containing oxymetazoline without any preservative, decreased the CBF after 15 min to 25%, but this effect was completely reversible. Nasivin® and Sinex® (Fig. 2), containing oxymetazoline and BAC as major constituents, caused a ciliary arrest after 15 min incubation, and this effect appeared to be irreversible.

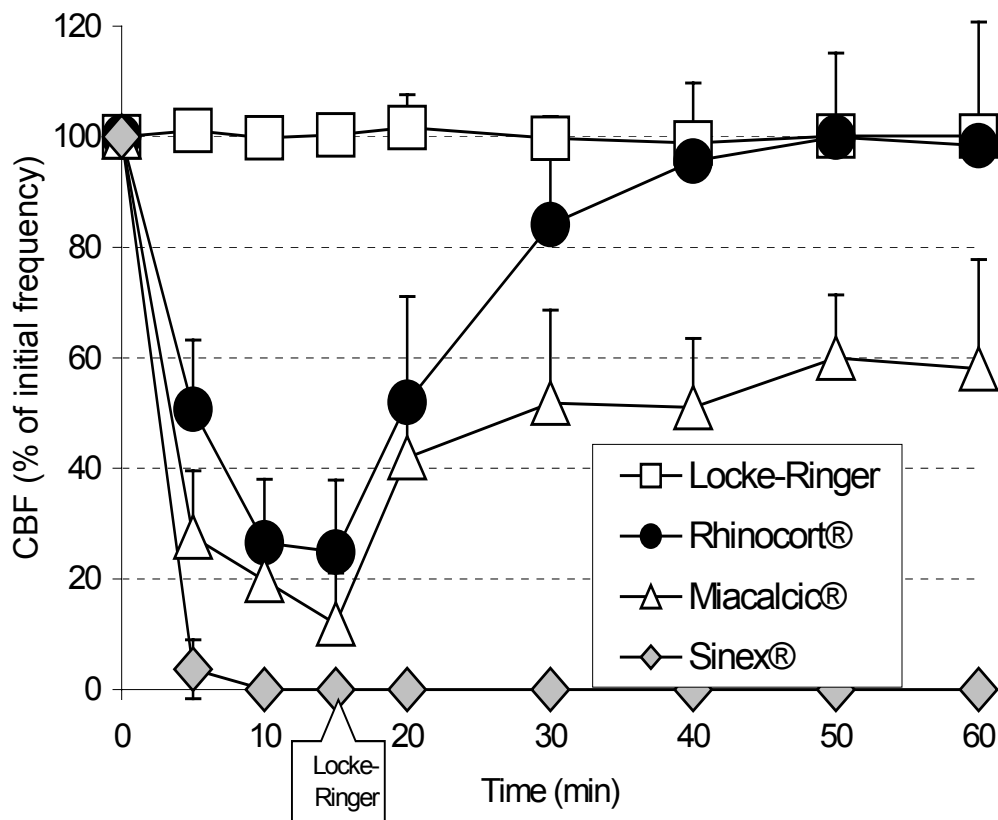


Figure 2. The effect of three nasal products on CBF. After 15 min incubation of the ciliated tissue in the nasal formulation, the reversibility of the CBF in Locke-Ringer solution was measured. The effect, after reversibility testing at 60 min, of Rhinocort® (black circle) is classified as ciliofriendly, that of Miacalcic® (white triangle) as cilio-inhibiting and that of Sinex® (gray rhombus) as ciliostatic. Locke Ringer (white box), the control solution, has no cilio-inhibiting influence. CBF is expressed as percentage of the initial frequency (100%) and data are mean + SD.

Otrivin® (containing xylometazoline, BAC and EDTA) and Otriven® (preservative-free xylometazoline) decreased the mean CBF to 21 and 18% after 15 min exposure. However, only the effect of the preservative-free Otriven® was completely reversible (see Table I).

Nasonex® showed no ciliary beating after 15 min, but the ciliated tissue regained its activity to $33 \pm 19\%$ at 60 min. Minrin® appeared to be ciliostatic,

showing complete and irreversible ciliary arrest within 5 min after exposure in all experiments (Fig. 3a, n=8).

As an illustration of the classification into three categories the profile of Rhinocort® , Miacalcic® and Sinex® are presented in Fig. 2.

Investigational Products:

The effects of some investigational nasal products (hydroxocobalamin, melatonin, midazolam and propranolol) are summarized in Table II.

Excipients:

The effects on CBF of a number of excipients (physiological saline, preservatives, buffers, etc.) are described in Table III. Sometimes the effect is ciliofriendly, but also a ciliostatic effect can be measured, as demonstrated in Fig. 3b for the phosphate buffer and the preservative chlorobutanol.

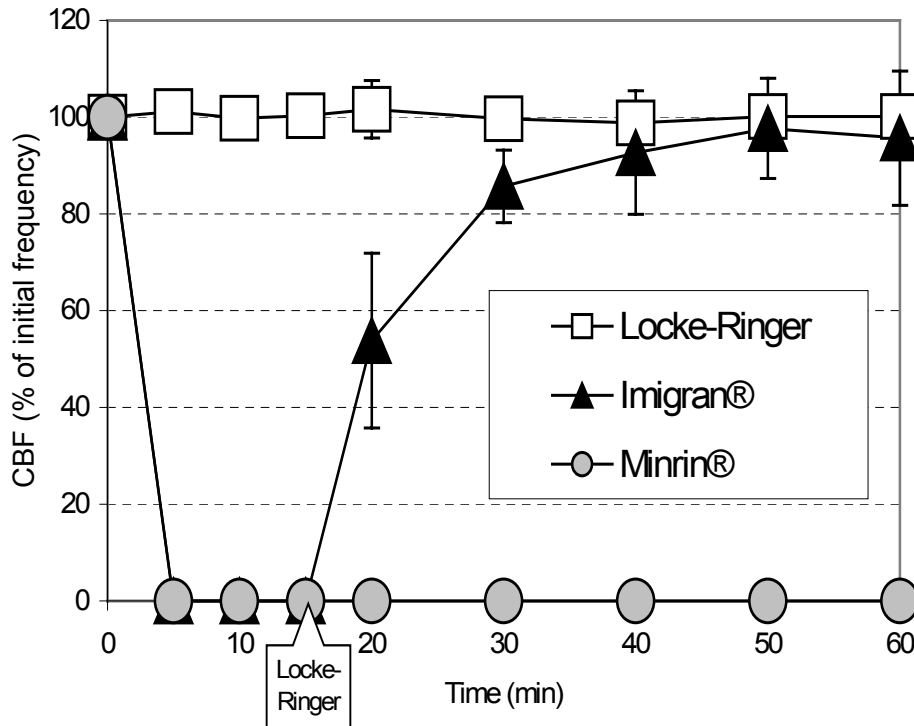
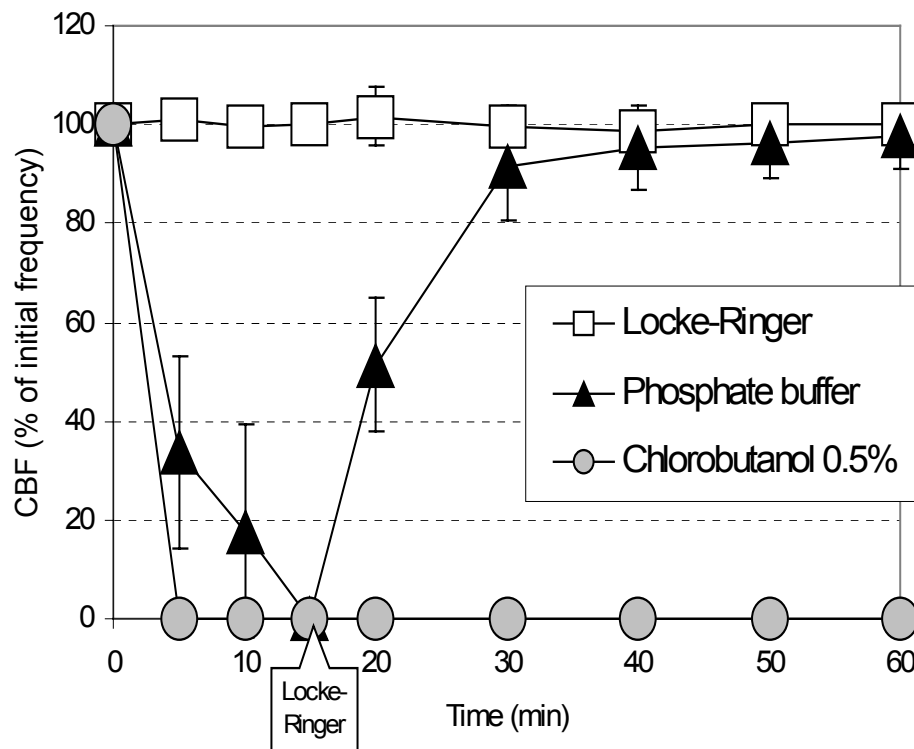
Figure
3aFigure
3b

Figure 3a (top) & 3b (below). Effects of Imigran® and Minrin® on CBF: contribution of formulation constituents. Effects of both nasal products can be explained by its constituents. The effect, after reversibility testing, of Imigran® (containing a phosphate buffer) (black triangle, 3a) is probably due to the buffer solution (black triangle, 3b). The ciliostatic effect of Minrin® (gray circle, 3a) is caused by its preservative chlorobutanol 0.5% (gray circle, 3b). CBF is expressed as percentage of the initial frequency (100%) and data are mean \pm SD.

Figure 4a

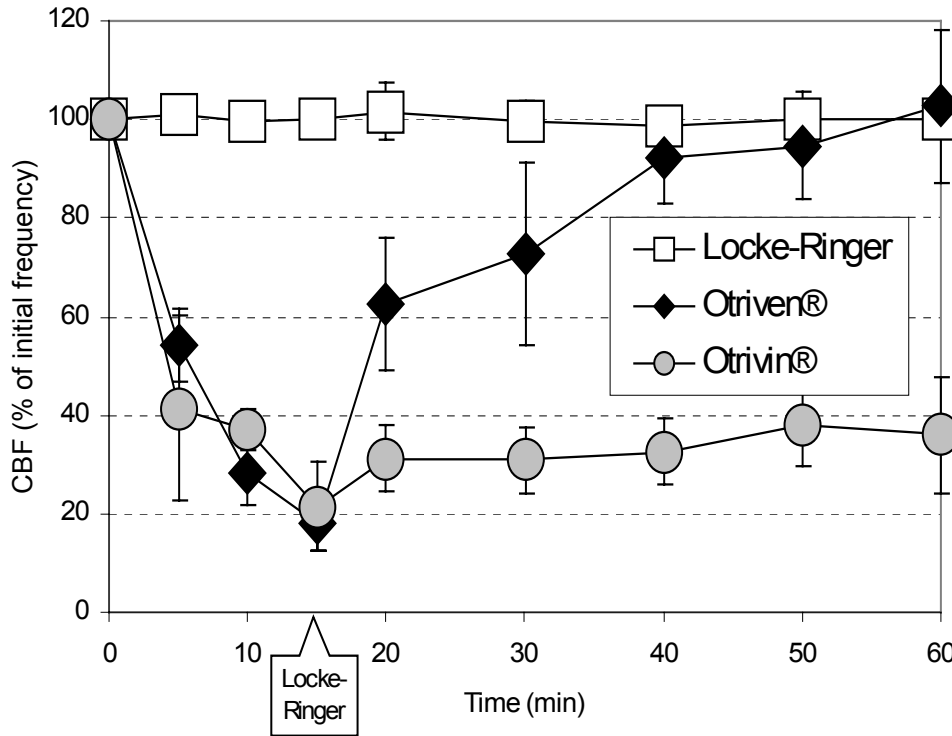


Figure 4b

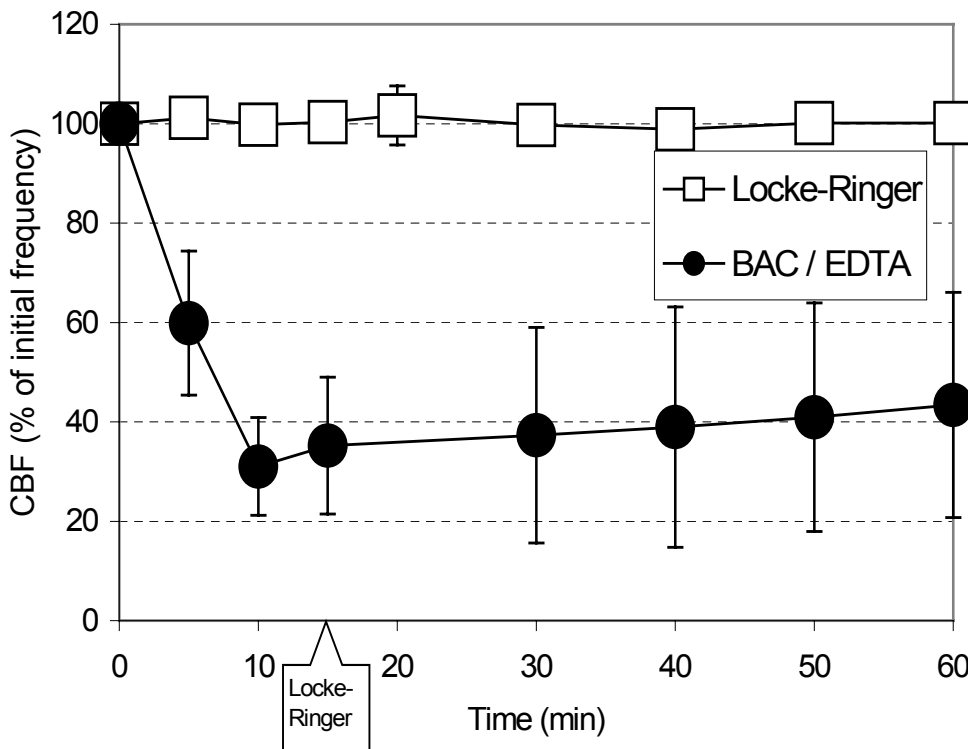


Figure 4a (top) & 4b (below). The difference between the effects of Otrivin® (*with preservative*) and Otriven® (*without preservative*) on CBF. The cilio-inhibiting effect of Otrivin® is likely to be caused by its preservative. Note the similar profile of Otrivin® (gray circle, 4a) and BAC 0.01%/ EDTA 0.1% (black circle, 4b) compared to the ciliofriendly effect of Otriven® (black rhombus, 4a), xylometazoline without any preservative. CBF is expressed as percentage of the initial frequency (100%) and data are mean \pm SD.

Table I. The effect of (non-) prescription nasal drug formulations on ciliary beat frequency (CBF) in vitro

CBF (% of initial frequency) after 15 min incubation in the test formulation (t=15) and after reversibility testing in Locke-Ringer solution until 60 min (t=60). Data are expressed as the mean (\pm SD) of 6 – 8 experiments. Classification according to Figure 1.

BAC= benzalkonium chloride; EDTA= sodium edetate; RAMEB= randomly methylated β -cyclodextrin

Nasal Product	Main Constituents	CBF t=15 (SD)	CBF t=60 (SD)	Classification
Aerodiol®	Estradiol, RAMEB	42 (7)	97 (8)	Ciliofriendly
Flixonase®	Fluticasone, BAC, Phenylethylalcohol	9 (5)	62 (11)	Cilio-inhibiting
Imigran®	Sumatriptan, Phosphate buffer	0 (0)	96 (14)	Ciliofriendly
Miacalcic®	Calcitonin, BAC	12 (9)	58 (20)	Cilio-inhibiting
Minrin®	Desmopressin, Chlorobutanol	0 (0)	0 (0)	Ciliostatic
Nasacort®	Triamcinolone acetonide, BAC, EDTA	38 (7)	78 (8)	Ciliofriendly
Nasivin®	Oxymetazoline, BAC, EDTA	2 (5)	4 (10)	Ciliostatic
Nasivin® pur	Oxymetazoline	25 (4)	97 (13)	Ciliofriendly
Nasonex®	Mometasone fuorate, BAC Phenylethylalcohol	0 (0)	33 (19)	Cilio-inhibiting
Otriven®	Xylometazoline, citrate, glycerol	18 (5)	103 (6)	Ciliofriendly
Otrivin®	Xylometazoline, BAC, EDTA	21 (9)	36 (12)	Cilio-inhibiting
Rhinocort®	Budesonide, Sorbate, EDTA	25 (13)	98 (22)	Ciliofriendly
Sinex®	Oxymetazoline, BAC, Chlorhexidine, EDTA, Camphor, Menthol, Eucalyptol	0 (0)	0 (0)	Ciliostatic
Control				
Locke-Ringer (LR)		100 (3)	100 (4)	Ciliofriendly

Table II. The effect of investigational nasal formulations on ciliary beat frequency (CBF) in vitro. For explanation: see legend of Table I

Investigational products	Main Constituents	CBF		Classification
		t=15 (SD)	t=60 (SD)	
Hydroxocobalamin 2.0%	Hydroxocobalamin, Locke-Ringer	90 (13)	88 (5)	Ciliofriendly
Hydroxocobalamin 1.2%	Hydroxocobalamin, Acetate buffer	0 (0)	79 (12)	Ciliofriendly
Melatonin 0.05%	Melatonin, Locke-Ringer	80 (12)	99 (4)	Ciliofriendly
Melatonin 0.2%	Melatonin, β -Cyclodextrin	42 (5)	102 (3)	Ciliofriendly
Midazolam 3.1%	Midazolam, Benzylalcohol, Propylene glycol	0 (0)	0 (0)	Ciliostatic
Propranolol 1.0%	Propranolol, Locke-Ringer	0 (0)	0 (0)	Ciliostatic

Table III. The effect of excipients on ciliary beat frequency (CBF) in vitro.

For explanation: see legend of Table I.

BAC= benzalkonium chloride; EDTA=sodium edetate; RAMEB= randomly methylated β -cyclodextrin

Excipient	CBF		Classification
	t=15 (SD)	t=60 (SD)	
NaCl 0.9%	74 (12)	95 (8)	Ciliofriendly
BAC 0.01%	54 (22)	70 (11)	Cilio-inhibiting
BAC 0.02%	52(27)	20(19)	Ciliostatic
BAC 0.01% / EDTA 0.1%	35 (14)	43 (23)	Cilio-inhibiting
Benzylalcohol 1% / Propylene glycol 25%	0 (0)	0 (0)	Ciliostatic
Chlorobutanol 0.5%	0 (0)	0 (0)	Ciliostatic
Phenylethylalcohol 0.5%	0 (0)	97 (12)	Ciliofriendly
Phosphate buffer (120 mM; pH 5.4)	0 (0)	98 (6)	Ciliofriendly
Potassium sorbate 0.2% / EDTA 0.1%	62 (9)	99 (5)	Ciliofriendly
RAMEB 2.0%	61 (17)	93 (6)	Ciliofriendly
Sodium acetate buffer (20 mM; pH 4.5)	0 (0)	88 (15)	Ciliofriendly

Discussion

The measurement of effects on CBF *in vitro* is an accurate and reproducible technique for testing formulations which can interfere with the normal cilia movement. On the basis of the results of this study, it is possible to classify nasal drug formulations on their effects on cilia movement *in vitro*.

However, it is important to emphasize that the effects of drugs and excipients as measured in this study, are only indicational for the effects of nasal drugs on cilia activity *in vivo*. To establish the actual local toxicity of nasal drugs, measuring CBF *in vitro* is probably too sensitive.^{10, 14} *In vitro* the excised ciliated tissue is totally immersed in the test formulation, while *in vivo* the viable ciliated epithelium is protected by a mucus barrier. Nevertheless, this *in vitro* method is a valuable tool for the development of safe nasal drug formulations and selection of safe excipients. It has been shown that the effects on the ciliated tissue of chicken trachea *in vitro* are quite similar to those on human ciliated tissue *in vitro*.^{15, 16} Moreover, use of a large number of animals (e.g. rats, rabbits) can be avoided, since one chicken trachea allows up to 20 *in vitro* cilia experiments.

In order to evaluate the outcome of the CBF and the reversibility testing we have made a classification in three categories. The classification of drugs and excipients compares in relative terms the toxicity potential of constituents of nasal drug formulations. Ciliofriendly and cilio-inhibiting formulations will give a reversible effect on the cilia, whereas ciliostatic formulations have a stronger and (almost) irreversible effect on CBF (Fig. 1 & 2).

In the present study we investigated widely-used nasal products, investigational formulations and a number of excipients used in these products. Locke-Ringer (LR) was selected as control solution, because LR does not influence ciliary activity in a time span of at least 60 min (Fig.2-4). Physiological saline is not a good control, because it has a mild inhibiting effect on CBF (Table III), as recently reported in this journal.¹⁸

Most nasal products also contain preservatives as a major constituent, which appeared to contribute substantially to the ciliostatic potential of the whole product. For example Minrin®, in a number of countries, containing chlorobutanol 0.5% as preservative, has a ciliostatic profile similar to that of the single preservative (compare Fig. 3a with 3b).

Also all products with BAC as a preservative have a cilio-inhibiting effect, most likely caused by the presence of this preservative. The corticosteroid nasal sprays tested in this study are either ciliofriendly (Nasacort®, Rhinocort®) or cilio-inhibiting (Flixonase®, Nasonex®). The difference

between these products is due to the presence of different preservatives and probably not to the different drug compounds. Additives (like NaCl, benzylalcohol, propylene glycol, acetate buffer, phosphate buffer) also have their effect on ciliated tissue, as demonstrated in Table III and Fig. 3. For example, hydroxocobalamin 1.2% nasal formulation containing acetate buffer (pH 4.5), resulted in a completely reversible ciliary arrest. This effect can be attributed to the acetate buffer (Table II and III). Similarly, the effect of Imigran® is mainly caused by the phosphate buffer (Table I and III, Fig.3a and b).

Xylometazoline and oxymetazoline have a similar effect on CBF.¹⁵ Nasivin® pur, oxymetazoline (*without* any preservative), has a ciliofriendly effect. However, Nasivin® and Sinex®, oxymetazoline with BAC and EDTA as main constituents, are classified as ciliostatic. The main reason for the ciliostatic effect is the high concentration of BAC, which was measured to be 0.02% w/v in both products. For the products with xylometazoline (Otrivin® and Otriven®) a similar explanation is feasible, as shown in Fig. 4. Additionally, Sinex® contains chlorhexidine, camphor, menthol and eucalyptol which also enhance the ciliostatic effect.¹³

It is clear that most nasal products have a reversible effect on the ciliated tissue classified as ciliofriendly (>75%) or cilio-inhibiting (25-75%). Only sometimes the drug itself (e.g. propranolol 1.0%) is irreversibly ciliostatic, but often the presence of the additives, especially preservatives, is the reason for the observed ciliostatic profile of nasal formulations. We recommend preservative-free formulations, especially those for chronic use. When prescribing products with a ciliostatic profile, the effects on the ciliated tissue should be taken into account and frequent use should be avoided.

Conclusion

This classification, evaluating the influence of nasal drug formulations on ciliary movement, is a valuable tool in the design of safe nasal drugs. The number of whole animal studies in vivo can be reduced substantially by using this in vitro screening technique.

The formulations and excipients investigated in this study demonstrate that the effect on ciliary movement of most drug formulations is due to the preservatives and/ or additives, and mostly not to the drug itself.

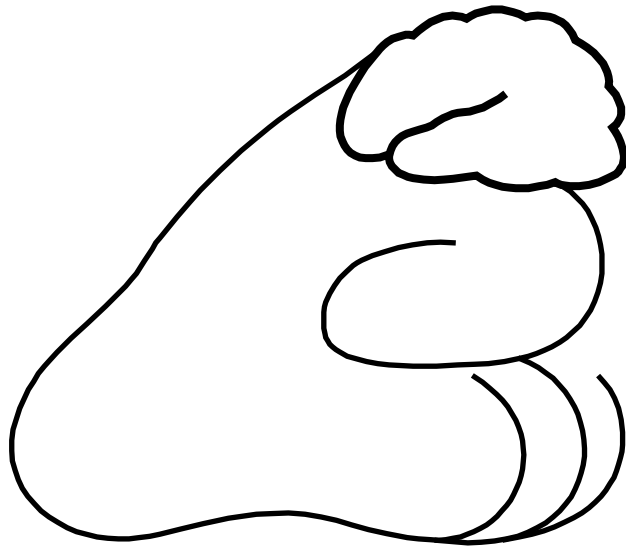
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SECTION IV

NASAL DRUG DELIVERY AND TRANSPORT TO THE CEREBROSPINAL FLUID AND BRAIN



Chapter 6

Quantitative determination of melatonin in human plasma and cerebrospinal fluid with high-performance liquid chromatography and fluorescence detection

Biomedical Chromatography 2000;14:306-310.

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This chapter has been included in this thesis to demonstrate the way we solved the problem of measuring extreme low CSF levels of one of the model compounds (melatonin), used in chapter 7 and 9.

Abstract

A validated new and precise reversed-phase high-performance liquid chromatographic method for the determination of melatonin in human plasma and cerebrospinal fluid, with 5-fluorotryptamine as internal standard, is described.

Liquid–liquid extraction with dichloromethane was performed under alkaline conditions. After evaporation of the organic solvent, the extract was dissolved in eluent and chromatographed on a base-deactivated octadecyl column, using an eluent composed of 650 mL potassium dihydrogenphosphate solution (0.07 mol/L water), adjusted to a pH of 3.0 with a 43% phosphoric acid solution, mixed with 350 mL methanol.

Fluorescence detection at an excitation wavelength of 224 nm and an emission wavelength of 348 nm was used for quantitation. Melatonin and 5-fluorotryptamine chromatographed with retention times of 5.3 and 9.3 min, respectively.

Mean recoveries of 96% ($n = 10$) and 95% ($n = 5$) were found for melatonin in plasma and cerebrospinal fluid respectively. 5-Fluorotryptamine was found to have a mean recovery of 90% ($n = 10$) and 82% ($n = 5$) in plasma and cerebrospinal fluid, respectively. The repeatability coefficients of variation for both melatonin and 5-fluorotryptamine in plasma were 4–5% [five different samples ($r = 5$) on two consecutive days ($n = 2$)], with reproducibility coefficients of 1.6–7% ($n = 2$, $r = 5$) and 0.9–4% ($n = 2$, $r = 5$) for melatonin and internal standard, respectively.

In cerebrospinal fluid the repeatability coefficient of variation of the extraction procedure was 5% ($n = 1$, $r = 5$) for melatonin and 7% ($n = 1$, $r = 5$) for 5-fluorotryptamine. The correlation coefficients of the calibration curves were 0.9998 ($n = 2$) in plasma at a concentration range of 0.108–25.9 ng/mL and 0.9994 ($n = 2$) at a concentration range of 0.108–25.9 ng/mL in cerebrospinal fluid. The limit of detection was determined at 8 pg/mL which enables to measure melatonin concentrations at physiological concentrations reached during daytime.

Introduction

The endogenous hormone melatonin *N*-acetyl-5-methoxytryptamine (Figure 1) is an amino acid derivative which is secreted by the pineal gland. It plays an important role in the regulation of the circadian sleep–wake cycle. Normal average physiologic plasma levels of melatonin during daytime hours are 10 pg/mL, increasing to an average of 60 pg/mL at night (Epstein 1997). Melatonin has been administered orally at dosages of 0.1–5 mg for jet lag and sleep disorders and at much higher doses for the treatment of cancer as single drug or in combination with immunomodulating drugs such as interleukin-2 (Epstein 1997). Besides oral administration, the drug is also administered by the intravenous or intramuscular route. However, there is little data about the concentrations reached in the effect compartment due to a high first-pass metabolism and the existence of natural barriers (blood–brain barrier) to melatonin absorption from the blood circulation to the central nervous system.

To study the melatonin uptake into the cerebrospinal fluid in humans after taking melatonin in different administration forms, an analytical method is warranted

to measure melatonin in human cerebrospinal fluid and in plasma.

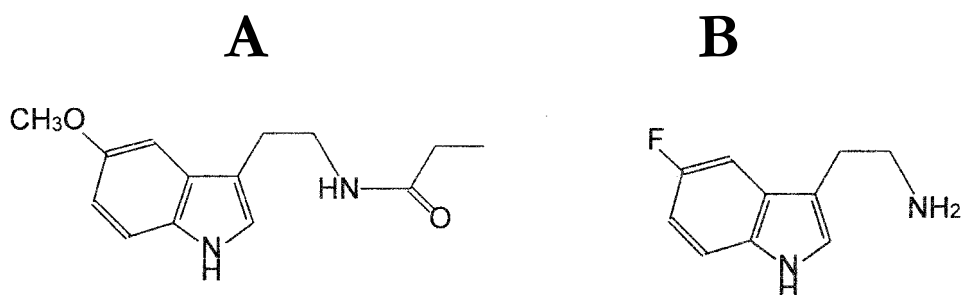


Figure 1. Chemical structures of melatonin (A) and 5-fluorotryptamine (B).

Several gas chromatography–mass spectrometric (Beck and Pevet 1984), Cattabeni *et al.*, 1972) and immunoassay methods (Leung, 1991; Yie *et al.*, 1993) have been reported for the determination of melatonin in biological tissues. More frequently HPLC methods with electrochemical (Chanut *et al.* 1998; Harumi *et al.* 1996; Hernandez *et al.* 1990; Goldman *et al.* 1980; Vieira *et al.* 1992; Azekawa *et al.* 1990; Lee Chin, 1990) or fluorometric detection (Lee Chin 1990; Vitale *et al.* 1996; Peniston-Bird *et al.* 1993; Bechgaard *et al.* 1998; Mills and Finlay 1991) have been described. Fluorescence capacity is characteristic for the indole nucleus of melatonin, which makes it possible to measure low melatonin levels without derivatization. Fluorescence detection

has the advantage over electrochemical detection of being highly selective and nondestructive.

In the current manuscript we present a validated new and highly sensitive reversed-phase high performance liquid chromatographic method with fluorescence detection for the determination of melatonin in plasma and cerebrospinal fluid, using 5-fluorotryptamine (Figure 1) as internal standard. The validation data of the assay in human plasma are presented and a system suitability test was performed to test the application in cerebrospinal fluid.

Materials and Methods

Drugs and chemicals. Melatonin and 5-fluorotryptamine were obtained from Sigma (Zwijndrecht, The Netherlands). Methanol (gradient grade), phosphoric acid (pro analysis), phosphoric acid 43%, dichloromethane and potassium dihydrogenphosphate (pro analysis) were purchased from Merck (Amsterdam, The Netherlands). Milli-Q ultra pure water was from a Millipore (Etten-Leur, The Netherlands) water delivery system. All melatonin and internal standard (5-fluorotryptamine) stock solutions were prepared in methanol and stored at appropriate temperatures.

Equipment. The chromatography system consisted of a Rheodyne 7125 injector, a Waters M515 pump at a flow rate of 1.0 mL/ min, a Millennium³² (version 3.05) chromatographic data system from Waters (Etten-Leur, The Netherlands) and a Jasco FP920 fluorescence detector from Jasco (Maarssen, The Netherlands).

Separation was performed on a 125 x 4.6 mm Supelcosil column packed with 5 mm C₁₈-base deactivated particles with a 20 x 4.6 mm Supelguard C₁₈-base deactivated guard column from Supelco (Zwijndrecht, The Netherlands).

The mobile phase was prepared by mixing 350 mL methanol with 650 mL of a solution which was composed of a potassium dihydrogenphosphate solution (0.07 mol/L water) adjusted to a pH of 3.0 with a 43% phosphoric acid solution. The solvent was filtered and degassed through a 0.22 µm filter from Millipore (Etten-Leur, The Netherlands).

Sample preparation. In a 10 mL disposable glass tube, 40.0 µL of a 0.3 µg/mL 5-fluorotryptamine internal standard solution in methanol was added to 1.0 mL plasma or cerebrospinal fluid sample, containing melatonin. A 100 mL volume of a 4 M sodiumhydroxide solution in water and 5 mL

dichloromethane were added. After 10 min of shaking at 240 min^{-1} , the solution was centrifugated for 5 min at 2700 g . The organic layer was transferred into a clean disposable glass tube and evaporated at 40°C under a nitrogen flow. The residue was dissolved in $250 \mu\text{L}$ eluent. A $20 \mu\text{L}$ volume of this solution was injected into the chromatographic system.

Optimization of the detection wavelength. The excitation and emission wavelengths were determined by recording an UV excitation spectrum of a melatonin solution in eluent into the fluorescence detector. At the wavelengths at which maximal absorption was observed, an emission-spectrum was assessed. The emission and excitation wavelengths at which maximal emission was observed were used for detection.

Validation of the method of analysis. Validation of the method was performed according to the procedure 'Validation of bioanalytical methods' (Manual of quality control, Department of Pharmacy, Academic Medical Center, University of Amsterdam).

Specificity and selectivity. For the examination on the presence of interfering endogenous components, human plasma and cerebrospinal fluid from six different drug-free volunteers was tested. These samples were pretreated according to the sample preparation procedure described above, apart from the addition of internal standard solution. A reference solution containing melatonin and 5-fluorotryptamine in plasma or cerebrospinal fluid was prepared and the chromatograms were compared with those of the blank solutions.

Recovery from plasma. Three serum standards with concentrations ranging over the limits of quantitation of the melatonin assay, were determined 10 times and compared with unpretreated reference solutions in eluent, prepared at similar concentrations as the standards. For the determination of the recovery of melatonin from human plasma, three standards containing 1.080, 6.480 and 12.96 ng/mL were assayed in quintuple and compared with reference solutions prepared in eluent with similar concentrations as the pretreated solutions.

The recovery of the internal standard 5-fluorotryptamine was determined in a similar way at the nominal concentration (13.06 ng/mL), half the nominal concentration and twice the nominal concentration.

Recovery from cerebrospinal fluid. One cerebrospinal fluid standard with a melatonin concentration of 6.480 ng/mL was assayed in quintuple and compared with a reference solution at a similar concentration. The internal standard recovery was performed concordingly at a concentration of 13.06 ng/mL.

Repeatability of extraction from plasma and cerebrospinal fluid. The samples used for the determination of the recovery from plasma were analyzed in two groups (each consisting of five samples) under varying conditions, such as the use of different chromatographic systems with same characteristics and on consecutive days. The concentrations of melatonin and 5-fluorotryptamine found in the plasma samples assayed under both conditions were individually used to calculate the repeatability in both groups of concentrations. The calculation of the repeatability of the extraction from the cerebrospinal fluid was performed once using the data achieved from the recovery test.

The repeatability is defined as:

$$\text{repeatabilityCV} = \frac{\sqrt{MS_{wg}}}{\text{mean}} \cdot 100\%$$

where MS_{wg} represents the mean square within both groups and CV the coefficient of variation. The mean square within groups was determined by the ANOVA test, performed with the statistical software program SPSS (version 6.1.3, SPSS Inc.).

Reproducibility of extraction from plasma. The concentrations found in the samples for the determination of the repeatability were used to calculate the reproducibility between the two data sets obtained on consecutive days. The variation between the two individual sets of results was determined, submitting the results to the ANOVA test. The mean square within groups and the mean

square between groups were calculated. The reproducibility is defined as:

$$\text{reproducibilityCV} = \frac{\sqrt{\frac{MS_{bg} - MS_{wg}}{n}}}{\text{mean}} \cdot 100\%$$

where M_{swg} represents the mean square within groups, M_{sbg} the mean square between groups and n the number of analysis of the sample quantified in one run.

Limit of quantitation. The lower limit of quantitation (LLQ) is defined as the concentration which can be determined with a given precision. The LLQ is appointed at the concentration equal to $S/N = 5$. The recovery and the reproducibility from plasma at this concentration was determined.

The higher limit of quantitation (HLQ) is defined as twice the highest concentration in human samples to be expected in the study.

Linearity. The linearity of the assay is the property of having a linear relationship between the melatonin concentration and the detector response of the method. Five standards with concentrations between the limits of quantitation were assayed twice for plasma and once for cerebrospinal fluid. The results were submitted to the Student t -test using the statistical program 'STATCAL' (STATCAL 6.50, University of Amsterdam, The Netherlands).

This program calculates the probability of the calibration curves order performing the Student t -test to polynoma ($y = A + Bx + Cx^2 + \dots$) with different degrees. For a linear relationship, no significance ($p < 0.05$) should be found for orders surpassing the first degree.

A calibration curve containing standards of 0.108, 1.08, 3.24, 6.48, 12.96 and 25.92 ng/mL melatonin and 13.06 ng/mL 5-fluorotryptamine as internal standard were used for the determination of the linearity of the curve in plasma. Standards with concentration 0.108, 1.08, 3.24, 6.48 and 25.92 ng/mL melatonin and 13.06 ng/mL 5-fluorotryptamine as internal standard were used for the determination of the linearity in cerebrospinal fluid.

Stability. Melatonin stock solutions in methanol and spiked liquor and plasma samples were stored at suitable temperatures and analyzed at appropriate time intervals.

Plasma concentration curve of melatonin in a human volunteer. A healthy 29-year-old male volunteer with a normal kidney and liver function took 5 mg of melatonin as an oral aqueous solution at $t = 0$. Melatonin plasma concentrations were determined before and 30, 75, 120, 270 and 450 min after taking melatonin.

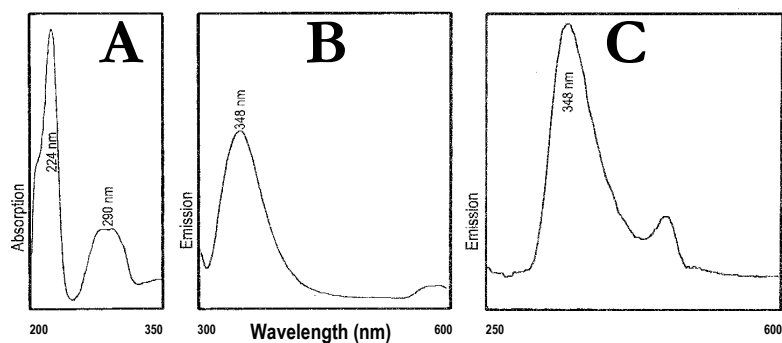


Figure 2. Excitation spectrum of melatonin (A). The emission spectra (B and C) were recorded at the maximal absorption bands of melatonin: 224 nm (B) and 290 nm (C).

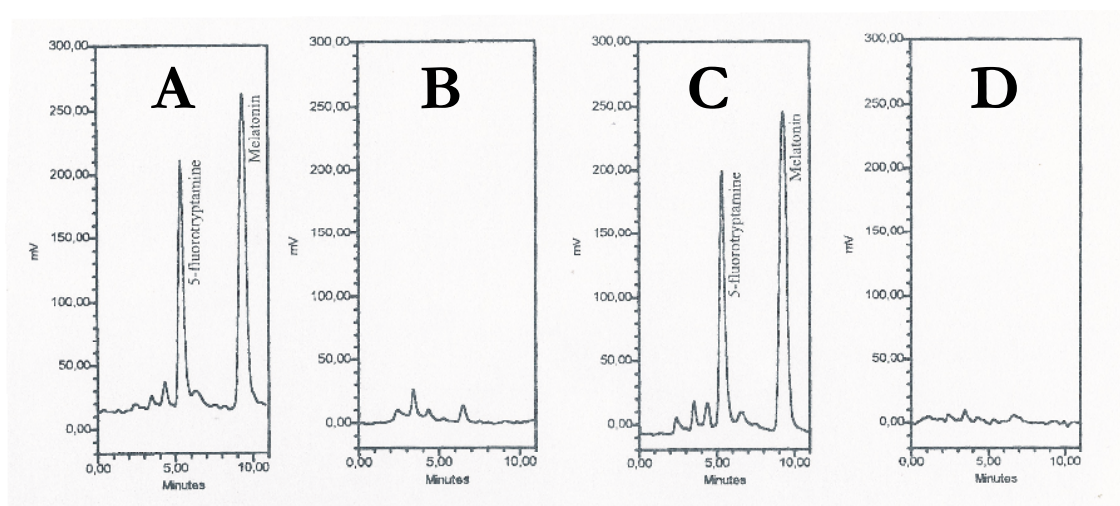


Figure 3. Chromatograms of a plasma extract containing 6 ng/mL melatonin and 5-fluorotryptamine (A), a blank plasma extract (B), a cerebrospinal fluid extract containing 6 ng/mL melatonin and 5-fluorotryptamine (C), and a blank cerebrospinal fluid extract (D).

Results

Optimization of the detection wavelength. Two maximum absorption bands were found in the UV spectrum of melatonin at 224 and 290 nm. Maximal emission wavelengths at 348 nm were obtained from both excitation wavelengths, achieving the highest emission intensity when using the excitation wavelength of 224 nm (Figure 2). Therefore, an excitation wavelength of 224 nm and an emission wavelength of 348 nm was chosen for detection in this study.

Specificity and selectivity. Melatonin and 5-fluorotryptamine chromatograph separately from each other and from endogenous components in plasma as well as in cerebrospinal fluid with retention times of 5.3 and 9.3 min, respectively. Representative chromatograms of blank plasma and cerebrospinal fluid spiked with melatonin and 5-fluorotryptamine and a chromatogram of blank plasma and cerebrospinal fluid are shown in Figure 3.

Recovery. Mean recoveries of 96% ($n = 10$) and 95% ($n = 5$) were found for melatonin in plasma and cerebrospinal fluid respectively. 5-Fluorotryptamine showed mean recoveries of 90% ($n = 10$) and 82% ($n = 5$) in plasma and cerebrospinal fluid respectively (Table 1).

Table 1. Recoveries, repeatability and reproducibility of the extraction of melatonin and 5-fluorotryptamine from plasma and cerebrospinal fluid (nd = not determined)

Compound	concentration (ng/ml)	recovery (%)	repeatability coefficient of variation (%)	reproducibility coefficient of variation (%)
melatonin in plasma	1.08	96	4	7
	6.48	98	5	5
	12.96	95	4	1.6
melatonin in cerebrospinal fluid	6.48	95	5	nd
5-fluorotryptamine in plasma	6.53	92	4	4
	13.06	88	4	1.1
	26.11	91	5	0.9
5-fluorotryptamine in cerebrospinal fluid	13.06	82	7	nd

Repeatability. The repeatability of melatonin and 5-fluorotryptamine from plasma ranged from 4% to 5% five different samples ($r = 5$) on two consecutive days ($n = 2$). In cerebrospinal fluid, the repeatability was found to be 5% ($n = 1$, $r = 5$) and 7% ($n = 1$, $r = 5$) for melatonin and 5-fluorotryptamine respectively (Table 1).

Reproducibility. The reproducibility of the extraction from plasma was 1.6–7% ($n = 2$, $r = 5$) and 0.9–4% ($n = 2$, $r = 5$) for melatonin and 5-fluorotryptamine respectively (Table 1).

Limit of quantitation. The lower limit of quantitation of melatonin was calculated at 8 pg/mL. The recovery from plasma at this value was

determined to be 103% ($n = 1$, $r = 5$) with a repeatability of 8% ($n = 1$, $r = 5$). The higher limit of quantitation was estimated at 25.92 ng/mL.

Linearity. The best curve fitting was obtained with first degree regression, when applying the Student t -test to the calibration points. The calibration curve of melatonin was found to have a mean linear correlation coefficient of 0.9998 ($n = 2$) in plasma and a mean correlation coefficient of 0.9994 ($n = 1$) in cerebrospinal fluid.

Stability. Melatonin stock solutions were found to be stable for at least 45 days (102% of the initial value). The concentrations in liquor, stored at 25, 4 and -20°C , were 84%, 63% and 105%, respectively, after 4 days. After a period of 52 days, 92% melatonin was found in the cerebrospinal fluid samples stored at -20°C .

Concentrations in plasma after 4 days were found to be 65%, 61% and 106% when storing the samples at 25, 4 and -20°C , respectively. An 81% recovery was found after 52 days when plasma samples were stored at -20°C .

Plasma concentration curve of melatonin in a human volunteer. A plasma concentration–time curve of a human volunteer after taking 5 mg melatonin orally is shown in Figure 4.

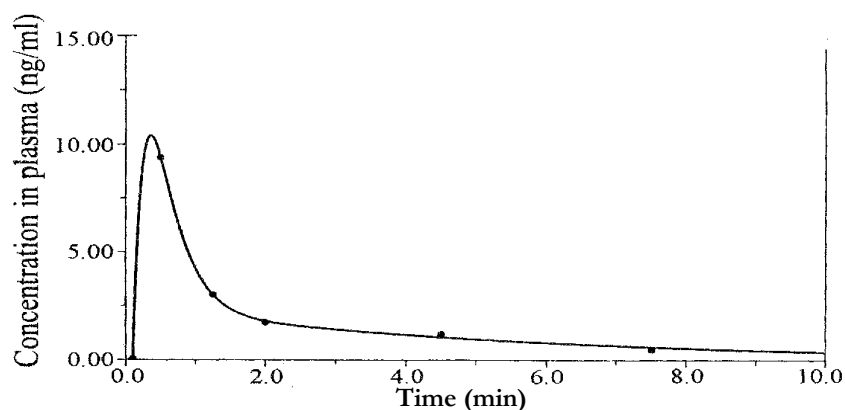


Figure 4. Plasma concentration–time curve of melatonin in a human subject, given 5 mg melatonin orally in an aqueous solution.

Discussion

We developed a new method for the determination of melatonin in human plasma and cerebrospinal fluid, using a liquid–liquid extraction procedure and HPLC in combination with fluorescence detection. As melatonin reaches very low concentrations in plasma and cerebrospinal fluid, it is necessary to apply a

very sensitive detection method and to use highly sensitive equipment. Fluorescence detection as applied in our study explores the fluorescence capacity of the indole nucleus of melatonin and enables to detect extreme low melatonin concentrations. With this method melatonin concentrations can be determined at physiological concentrations reached during daytime of 8 pg/mL. The achieved sensitivity of <10 pg/mL is sufficient for our study. However further increase of the sensitivity to concentrations as low as 1 pg/mL could be measured by alteration of the method; dissolving the residue in 100 mL instead of 250 mL after evaporation of organic extraction solvent and injecting 60 mL into the HPLC system instead of the 20 mL are suggested to enhance the sensitivity. Fluorescence detection was optimized for the excitation and emission wavelengths. Few endogenous compounds, except for some tryptamine derivatives such as serotonin and tryptamine, were detectable in human blank plasma and cerebrospinal fluid at the wavelengths used. However, these substances all chromatographed separately from melatonin and 5-fluorotryptamine (data not shown).

The results of the tests performed of the system's suitability for determination of melatonin in cerebrospinal fluid correlated well with the results obtained from the validation of the melatonin assay in plasma. Therefore the quantitation can be performed in a similar way in both plasma and in cerebrospinal fluid. In comparison with other HPLC assays described, mainly developed to measure melatonin in the pineal gland (Harumi *et al.*, 1996; Hernandez *et al.*, 1990; Azekawa *et al.*, 1990; Lee Chin, 1990; Vitale *et al.*, 1996) our method offers comparable sensitivity in plasma and cerebrospinal fluid [1–60 pg on the column (Chanut *et al.*, 1998; Goldman *et al.*, 1980; Harumi *et al.*, 1996; Hernandez *et al.*, 1990; Lee Chin, 1990; Leung, 1991; Mills and Finlay, 1991; Peniston-Bird *et al.*, 1993; Vieira *et al.*, 1992; Vitale *et al.*, 1996) and has the advantage of making use of an internal standard, which results in less variability of the assay. Furthermore the extraction procedure offers an easy practicable and faster alternative for the commonly used solid phase extractions for determination of melatonin in plasma and serum.

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Chapter 7

Direct access of drugs to the human brain after intranasal drug administration?

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Abstract

Objective/Hypothesis It is suggested that intranasal drug delivery could be used to administer drugs directly to the brain, bypassing the blood-brain barrier. Real evidence of this new route of drug transport is still missing because of lacking intranasal- intravenous comparison.

This study evaluates drug CSF levels in patients after IN and IV administration of two test formulations. Our aim is to investigate the possibility of direct transport of drugs from the olfactory area to the CSF in human volunteers.

Study Design Pharmacokinetic study in patient volunteers.

Methods Eight patients with an external cerebrospinal drain were recruited. They received either a hydrophilic hydroxocobalamin or a lipophilic melatonin formulation and the drug IN on the first day and the same drug IV on the second day. Blood samples and CSF samples were collected just before and at 5, 10, 20, 30, 40, 60, 120 and 180 minutes after drug administration. Concentration-time curves of the plasma and CSF levels were compared after IN and IV administration.

Results The uptake of hydroxocobalamin into the CSF follows exactly the same pattern as the uptake in blood after IN and IV. The melatonin CSF uptake in each patient during 180 minutes after IN and IV administration was the same, whether the drug was administered IN or IV. Both results suggest no additional transport from the nose direct to the CSF.

Conclusion We found no evidence of direct transport of the drugs from the nose to the CSF.

Key words: nasal drug delivery, melatonin, hydroxocobalamin, central nervous system, blood-brain barrier.

Introduction

New ways to circumvent the blood-brain barrier could be useful in the treatment of CNS disorders or in the prevention of a disorder (e.g. treatment of a vitamin B₁₂ deficiency to avoid the development of AD¹). For more than 30 years studies, mainly in animals, have proposed the direct transport of a variety of compounds directly from the nose to the CSF after intranasal (IN) administration^{2,3}. A recent report suggests that “sniffing neuropeptides” may lead to an accumulation of these peptides, such as melanocortin and insulin, in the CSF within 80 minutes⁴. The results suggest that small amounts of peptide molecules travel to the CSF via the olfactory region, but the authors admitted that the data cannot establish that IN administration results in greater uptake in the CSF than does IV administration. Moreover, 20 years ago in experiments with other neuropeptides in dogs, no direct or facilitated transport from nose to the CSF could be demonstrated⁵. Obviously the nose-to-brain transport pathway hypothesis is still controversial. Solid human data are meagre. In this paper we present data in patients with a CSF drain regarding the ‘nose to brain’ transport of drugs comparing uptake by CSF after IN vs IV administration.

Materials and methods.

We recruited patients from the Neurosurgery Department. We selected melatonin (lipophilic, MW 232) and hydroxocobalamin (vitamin B₁₂, hydrophilic, MW 1346) as model compounds because for both drugs kinetics of nasal absorption in human subjects have been documented^{6,7} and they are considered safe in the doses used. The study protocol was approved by the Medical Ethical Committee of the University Hospital of Amsterdam and all patients gave written informed consent.

Three patients (two women, one man, 42-54 years of age) received melatonin IN (0.4 mg, one puff of 0.2 mg=100µl in each nostril) and IV (0.2 mg) the consecutive day. Five patients (Four women, one man, 49-52 years of age) received hydroxocobalamin IN (1.5 mg, one puff of 0.75 mg= 70µl in each nostril) and IV (0.075 mg) the consecutive day. The IV administration (drug dissolved in 100 ml saline solution) was done by infusion over 15 minutes to mimic the time for nasal absorption. Nasal doses were given by one puff in each nostril using unit-dose nasal sprays (Pfeiffer, Radolfzell, Germany). They were weighted prior and following administration to ensure given doses.

During spraying patients were in a horizontal position with hyperextension of the neck, which was maintained for 10 minutes.

Blood samples (indwelling arterial forearm cannula) and CSF samples (cisternal or lumbar CSF drain tap) were taken at $t = 0, 5, 10, 20, 30, 40, 60, 120, 180$ minutes.

Melatonin was determined by a validated high-performance liquid chromatographic method with fluorescence detection⁸. The coefficient of variation (CV) is 4-5% for low and high range levels of melatonin. The hydroxocobalamin concentrations were determined in plasma and in the cerebrospinal fluid by radioimmunoassay (Solid Phase No Boil Dual Kit, Diagnostic Products Corp., Los Angeles, CA USA). The CV is 4% for high levels and 9% for low levels of hydroxocobalamin.

Results.

The maximum plasma concentrations of *melatonin* in the systemic circulation were measured in the sample taken 10 min after IV administration and five min after IN administration. The melatonin CSF uptake in each patient during 180 minutes after IN and IV administration was the same, whether the drug was administered IN or IV (table1a). We calculated also the ratio of the uptake of melatonin in the CSF after IN in relation to the concentrations in plasma after IV (CSF ratio) and found no additional transport to the CSF after IN (table1a).

The maximum plasma concentrations of *hydroxocobalamin* in the systemic circulation after IV were found after 20-30 min in all subjects. The time to reach the maximum hydroxocobalamin levels after nasal absorption varied, but the main fraction had been absorbed within 30 minutes. The CSF ratio for hydroxocobalamin could not be calculated for each patient, because increases in CSF levels of hydroxocobalamin were sometimes very low, often less than 10 pmol/l and very close to the detection limit. The extreme low CSF levels were counted as zero. Therefore we calculated the CSF ratio on the mean AUC plasma and mean AUC csf values of the five patients. The mean AUC csf/ AUC plasma ratio's after IN and IV administration of hydroxocobalamin are the same (0.0049), which indicates no additional transport of hydroxocobalamin from the nose to the CSF (table 1b). The uptake of hydroxocobalamin into the CSF follows exactly the same pattern as the uptake in blood after IN and IV, with a time lag of about 30 minutes (figure 1). It seems plausible to suggest that this time is needed to pass the blood-brain barrier.

Table 1 a. Melatonin uptake (as AUC) in CSF and plasma per patient (X- Z) after IN and IV administration of melatonin.

Melatonin	AUC csf IN (pg/ml).min	AUC plasma IN (pg/ml).min	AUC csf IV (pg/ml).min	AUC plasma IV (pg/ml).min	CSF RATIO*
patient X	127,100	345,300	106,400	253,200	0.88
patient Y	306,300	506,300	311,600	184,300	0.36
patient Z	484,000	299,800	525,600	286,200	0.88
Mean	305,800	383,800	314,533	241,233	0.71
mean AUC csf/ AUC plasma:		IN: 0.80		IV: 1.30	

Table 1 b. Hydroxocobalamin uptake (as AUC) in CSF and plasma per patient (P-T) after IN and IV administration of hydroxocobalamin.

Hydroxo- cobalamin	AUC csf IN (pmol/l).min	AUC plasma IN (pmol/l).min	AUC csf IV (pmol/l).min	AUC plasma IV (pmol/l).min	CSF RATIO*
patient P	0	308,600	0	739,000	
patient Q	2,440	91,180	3,670	525,300	
patient R	780	121,700	3,965	487,000	
patient S	0	351,400	480	538,700	
patient T	2,850	354,300	7,190	842,300	
Mean	1,214	245,436	3,061	626,460	1.0
mean AUC csf/ AUC plasma:		IN: 0.0049		IV: 0.0049	

Table 1: Uptake of melatonin (1a) and hydroxocobalamin (1b) expressed as area under the curve (AUC) from 0-180 min using the trapezoid method.

$$*CSF \text{ ratio} = \frac{AUC \text{ csf IN}}{AUC \text{ plasma IN}} \quad / \quad \frac{AUC \text{ csf IV}}{AUC \text{ plasma IV}}$$

When the CSF uptake is larger after IN administration the CSF ratio >1.

The calculated CSF ratio in patient X-Z is smaller than 1 for all three patients, demonstrating no additional transport of melatonin from the nose to the CSF.

The calculated mean CSF ratio for patient P-T is 1, because the mean AUC csf/ AUC plasma ratios are equal, indicating no extra transport to the CSF after IN administration of hydroxocobalamin. IN= intranasal.

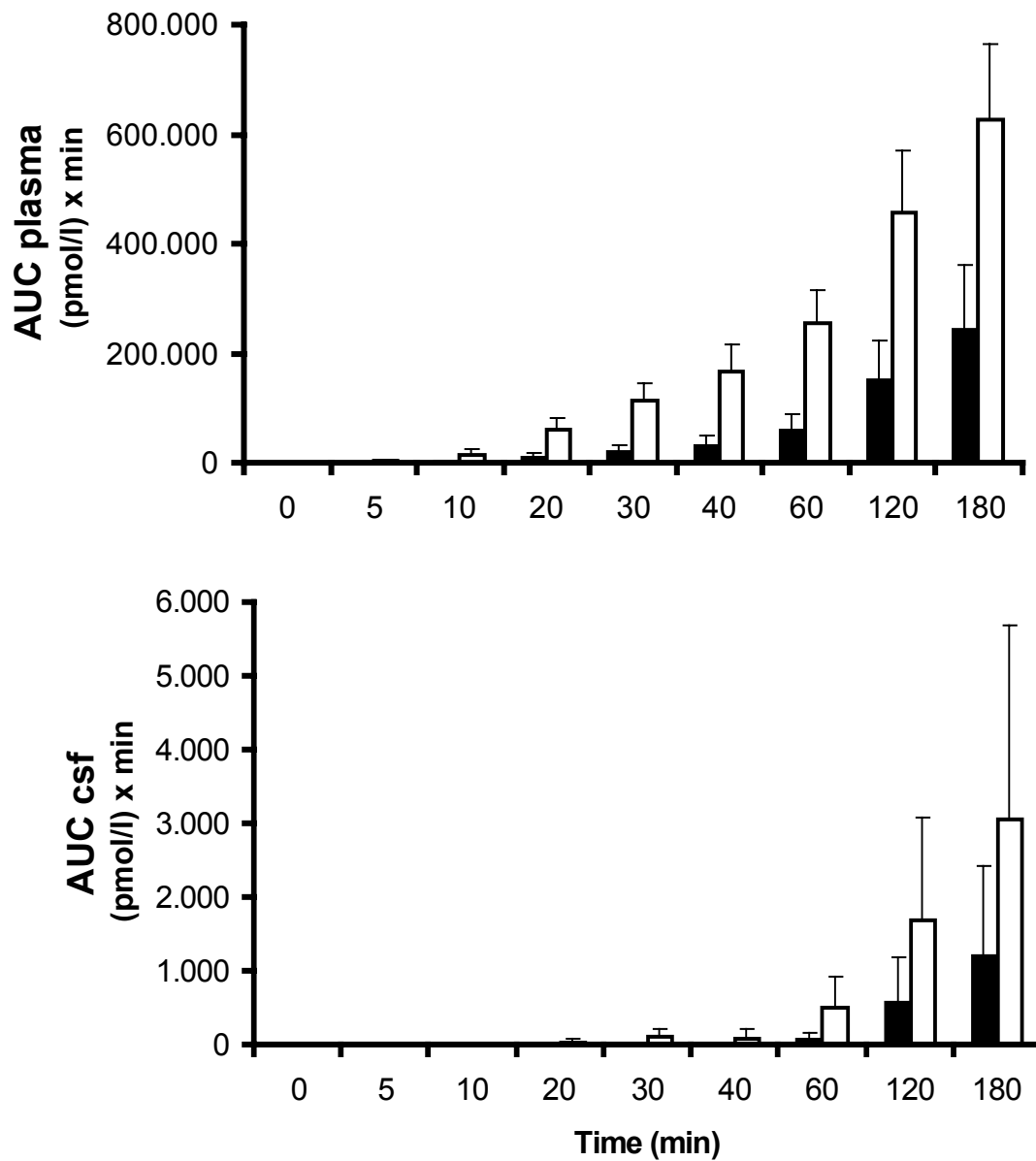


Figure 1. Hydroxocobalamin accumulation (+/- SD) expressed as area under the curve (AUC) in plasma, top figure (AUC plasma IN/ IV) and CSF, figure below (AUC csf IN/ IV). The uptake of hydroxocobalamin in the CSF after IN (black bars) shows exactly the same pattern as after IV (white bars), indicating transport to the CSF via the blood-brain barrier. IN= Intranasal.

Discussion.

The results demonstrate that nasal administration of melatonin and hydroxocobalamin leads to a rapid rise in blood and CSF levels, but they do *not* demonstrate a direct transport from nose to CSF. CSF turnover rate has not been included in the calculations because all data were analyzed in an intraindividual comparison and therefore differences in CSF turnover rate should not influence the individual results. Endogenous levels of melatonin and cobalamin could have influenced the results, but the endogenous levels in blood and CSF are negligible compared to the high levels achieved during our experiments. Inclusion criteria were strict and the population suitable for this study (CSF drain, fully conscious and two days participation) is small. Nevertheless the results are at least indicative for hydrophilic and lipophilic drug transport to the CSF. Similar results are being seen in our concurrent rat studies with a comparable protocol.

Although several animal studies^{2,3} and a recent human study⁴ have suggested a nose to brain pathway, we found *no* extra transport from nose to CSF. What could be the explanation for the different results obtained with our study design and the human study that suggested nose to brain transport for peptides?⁴ Firstly, in our experience the nasal cavity can accommodate only a volume of about 100 microliter of fluid per nostril. In the neuropeptide study² the various formulations were given by repeated puffs of an unrevealed volume in each nostril every 30-45s. Secondly, we investigated two non-peptide drugs that are better absorbed into the systemic circulation than are peptides. It is possible that poor systemic absorption means that more drug is available for direct transport from the olfactory area to the CSF, but for a real proof of direct nose-to-CSF transport an intraindividual comparison of CSF levels after IN and IV administration is required. That comparison was missing in the neuropeptide study⁴. Perhaps the method we used will lead to new studies with other drugs and will answer the question whether for a specific drug a direct nose to brain pathway in humans does exist.

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Chapter 8

Hydroxocobalamin uptake into the cerebrospinal fluid after nasal and intravenous delivery in rats and humans

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Abstract

The possibility of direct transport of hydroxocobalamin from the nasal cavity into the cerebrospinal fluid (CSF) after nasal administration in rats was investigated and the results were compared with a human study.

Hydroxocobalamin was given to rats ($n = 8$) both intranasally (214 $\mu\text{g}/\text{rat}$) and intravenously (49.5 $\mu\text{g}/\text{rat}$) into the jugular vein using a Vascular Access Port. Prior to and after drug administration blood and CSF samples were taken and analysed by radioimmunoassay.

The $\text{AUC}_{\text{CSF}}/\text{AUC}_{\text{plasma}}$ ratio after nasal delivery does not differ from the ratio after intravenous infusion, indicating that hydroxocobalamin enters the CSF via the blood circulation across the blood-brain barrier. This same transport route is confirmed by the cumulative AUC-time profiles in CSF and plasma, demonstrating a 30 min delay between plasma absorption and CSF uptake of hydroxocobalamin in rats and in a comparative human study.

The present results in rats show that there is no additional uptake of hydroxocobalamin in the CSF after nasal delivery compared to intravenous administration, which is in accordance with the results found in humans. This indicates a predictive value of the used rat model for the human situation when studying the nose to CSF transport of drugs.

Keywords: hydroxocobalamin, intranasal delivery, intravenous infusion, cerebrospinal fluid, rat, human

Introduction

With the growing number of patients suffering from central nervous system (CNS) diseases a suitable approach for drug targeting to the brain becomes more and more important. The blood-brain barrier (BBB) hampers drugs to access the CNS and therefore enables a direct therapy for such diseases. In the last decades the nasal administration route has gained much interest in this respect, because the olfactory neurones connect the nasal cavity directly with the brain and the surrounding cerebrospinal fluid (CSF). Dyes, viruses, metals, proteins, and small molecular weight drugs have been investigated on nose-brain/CSF transport in animals and men (Mathison *et al.*, 1998; Illum, 2000). However, the feasibility of the nose-brain pathway for drug targeting to the brain and CSF is still controversial. In rats most of the investigated lipophilic drugs like the steroid hormone hydrocortisone (Van den Berg *et al.*, 2002b), a serotonin antagonist (Dahlin and Björk, 2000) and a cognition enhancing drug (Hussain *et al.*, 1990) are taken up into the CSF following absorption into blood and subsequent crossing the BBB. This is in contrast to a number of hydrophilic drugs like cephalexin (Sakane *et al.*, 1991), the anti-HIV agents zidovudine (Seki *et al.*, 1994) and D4T (Yajima *et al.*, 1998), dopamine (Dahlin *et al.*, 2001) and L-dopa butylester (Kao *et al.*, 2000), which have been found to be directly transported into the CSF after nasal administration in rats. Moreover, direct transport of the lipophilic drugs hydroxyzine and lidocaine (Chou and Donovan, 1997; Chou and Donovan, 1998) has also been reported in animals.

Human pharmacodynamic studies mainly suggested direct uptake of hydrophilic, high molecular weight peptide drugs into the brain after nasal delivery (Fehm *et al.*, 2000). These observations were based on differences in event related brain potentials following an auditory odd ball task, whereas pharmacokinetic evidence was not provided.

In a recently published human study in neurosurgery patients with a CSF drain the hydrophilic and high molecular weight drug hydroxocobalamin (vitamin B₁₂ analogue, MW = 1346 g/mol, aqueous solubility 10 % w/v (Merkus, 1998)) was tested on nose-CSF transport (Merkus *et al.*, 2003). This compound was chosen, because as a hydrophilic compound it is relatively well absorbed after nasal delivery (bioavailability is 5.4 %; Van der Kuy *et al.*, 2001) and safe to be used in humans (Van Asselt *et al.*, 1998; Lonterman *et al.*, 2000). However, increases of CSF levels of hydroxocobalamin were sometimes very low and near the detection limit of the radioimmunoassay (Merkus *et al.*, 2003), although it was tried to increase the hydroxocobalamin concentration by evaporation of the CSF samples according to Nijst *et al.* (1990). Therefore,

it was decided in the present study to increase the dose of hydroxocobalamin by using in rats the same formulation as in the human study. This resulted in a relatively high dose (30-fold higher per kg bodyweight than in humans) to ensure that the hydroxocobalamin levels in the CSF were well above the detection limit of the radioimmunoassay. The rat experiments were performed using a rat model (Van den Berg *et al.*, 2002a; Van den Berg *et al.*, 2002b), which allows simultaneous and serial CSF and blood sampling and also the comparison of intranasal and intravenous drug delivery in the same animal. The aim of the present paper was to study the CSF uptake of hydroxocobalamin after intranasal and intravenous administration in rats and to compare the results with that of the human study (Merkus *et al.*, 2003).

Materials and Methods

Materials

Hydroxocobalamin chloride was from BUFA B.V. (Uitgeest, The Netherlands) and povidone iodine from Sigma Chemical (St. Louis, MO, USA). Janssen Pharmaceutica (Beerse, Belgium) supplied Hypnorm[®] (fentanyl citrate 0.315 mg/ml, fluanisone 10 mg/ml). Dormicum[®] (midazolam, 5 mg/ml) was from Genthon B.V. (Nijmegen, The Netherlands) and Temgesic[®] (buprenorphine, 0.3 mg/ml) from Schering-Plough (Maarssen, The Netherlands). All other reagents were of analytical grade.

Hydroxocobalamin Formulations

The hydroxocobalamin formulation for nasal delivery consisted of hydroxocobalamin chloride (11 mg/ml), sodium acetate (2.7 mg/ml) and sodium chloride (7.0 mg/ml) dissolved in Millipore[®] water, and the pH was adjusted at 4.5 with hydrochloride (Merkus, 1998). For intravenous infusion hydroxocobalamin was dissolved in sterile saline (11 µg/ml).

Animals

Male Wistar rats (Charles River, Someren, The Netherlands) were used, weighing 330 – 470 g at the start of the experiments. The animals ($n = 8$) were housed 2 per cage, with free access to food and water and a 12-h light/dark cycle. The animal experiments were approved by the Ethical Committee for Animal Experiments (Leiden University, Leiden, The Netherlands). All rats were used for intranasal and intravenous treatment in a cross-over design.

Implantation of Vascular Access Port

The animals were provided with a Vascular Access Port (VAP) as described before (Van den Berg *et al.*, 2002b). Briefly, the rats were anaesthetised with Hypnorm[®] (0.5 ml/kg) and Dormicum[®] (0.5 ml/kg) intramuscularly. Two incisions were made, one at the level of the lower ribs to create a pocket for inserting the VAP (Access Technologies, Skokie, IL, USA) and one in the neck to cannulate the jugular vein. The VAP, attached to a silicone catheter (ID 0.5 mm, OD 1.0 mm), was fitted into the pocket, and the catheter was tunnelled underneath the skin from the pocket to the second incision in the neck and inserted into the jugular vein. As post-operative care Temgesic[®] (0.3 ml/kg, intramuscularly) was given for pain relief. The rats were allowed to recover 1 week before starting the experiments. To avoid blockage of the catheter, the VAP was flushed weekly with heparin solution (400 µl; 400 IU/ml).

Nasal and Intravenous Delivery of Hydroxocobalamin

Prior to drug administration the rats were anaesthetised as described above and fixed in a stereotaxic frame (model 51600, Stoelting, Wood Dale, IL, USA) using the supine-70° angle position (Van den Berg *et al.*, 2002a). The animals were kept anaesthetised throughout the experiment, if necessary top op anaesthesia was given. For intranasal administration of the hydroxocobalamin formulation, a polyvinylchloride (PVC) tube (ID 0.5 mm, OD 1.0 mm) attached to a Hamilton syringe was inserted into the left nostril of the rat for about 2 cm. The nasal hydroxocobalamin dose (214 µg/20 µl/rat) was delivered by gently pushing the plunger of the syringe and after delivery the PVC tube was removed.

Subsequently, hydroxocobalamin was administered to the rats by intravenous infusion (49.5 µg/rat). The infusion rate (30 µl/min for 150 min) was chosen in such a way to simulate the observed maximal hydroxocobalamin plasma levels after intranasal delivery. This infusion rate was determined by giving the rats ($n = 3$) an intravenous bolus injection of the vitamin as described previously (Van den Berg *et al.*, 2002b).

Prior to and following hydroxocobalamin delivery, blood and CSF samples were taken until 240 min after administration. Fifteen blood samples were taken at $t = 0, 2.5, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 210$ and 240 min and 11 CSF samples were taken at $t = 0, 15, 30, 45, 60, 90, 120, 150, 180, 210,$ and 240 min. Each rat received both the nasal and the intravenous treatment. Between experiments the animals were allowed to recover for one week.

Blood and CSF sampling

Blood samples (200 μl) were taken from the tail vein using heparinised tubes (Microvette[®] CB 100/200, Sarstedt, Nümbrecht, Germany). Samples were centrifuged (15 min at 14.000 rpm; ambient temperature) and the obtained plasma was stored at 4°C until analysis.

For CSF sampling a cisternal puncture was performed as described before (Van den Berg *et al.*, 2002a). Briefly, rats were anaesthetised and fixed in a stereotaxic frame as mentioned above. The cisternal puncture was performed 5.2 – 6.5 mm ventrally from the occipital crest, dependent on the rat's weight. After the puncture, one drop of CSF was microscopically examined on erythrocyte contents; the experiment was continued when the erythrocyte contamination was less than 500 cells/ μl (< 0.01 % of normal blood content). Following intranasal or intravenous drug administration, CSF samples (about 30 μl) were taken and directly collected in pre-weighed radioimmunoassay tubes and stored at 4°C until analysis.

Hydroxocobalamin Analysis

Plasma and CSF samples were analysed on hydroxocobalamin by radioimmunoassay (Dualcount Solid Phase No Boil Assay, DPC, Los Angeles, CA, USA) with a detection limit of 25 pmol/L. The analysis was performed according to the manufacturer's protocol. When calculating the hydroxocobalamin concentrations for the CSF samples, the sample volumes were taken into account.

Data Analysis

To determine the contribution of the nose-CSF pathway to the hydroxocobalamin uptake into CSF, the $\text{AUC}_{\text{CSF}}/\text{AUC}_{\text{plasma}}$ ratios were calculated for each route of administration. The area under the concentration-time curve (AUC) values (0-240 min) were calculated using the trapezoidal rule. All AUC values and $\text{AUC}_{\text{CSF}}/\text{AUC}_{\text{plasma}}$ ratios were calculated per individual animal before determining the mean value. Data were analysed according to the paired Student's t-test, using the computer program SPSS version 8.0 for Windows.

Results

Hydroxocobalamin was administered intranasally (214 µg/rat) and by intravenous infusion (49.5 µg/rat) to the same set of rats to determine the relative uptake of this vitamin analogue into CSF after nasal delivery compared to intravenous administration. The plasma concentration-time profiles (Fig. 1a) show a slow and prolonged absorption of hydroxocobalamin after nasal delivery, reaching maximal plasma levels of 192 ± 53 nmol/L (mean \pm sd) at 150 min after administration. This was simulated by intravenous infusion of hydroxocobalamin, resulting in similar plasma profiles (Fig. 1a). The observed hydroxocobalamin concentrations in CSF following both intranasal and intravenous delivery increased slowly, but did not reach a maximum within the sampling period of 240 min (Fig. 1b).

	Intranasal	Intravenous
Rats^a		
AUC _{CSF} (nmol*min/L)	166 \pm 105	202 \pm 148
AUC _{plasma} (nmol*min/L)	31272 \pm 8000	32086 \pm 5284
AUC _{CSF} /AUC _{plasma} (%)	0.5 \pm 0.2	0.6 \pm 0.4
Humans^b		
AUC _{CSF} /AUC _{plasma} (%) ^c	0.5	0.5

Table I AUC_{CSF}/AUC_{plasma} ratios of hydroxocobalamin

Data are presented as mean \pm sd, ^a $n = 8$, ^b $n = 5$ (Merkus *et al.*, 2003, chapter 7)

^c Ratio of mean AUC values

As stated in Table I, the distribution of the drug over CSF and plasma after intranasal delivery (0.5 ± 0.2 %) was not significantly different ($p = 0.57$) from that following intravenous infusion (0.6 ± 0.4 %) in rats, which is similar to the results found in humans (Table I). Besides, the hydroxocobalamin uptake into CSF followed the same pattern as the absorption in plasma after intranasal and intravenous delivery, which is demonstrated by the cumulative AUC values plotted against time (Fig. 2). This is also in accordance with the results observed in the human study (Fig. 3). In both species the uptake of hydroxocobalamin in CSF showed a lag time of about 30 min after absorption in plasma.

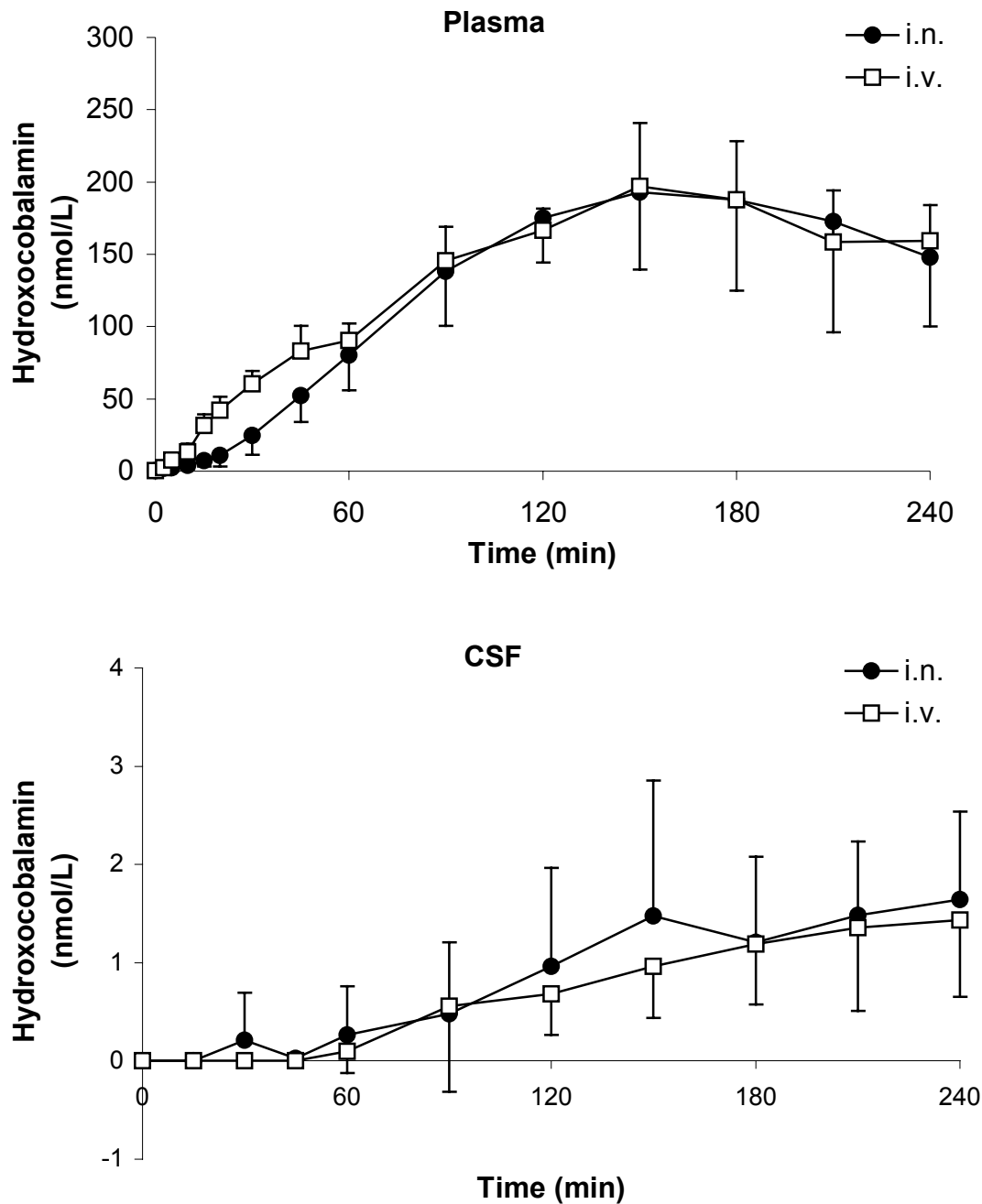


Figure 1 Hydroxocobalamin concentrations in plasma (top figure) and CSF (bottom figure) after intranasal delivery (i.n.; 214 $\mu\text{g}/\text{rat}$) and intravenous infusion (i.v.; 49.5 $\mu\text{g}/\text{rat}$) in rats. Results are expressed as mean \pm sd of 8 animals.

Discussion

In the present study the distribution of hydroxocobalamin over CSF and plasma after intranasal administration is compared to that following intravenous infusion in rats. The observed similarity in distribution profiles after both delivery routes demonstrates no direct hydroxocobalamin transport to the CSF from the nasal cavity. These results are consistent with a human study, in which the same hydroxocobalamin formulation has been tested using a comparable experimental set-up (Merkus *et al.*, 2003). The observed $AUC_{\text{CSF}}/AUC_{\text{plasma}}$ ratios after intranasal and intravenous administration are similar in both species (Table I), just like the cumulative AUC-time profiles of hydroxocobalamin in plasma and CSF (Fig. 2 and 3). Also, the lag time of about 30 min between the plasma absorption and CSF uptake of hydroxocobalamin indicates that this hydrophilic drug is taken up into the CSF subsequent to passage of the BBB, and not by direct transport from the nasal cavity into the CSF.

It should be noted that the rat and human study show remarkable differences in the time to reach plasma C_{max} values of nasal hydroxocobalamin, being about 150 min in rats (Fig. 1a) and about 30 min in men (Merkus *et al.*, 2003). In the rat study hydroxocobalamin is delivered intranasally in anaesthetised rats, whereas in the human study this vitamin is administered in conscious patients. It is well known that anaesthetics, due to their inhibitory effect on the nasal mucociliary clearance, prolong the residence time of the formulation in the nasal cavity and therefore the absorption phase of the administered drug (Hussain *et al.*, 1997; Mayor and Illum, 1997). Obviously, the very high nasal dose used in rats compared to the human study and/or the slow mucociliary clearance in the experimental conditions of the rat study causes a nasal absorption in rats that is slower than in humans. In order to exclude possible oral absorption, the nasally administered dose was also instilled at the back of the throat in rats to simulate possible swallowing of the formulation after intranasal delivery, and in these studies no hydroxocobalamin absorption in plasma was found (data not shown). This is supported by a study in human volunteers, in which the reported oral hydroxocobalamin bioavailability is negligible (Van der Kuy *et al.*, 2000).

The safety of the used hydroxocobalamin formulation was tested previously in vitro and classified as cilio-friendly (Merkus *et al.*, 2001). This formulation was also investigated in vivo during 4 weeks in geriatric patients ($n = 21$), and no adverse effects were reported (Lonterman *et al.*, 2000).

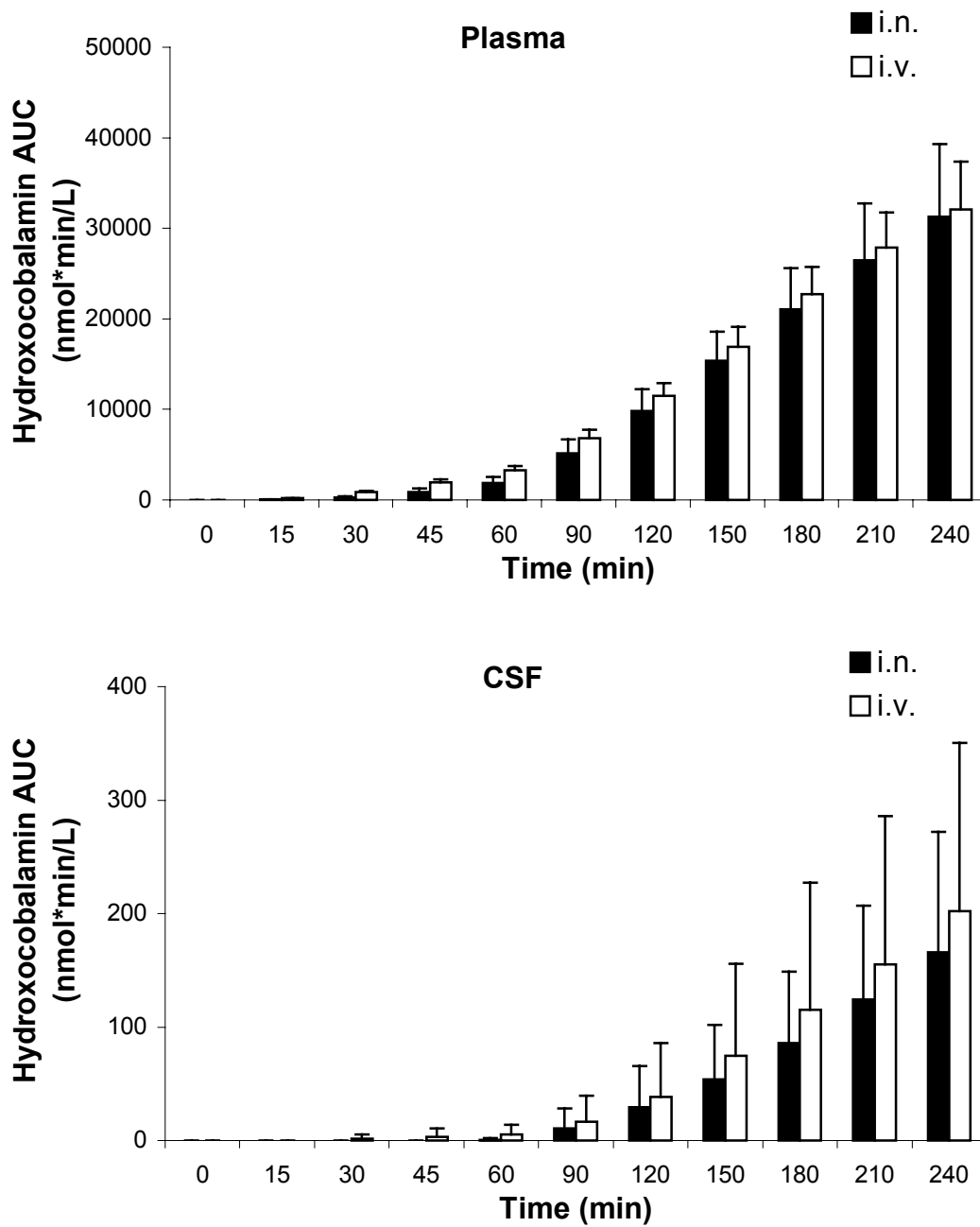


Figure 2 Hydroxocobalamin cumulative AUC values in plasma (top figure) and CSF (bottom figure) after intranasal delivery (i.n.; 214 $\mu\text{g}/\text{rat}$) and intravenous infusion (i.v.; 49.5 $\mu\text{g}/\text{rat}$) in rats. Results are expressed as mean \pm sd of 8 animals.

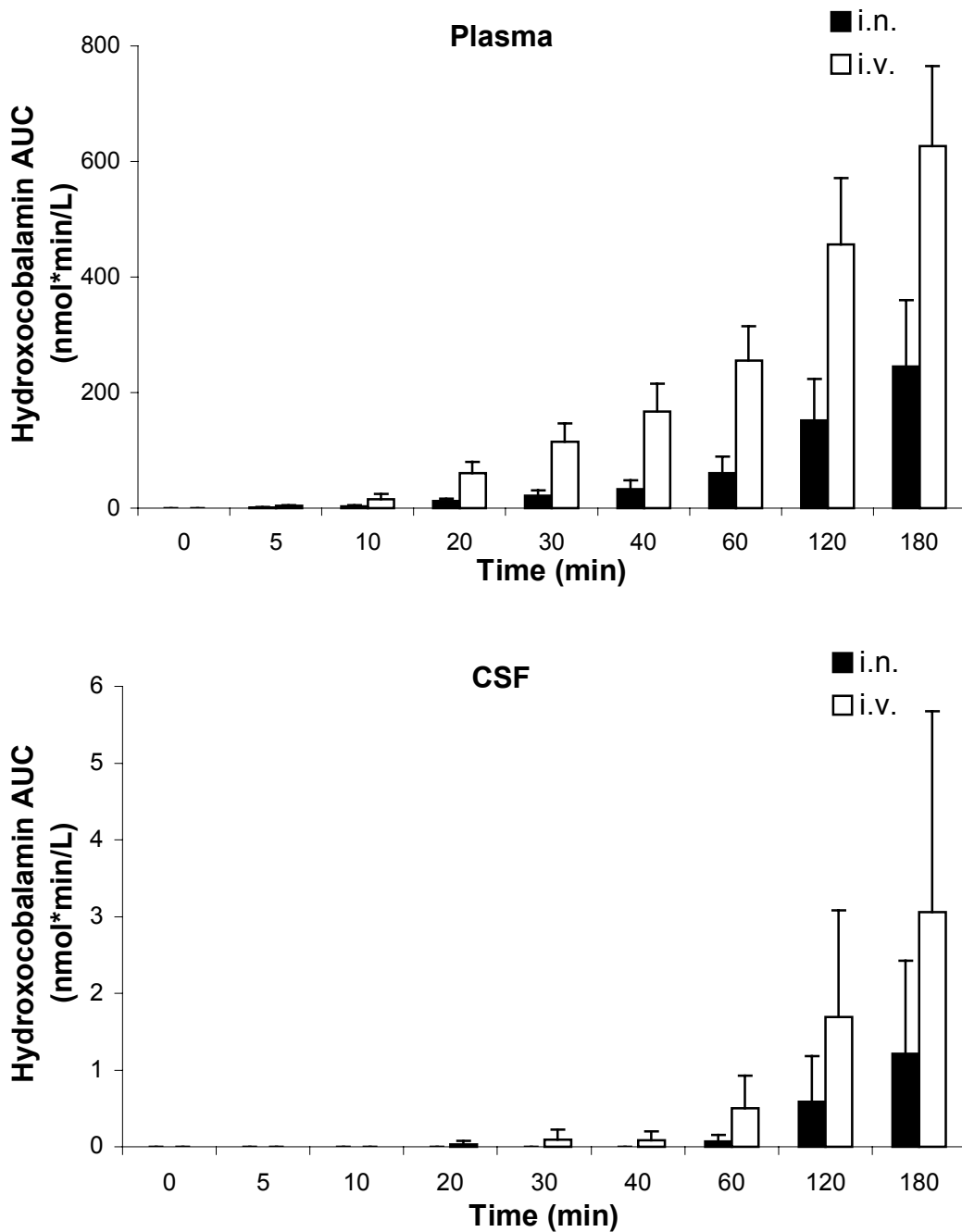


Figure 3 Hydroxocobalamin cumulative AUC values in plasma (top figure) and CSF (bottom figure) after intranasal delivery (i.n.; 1500 µg/subject) and intravenous infusion (i.v.; 75 µg/subject) in humans. Results are expressed as mean ± sd of 5 subjects; adapted from Merkus *et al.* (2003, chapter 7).

The present results are in contrast to the reported human studies using nasal vasopressin (Pietrowsky *et al.*, 1996a; Born *et al.*, 2002), angiotensin II (Derad *et al.*, 1998), cholecystokinin-8 (CCK-8) (Pietrowsky *et al.*, 1996b) and MSH/ACTH₄₋₁₀ (Fehm *et al.*, 2001; Born *et al.*, 2002). These are all hydrophilic peptide drugs with a molecular weight in the range of 1000 – 1600 g/mol, which is comparable to that of hydroxocobalamin (MW = 1346 g/mol). In the pharmacodynamic study by Fehm *et al.* (2001) and the pharmacokinetic study by Born *et al.* (2002) intranasal delivery of MSH/ACTH₄₋₁₀ and vasopressin in humans was compared with placebo treatment only, and therefore no proof for a direct nose-brain/CSF route was given. Born *et al.* (2002) claimed some nose to CSF transport after nasal administration of MSH/ACTH₄₋₁₀, because they could not find any absorption of this peptide in the systemic circulation. However, uptake of this peptide into CSF was only observed after delivery of the 10 mg and not after the 5 mg dose. The vasopressin uptake in CSF determined after nasal administration was attributed to a combination of direct nose to CSF and BBB transport (Born *et al.*, 2002). Nevertheless, this needs to be confirmed by delivery of vasopressin via the intravenous route. The pharmacodynamic studies on vasopressin (Pietrowsky *et al.*, 1996a), angiotensin II (Derad *et al.*, 1998) and CCK-8 (Pietrowsky *et al.*, 1996b) investigated intranasal and intravenous administration of these peptides. The observed differences in event related brain potentials suggest a direct entry of the delivered drugs from the nasal cavity into the central nervous system. Such a direct transport route was not evident from the present study examining the distribution of hydroxocobalamin over plasma and CSF after intranasal and intravenous delivery in rats. This could be explained by the fact that this vitamin analogue is better absorbed into the systemic circulation than the above mentioned neuropeptides. Therefore, more of the neuropeptides is left in the nasal cavity compared to hydroxocobalamin to be transported via the olfactory neurones into the CSF. Secondly, in some of the studies the nasal formulation was administered by giving repeated puffs during (Derad *et al.*, 1998; Born *et al.*, 2002), instead of a single administration as used in the present and in the patient study (Merkus *et al.*, 2003).

In conclusion, the AUC_{CSF}/AUC_{plasma} ratios after intranasal and intravenous administration of hydroxocobalamin in rats and humans demonstrate no direct nose-CSF transport of this hydrophilic and high molecular weight drug. The results indicate also the predictive value of the used rat model for the human situation concerning nose-CSF transport of drugs.

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Chapter 9

Uptake of melatonin into the cerebrospinal fluid after nasal and intravenous delivery: Studies in rats and comparison with a human study

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Abstract

Purpose. To investigate the possibility of direct transport of melatonin from the nasal cavity into the cerebrospinal fluid (CSF) after nasal administration in rats, and to compare the animal results with a human study.

Methods. Rats ($n = 8$) were given melatonin both intranasally in one nostril (40 $\mu\text{g}/\text{rat}$) and intravenously by bolus injection (40 $\mu\text{g}/\text{rat}$) into the jugular vein using a Vascular Access Port. Just before and after drug administration blood and CSF samples were taken and analysed by HPLC.

Results. Melatonin is quickly absorbed in plasma ($T_{\text{max}} = 2.5$ min) and shows a delayed uptake into CSF ($T_{\text{max}} = 15$ min) after nasal administration. The melatonin concentration-time profiles in plasma and CSF are comparable to those after intravenous delivery. The $\text{AUC}_{\text{CSF}}/\text{AUC}_{\text{plasma}}$ ratio after nasal delivery (32.7 ± 6.3 %) does not differ from the one after intravenous injection (46.0 ± 10.4 %), which indicates that melatonin enters the CSF via the blood circulation across the blood-brain barrier. This demonstrates that there is no additional transport via the nose-CSF-pathway. These results resemble the outcome of a human study.

Conclusions. The present results in rats show that there is no additional uptake of melatonin in the CSF after nasal delivery compared to intravenous administration. This is in accordance with the results found in humans, indicating that animal experiments could be predictive for the human situation when studying nose-CSF transport.

Key words: melatonin, intranasal, intravenous, cerebrospinal fluid, rat, human

Introduction

The main problem in the development of neuro-active compounds is the passage of these drugs across the blood-brain barrier (BBB). This tight barrier protects the brain from exogenous compounds including drugs (1). Several methods have been investigated to open or manipulate the BBB (2) to enable drugs passing from the blood circulation into the brain. Nevertheless, these methods did not give a satisfying solution to the problem of brain targeting. Circumventing the BBB by targeting via the nose to brain pathway has been suggested as a possible alternative way to reach the brain and the surrounding cerebrospinal fluid (CSF) (3, 4).

The neuronal connection between the nasal cavity and the CSF and brain has been extensively investigated on the possibility for brain targeting of drugs. Animal and human studies have been performed providing pharmacokinetic (PK) (5-7) and pharmacodynamic (PD) data (8-13), respectively. In human studies hormones and peptide drugs were tested, mainly monitoring PD effects. Arginine-vasopressin (9), cholecystokinin-8 (13), adrenocorticotropin (ACTH) 4-10 (10) and insulin (12) increased brain potentials after nasal delivery compared to intravenous administration. Nasal delivery of angiotensin II increased both norepinephrine and vasopressin release, which was opposite to the effects after intravenous administration (11). These effects after nasal angiotensin II administration show similarities with the results after intracerebroventricular delivery in rats (14, 15), suggesting that nasal administration of angiotensin II induces a direct central effect.

Animal studies give a PK support for drug targeting via the nose-brain/CSF pathway. The influence of physicochemical factors like molecular weight, ionisation degree and lipophilicity on nose-brain transport has been investigated in animals (5). A large number of animal studies with low molecular weight drugs as hydroxyzine (6), dopamine (16), cephalexin (17), anti-HIV agents as D4T (18) and zidovudine (19), metals (20), viruses (21, 22), steroid hormones (23) and polypeptides (24, 25) claim that the nasal route of drug administration offers direct access to the brain and CSF in animals.

The key-question is still whether this direct transport route is really effective or not. To verify the actual feasibility of this novel approach, it is necessary to compare animal studies with human data. In order to extrapolate the results from animals to humans, the studies mentioned above need to be complemented with human PK and animal PD data. This difference in

available data between animals and men is due to practical reasons. It is more difficult to sample human CSF than to monitor PD effects in human subjects, while the contrary holds for animal studies. A recent *Neurology* paper describes for the first time the uptake of two model compounds in blood and CSF after nasal and intravenous delivery in the same human being. In neurosurgery patients with a CSF drain it was possible to investigate the nose-CSF pathway of the low molecular weight and lipophilic substance melatonin and the high molecular weight and hydrophilic molecule hydroxocobalamin, both serving as model compounds (26, Chapter 7). Due to the strict inclusion and exclusion criteria only three subjects could be investigated. In order to substantiate the results of this human study, in the present paper the same melatonin formulation was investigated in rats ($n = 8$) using a comparable experimental set-up. Furthermore, such a comparison can provide a basis for extrapolating the results of nose-CSF studies from animals to men.

Materials and Methods

Materials

Melatonin (LogP = 1.2 (27)) was from Biosynth AG (Staad, Switzerland), povidone iodine from Sigma Chemical (St. Louis, MO, USA) and β -cyclodextrin from Wacker-Chemie (Krommenie, The Netherlands). Ethanol (96%) of analytical grade was from Merck (Darmstadt, Germany). Sterile saline (0.9 % NaCl) and heparin (400 IU/ml) were obtained from the Hospital Pharmacy of Leiden University Medical Centre (Leiden, The Netherlands). Janssen Pharmaceutica (Beerse, Belgium) supplied Hypnorm[®] (fentanyl citrate 0.315 mg/ml, fluanisone 10 mg/ml). Dormicum[®] (midazolam, 5 mg/ml) was from Genthon B.V. (Nijmegen, The Netherlands). Nembutal[®] (pentobarbital sodium, 60 mg/ml) was purchased from Sanofi Sante Nutrition Animale (Libourne, France) and Temgesic[®] (buprenorphine, 0.3 mg/ml) from Schering-Plough (Maarsse, The Netherlands). Dichloromethane and KH_2PO_4 were from J. Baker (Deventer, The Netherlands), and acetonitrile was from Biosolve LTD (Valkenswaard, The Netherlands). All other reagents were of analytical grade.

Melatonin Formulations

The melatonin formulation for nasal delivery consisted of melatonin (2.0 mg/ml) and β -cyclodextrin (7.5 mg/ml) dissolved in saline (28). This formulation also contained benzalkonium chloride (0.01 % w/v) and EDTA

(0.1 % w/v) as preservatives. A 10-fold lower concentration was used for intravenous bolus injection.

Animals

Male Wistar rats (Charles River, Someren, The Netherlands) were used, weighing 330 – 465 g at the start of the experiments. The animals ($n = 8$) were housed (2 per cage) with free access to food and water with a 12-h light/dark cycle. At the end of the experiments the animals were euthanised with an overdose of Nembutal[®] (1 – 2 ml, intraperitoneally). All animal experiments were approved by the Ethical Committee for Animal Experiments (Leiden University).

Nasal and Intravenous Delivery of Melatonin

Prior to drug administration rats were anaesthetised with Hypnorm[®] (0.5 ml/kg) and Dormicum[®] (0.5 ml/kg) intramuscularly and fixed in a stereotaxic frame (model 51600, Stoelting, Wood Dale, IL, USA) using the supine-70° angle position (29). For intranasal administration of the melatonin formulation, a polyvinylchloride (PVC) tube (ID 0.5 mm, OD 1.0 mm) attached to a Hamilton syringe was inserted into the left nostril of the rat for about 2 cm. The nasal melatonin dose (40 µg/20 µl/rat) was delivered by gently pushing the plunger of the syringe. After delivery of the formulation the PVC tube was removed.

For the intravenous bolus injection the rats were provided with a Vascular Access Port (VAP) as described before (30). The intravenous melatonin formulation (40 µg melatonin/200 µl/rat) was administered using a 1 ml syringe attached to a Huberpoint needle. Subsequently, the VAP was rinsed with 500 µl saline to make sure that the entire formulation had entered the blood stream.

Prior to and following melatonin delivery, blood and CSF samples were taken until 120 min after administration. Each rat received both the nasal and intravenous treatment. Between experiments the animals were allowed to recover for one week.

Blood and CSF sampling

Blood samples (200 µl) were taken from the tail vein using heparinised tubes (Microvette[®] CB 100/200, Sarstedt, Nümbrecht, Germany) and the samples were stored at 4°C until analysis.

For CSF sampling a cisternal puncture was performed as described before (29). Briefly, rats were anaesthetised and fixed in a stereotaxic frame as

mentioned above. The cisternal puncture was performed 5.2 – 6.5 mm ventrally from the occipital crest, dependent on the rat's weight. After the puncture, one drop of CSF was microscopically examined on erythrocyte contents; the experiment was continued when the erythrocyte contamination was less than 500 cells/ μl ($< 0.01\%$ of normal blood content). Following intranasal or intravenous drug administration, CSF samples (about 30 μl) were taken and directly collected in pre-weighed HPLC vials and the volume was added up to 180 μl with Millipore[®] water. All samples were analysed the same day.

Melatonin Analysis

Blood samples were pretreated as follows. Blood samples were centrifuged (15 min at 14.000 rpm; ambient temperature) and the obtained plasma (100 μl) was extracted with dichloromethane (2 ml) by shaking at 1000 rpm (Vibrax, type VXR; Fisher Scientific, 's Hertogenbosch, The Netherlands) for 10 min. The two-phase system was centrifuged (5 min at 3000 rpm; ambient temperature) and the organic phase was pipetted into other tubes. Then dichloromethane was evaporated under a mild nitrogen stream at 35° C, and the residue was dissolved in 250 μl mobile phase (10 mM KH_2PO_4 (pH 3.0) : acetonitrile = 73 : 27). Plasma and CSF samples were analysed on melatonin as described previously (31). Briefly, samples were analysed by isocratic HPLC consisting of a Jasco PU-980 pump (Jasco, B&L systems, Zoetermeer, The Netherlands), a Chromspher C_{18} column (100 x 3.0 mm) with 5 μm sized particles (Varian BV, Houten, The Netherlands) using a flow of 1.0 ml/min and fluorescence detection ($\lambda_{\text{ex}} = 224$ nm, $\lambda_{\text{em}} = 348$ nm; Jasco 821, B&L systems, Zoetermeer, The Netherlands) with a detection limit of 8 pg/ml.

Data Analysis

The area under the concentration-time curve (AUC) values (0-120 min) were calculated using the trapezoidal rule. The CSF ratio was determined according to Equation 1. This ratio is a measure for CSF uptake after nasal delivery related to the uptake after intravenous administration (26). All AUC values and CSF ratios were calculated per individual animal before determining the mean value. Data were analysed according to the paired Student's t-test, using the computer program SPSS version 8.0 for Windows.

$$CSF \text{ ratio} = \frac{AUC_{CSF, in}}{AUC_{plasma, in}} \bigg/ \frac{AUC_{CSF, iv}}{AUC_{plasma, iv}} \quad \text{Equation 1}$$

Results

In eight rats melatonin (40 µg/rat) was administered intranasally and subsequently intravenously. Following intranasal administration, the plasma C_{max} for melatonin was observed in the first sample taken after delivery ($t = 2.5$ min) which was similar after intravenous bolus injection. Both routes showed comparable plasma concentration-time profiles of melatonin (Fig. 1a). The uptake of melatonin into the CSF was delayed for about 10 – 15 min compared to the absorption in plasma after intranasal and intravenous delivery (for both routes: $T_{max} = 15$ min; Fig. 1b). In CSF the uptake phase was similar for the intranasal and the intravenous route of administration, reaching mean C_{max} values of 18 ng/ml. This value was 3.5-5 fold lower than the C_{max} found in plasma (64 ± 37 and 87 ± 30 ng/ml after intravenous and intranasal administration, respectively; Fig. 1).

Table I. AUC_{CSF}/AUC_{plasma} ratios and the CSF ratio of melatonin

	Intranasal	Intravenous
Rats^a		
AUC_{CSF} (ng*min/ml)	774 ± 133	1069 ± 313
AUC_{plasma} (ng*min/ml)	2429 ± 576	2310 ± 400
AUC_{CSF}/AUC_{plasma} (%)	32.7 ± 6.3	46.0 ± 10.4
CSF ratio (Eq. 1)	0.76 ± 0.31	
Humans^b		
CSF ratio (Eq. 1)	0.71 ± 0.30	

Data are presented as mean \pm sd, ^a ($n = 8$), ^b ($n = 3$) (26, Chapter 7)

Table I gives an overview of the AUC values in plasma and CSF after intranasal and intravenous melatonin delivery, the AUC_{CSF}/AUC_{plasma} ratios and the CSF ratio. The calculated CSF ratio (0.76 ± 0.31) shows that the relative uptake of melatonin into the CSF after nasal delivery is not

significantly different from the uptake after intravenous injection. This ratio is smaller than 1, which indicates that there is no additional transport from the nasal cavity into the CSF. The CSF ratio found in rats is similar to that obtained in humans, as is also shown in Table I.

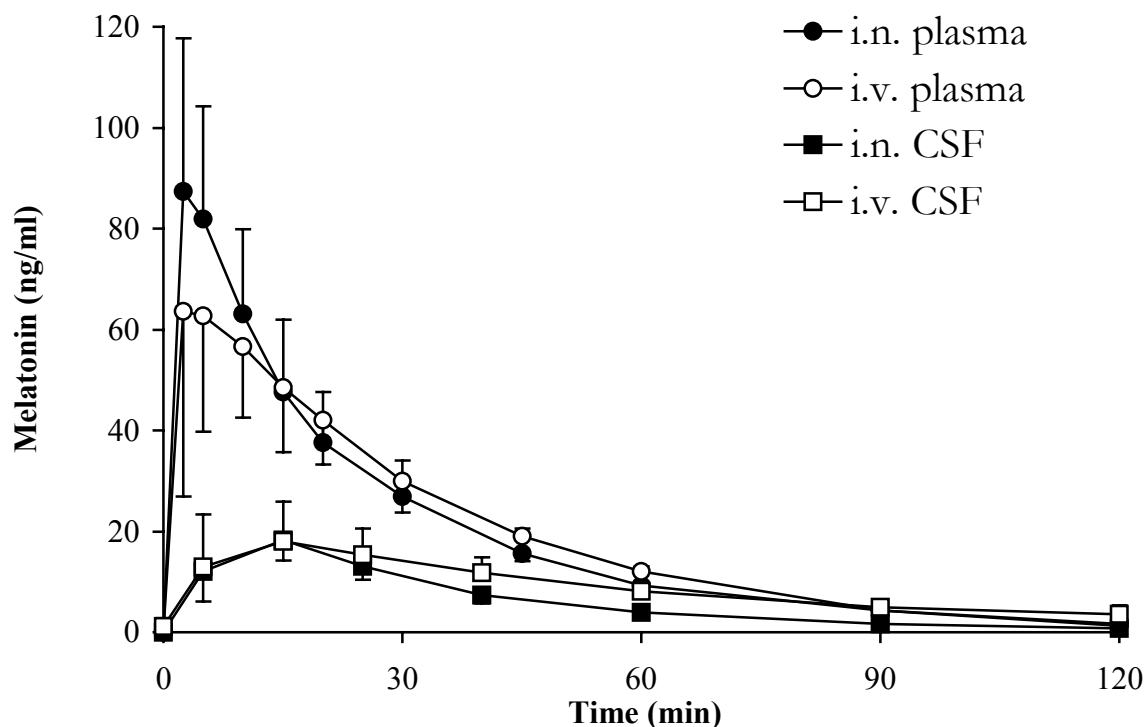


Figure 1. Plasma and CSF concentrations after intranasal (i.n.) and intravenous (i.v.) delivery of melatonin (40 μ g/rat) in rats. Results are expressed as mean \pm sd ($n = 8$).

Discussion

The present study demonstrates that nasal delivery of melatonin in rats does not result in additional uptake of this lipophilic/low molecular weight drug (MW = 232 g/mol) into the CSF via the nose-CSF pathway compared to intravenous administration. This is in contrast to some earlier reported rat studies with low molecular weight lipophilic and hydrophilic compounds (16, 17, 23). In these studies the drug concentrations in CSF after intranasal and intravenous delivery were determined at 1-2 time points only, which gives limited information about the CSF uptake of a drug and may therefore be misleading. Possible discarding of CSF samples contaminated with blood was also not reported. Blood contamination in CSF may lead to false positive

conclusions. Nevertheless, in a previous study from our laboratory another lipophilic and low molecular weight drug, hydrocortisone, was evaluated for nose-CSF transport in rats (30). When comparing the AUC_{CSF}/AUC_{plasma} ratios after intranasal and intravenous delivery for this steroid hormone, no direct nose-CSF transport was observed. These findings are supported by studies with other lipophilic drugs such as the serotonin antagonist (S)-UH-301 (7), a cognition enhancer (32) and the antihistamine triprolidine (6). A lack of direct nose-CSF transport was also reported for the hydrophilic vitamin B₁₂ analogue hydroxocobalamin, which was studied in the same rat model as described here (33).

The present rat studies show results similar to a human study (Table I; (26)), in which, the same melatonin formulation is tested. The administered melatonin dose in rats is relatively high in comparison with the human study on a mg/kg basis: about 20- and 40-fold higher for intranasal and intravenous administration, respectively. If the same dose (mg/kg) for humans would be used for rats, the melatonin concentrations in plasma and particularly in CSF would have been below the limit of detection of the used HPLC assay. Therefore, in the present rat study the same melatonin formulation but at a higher dose (40 µg/rat) was used. Similar to this rat study, all human subjects received two melatonin treatments (intranasally and intravenously (26)) and in both species melatonin is rapidly absorbed in the blood circulation after nasal delivery ($T_{max} = 2.5$ and 5 min for rats and humans, respectively). The relative uptake of melatonin into the CSF after nasal delivery compared to intravenous administration is comparable in rats and humans, which is evident from the calculated CSF ratios (Table I).

It should be noted that large interspecies differences exist in the anatomy, especially with respect to the shape of the nasal cavity and the relative sizes of the olfactory and respiratory epithelia. In rats about 50 % of the nasal cavity is covered with olfactory epithelium, whereas in humans this is only 8 % (34). Therefore, for compounds that are taken up via the olfactory epithelium, a difference in CSF ratio between rats and humans can be expected. Our study shows however no direct or extra transport of melatonin from the nose to the CSF. Obviously, there is no transport via the olfactory area and in both species the observed fast nasal absorption takes place via the respiratory epithelium that is highly vascularised and easily permeable for the low molecular weight lipophilic compound melatonin.

In conclusion, no additional transport from the nasal cavity to the CSF is found after intranasal and intravenous administration of melatonin in rats. Furthermore, the results of the present rat studies and the reported human study (26, Chapter 7) offer an opportunity to compare animal and human PK data, obtained by using the same drug formulation and a resemblance in experimental methods. Comparison of these two studies demonstrates that for nose-CSF transport of melatonin rat experiments can be predictive for human studies. To strengthen the basis for extrapolation from animal data to the human situation, more nasal drug formulations need to be investigated in both animals and men.

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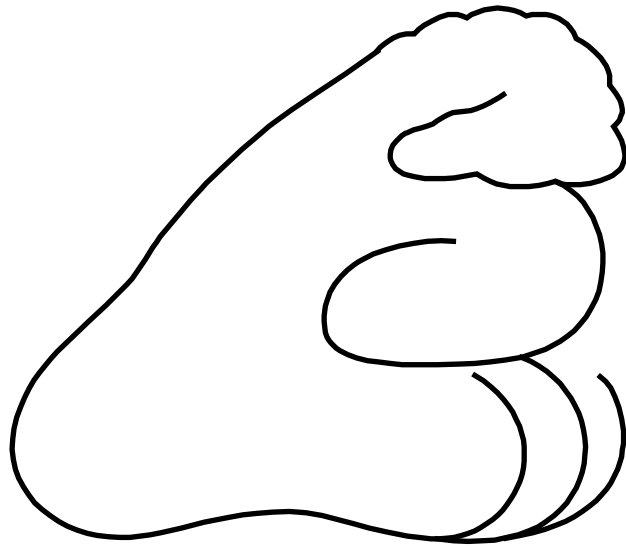
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SECTION V

GENERAL DISCUSSION AND SUMMARY



Chapter 10

Discussion and Conclusions

1. Introduction	160
2. (How) do nasal drugs reach the middle meatus?	160
2.1 Relevance of nasal deposition studies	160
2.2 Research methods in nasal deposition studies	162
2.3 Best technique and anatomy	163
2.4 Head position	164
2.5 Device	165
2.6 Future deposition research	165
2.7 Conclusions	166
3. Are nasal drugs potentially harmful to the cilia?	166
3.1 Preservatives and clinical relevance	167
3.2 Saline and Locke Ringer solution	168
4 Do nasal drugs have a direct route to the CSF?	168
4.1 Proof of ‘nose to brain/CSF’ pathway	168
4.2 Research methods in nose to CSF/brain transport	170
4.3 Nose-brain/CSF transport in perspective	171
4.4 Future research of nose to CSF/brain drug transport	171
Addendum 1	173
References	175

1. Introduction

The investigations described in this thesis contribute to a better understanding of nasal drug delivery. Many topics in this field are suitable for further research, but as pointed out in the *General Introduction* we selected three current questions that are of scientific interest at the present time. In *Section II* the available data about improvement of local nasal treatment is discussed and an attempt is made to answer the question how nasal drugs can reach the middle meatus. In *Section III* the in vitro toxicological aspects of some nasal drugs are tested and the effect of individual drugs and formulation excipients on ciliary movement is evaluated. Finally, the recent claim that nasal drugs can be transported directly via the olfactory neurons to the CSF/brain is investigated in humans and rats by comparing the intranasal and intravenous transport of some model drugs (*Section IV*). This will answer the question whether this potential transport route is existing at all or how effective this direct transport circumvents the blood-brain barrier (BBB).

The results of the various investigations have been discussed separately in the individual chapters, a more general discussion and conclusion of the three studied topics is presented below.

2. (How) do nasal drugs reach the middle meatus?

2.1 Relevance of nasal deposition studies

Millions of people use a nasal spray or drops on a daily basis for various reasons and some of these treatments are very effective. In that perspective it seems questionable to what extent deposition research is important.

Targeted deposition of nasally applied drugs is only important in some conditions and to a certain extent, depending on indication and efficacy of the prescribed drug. In (allergic) rhinitis patients the corticosteroid or decongestant nasal drug have to be effective at least on the easily accessible inferior turbinate, but in general a wide spread of the drug is aimed for. In intranasal systemic drug delivery only a few studies have been done on the influence of deposition on nasal drug absorption. For instance, for a well absorbed compound like nicotine, the nasal site of deposition appeared not to influence the nasal bioavailability⁴⁴. In contrast, in patients with chronic recurrent rhinosinusitis and/ or nasal polyposis, deposition in the middle meatus area is of crucial importance⁷². Recurrence of disease is likely to develop if the corticosteroids do not reach the middle meatus area in these two conditions. In addition, given the fact that corticosteroid treatment is very

successful, patients who *not* experienced an adequate effect of their nasal corticosteroid treatment in chronic rhinosinusitis or nasal polyposis will probably benefit the most from these drug deposition studies.

A rough estimation of the number of patients without significant effect of corticosteroids in the treatment of chronic rhinosinusitis with/without nasal polyposis would be around 40 000 chronic rhinosinusitis patients and 24 000 nasal polyposis patients in the Netherlands (see footnote^a).

The question “how the middle meatus is reached” is also important in an other current issue: the use of corticosteroid *drops* as compared to ‘the usual *spray*’ in the treatment of nasal polyposis^{6,47,64}. Corticosteroid drops are preferred over a corticosteroid spray in the treatment of moderate nasal polyposis, because of their proven efficacy^{6,32,37,64}. If this efficacy is due to the technique of administration, the dosage difference^b, clearance difference^{19,36} or the difference in formulation between corticosteroid drops or spray^c remains unknown.

Finally, in the question if deposition studies are essential, the fact that “up to 50% of the administered drug will not pass the vestibule and valve area and be lost by dripping out or cleaning of the nose”^{38,57,76} & Section II leaves enough room for future attempts to improve the deposition of the drug.

Footnote a: Estimation of the number of patients is based on following data: The

Netherlands has a population of 16 million people. Chronic recurrent rhinosinusitis has a prevalence of around 5% (Canada) [Chen 2003]. Nasal polyposis has a prevalence of around 3 % (2-4%) [Mygind 2000]. Assumptions: 50% of the diagnosed patients use a nasal corticosteroid and 10% of the drug treatment is not effective. Using these figures for the Dutch population: 800 000 people have *chronic rhinosinusitis* of which half use nasal corticosteroids of which in around 40 000 cases treatment is not effective. Also: 480 000 have *nasal polyposis* of which 240 000 use nasal corticosteroids of which in 24 000 cases treatment is not effective.

Footnote b: Fluticasone propionate drops are prescribed in a daily dosage of 400-800µg and fluticasone propionate spray in a daily dosage of 200-400µg (GlaxoSmithKline, Zeist, the Netherlands)

Footnote c: Fluticasone propionate spray (Flixonase® GlaxoSmithKline, Zeist, the Netherlands) contains phenylethylalcohol and benzalkonium chloride, whereas fluticasone propionate drops (Flixonase nasules® GlaxoSmithKline) does not contain a preservative.

2.2 Research methods in nasal deposition studies

Several studies have been conducted in the past years to investigate the ‘best technique’ of intranasal corticosteroid use. Nevertheless, in a recent and thorough review of these studies the American Academy of Otolaryngology-Head and Neck surgery could not draw definite conclusions how to use intranasal steroid sprays best⁸. The reason why this consensus is still missing, is most likely due to the variable research methods. This makes comparison difficult, and preclude, along with other factors, a definite advice of the best deposition technique.

As first and most important remark, we find it striking that pathological conditions, like rhinosinusitis and nasal polyposis, are only tested once in relation to topical nasal drug deposition⁷⁷, even though these conditions are the main reason for this type of treatment. It is therefore questionable whether the deposition investigations, all done on healthy volunteers, are predicting or simulating what would happen in a patient with severe nasal pathology.

Secondly, nearly all deposition investigations are slightly or completely different and therefore hard to compare. Moreover, they all have their drawbacks. We, like in all other endoscopic studies^{39,46,50,76}, had to exclude patients with a septal deviation obstructing direct view of middle turbinate. Others advocate or use decongestants and local analgesics^{1,39,73}. Ragavan et al. used a non-physiological cadaver study to prove the best head position⁶⁷, which of course is purely indicational.

The research methods can be roughly divided into endoscopic evaluation, patty count and nuclear scanning (addendum 1). In an *endoscopic evaluation* the researcher gets a true view of the middle meatus area, but the main drawback are the non-quantitative outcomes, the merely anterior view of the 3d middle meatus and the difficulty to follow the deposition over time. *Patty count* is the only real quantitative measurement, but the use of premedication with topical decongestant/analgesic weighs heavily on the relevance of the quantitative outcomes of this fairly uncomfortable method. The use of *nuclear scans* to evaluate the distribution of radiolabeled particles is useful to follow a rough distribution over time, but not recommendable to qualify or quantify an exact middle meatus deposition^{5,61}. In addition, most human deposition studies use different subjects to compare different techniques. This is to our opinion one of the main reasons why contradictory results were published (addendum 1). We used a single-blinded endoscopic video analysis (chapter 3 and 4) in an intra- and inter-individual comparison of 10 healthy volunteers, to locate the

amount of dyed test formulation after using 7 different techniques of administration. This method to investigate the ‘best technique’ and ‘influence of anatomy’ on topical nasal drug delivery was well possible, but not optimal. One of the most difficult parts was the inclusion and compliance of the volunteers, as they had to come seven different days and received only a small reimbursement. The endoscopy had not been pleasant in all cases and local irritation and congestion sometimes rendered a good view of the middle meatus difficult. Still we advocate not using topical decongestants and analgesics as they could alter the outcome³¹. Furthermore, we planned to analyze pictures of the middle meatus³⁸, but in the progress of the study (140 videos in total) we decided to incorporate whole videos⁷⁶ in the analysis as they contained much more valuable data. During the analysis we discovered the possible influence of the individual anatomy. If we had known that influence, we would have tested fewer techniques and more volunteers or would have repeated several tests in each volunteer.

2.3 Best technique and anatomy

Our goal to find the ‘best’ technique of topical nasal drug delivery by investigating 7 techniques in 10 volunteers, did not show any significant superior technique (chapter 3). When analyzing the anatomical differences between the subjects (chapter 4) it became clear why we were unable to find such a best technique: our results are only suggestive, explaining the lack of one single best technique in all patients. We conclude therefore that *individual anatomy necessitates an individual nasal drug delivery technique*. In this perspective a single best technique of local drug delivery, as many have tried to determine (Section I: 1.4.4 & 1.5.2 and addendum 1), is an unrealistic goal. There is no such thing as one best technique and Section II has opened a new, a more individual, look on optimizing local drug delivery.

In 2002, Homer et al., was the first to compare different techniques within one subject (intra-individual comparison of spray vs. drops)³⁹. They discovered that there is no superiority of either drops or spray, but an optimal technique per individual. Not knowing which factors are of influence, they did emphasize that individual factors do point out the optimal technique for topical nasal drug delivery. Independently of the administration technique used, it became clear to us that even a minor obstruction in a nostril can alter the drug deposition. These findings are confirming the results of Dowley et al³¹, who demonstrated that a congested inferior turbinate causes a diminished drug deposition and of Weber⁷⁶, who showed an improved spread due to a

decongested turbinate and a worse middle meatus deposition in patients with a slight septal deviation.

As stated earlier (paragraph 2.1) this more individual approach would only be practicable in nasal polyposis or chronic rhinosinusitis patients with unsatisfactory results on initial corticosteroid treatment and in an ENT outpatient clinic. When the initial topical steroid treatment of chronic rhinosinusitis or nasal polyposis fails and there are no signs of potential complications, the technique of spraying or drop administration can be reconsidered. The EAACI (European Academy of Allergology and Clinical Immunology) recently published³² a clear management scheme of chronic rhinosinusitis and nasal polyposis in which this suggestion could be considered, but our results are far from evidence based and still have to be proven valuable in the future.

2.4 Head position

In the investigations as described in chapter 3 and 4 we tried to determine the best head position for drug deposition to the middle meatus. In this study, we tested 4 head positions HUR (head up right), HDF (head down and forward), LHL (lateral head low) and LHB (lying head back) as explained and drawn in the *General Introduction*. Significant results in favor of one head position could not be found, but a trend in favor of two positions was seen. Nasal obstructions seemed more successfully bypassed in the lateral ways of administration, like the LHL and LHB head position (chapter 4). After comparing head positions using different devices, also a trend in favor of the LHL and LHB position was seen over the HDF position (chapter 3) which matches the findings of Kayarkar et al⁴⁶ and Kubba et al⁵⁰. Again in favor of the lateral head positions is the fact that in our and other studies the HDF position was the least comfortable^{45,46,49,50}.

In chronic rhinosinusitis patients, head positions are only investigated in one study. This study by Wilson et al., did unfortunately compare the HB (head back) and the HDF position on efficacy and they advocated the HDF position⁷⁷. The value of this result is nowadays less important, because the HB position is considered ineffective as it initiates a quick slide of the drug to the throat.

Although statistical significant figures are lacking, it seems reasonable to conclude that the LHL and LHB head position have the potential of being most successful and comfortable in topical drug delivery. Once more, it has to be stressed that this conclusion, based on results in healthy volunteers, still has to be confirmed in patient studies.

2.5 Device

Our studies were not primarily designed to compare devices and we believe that device preferences are depending on the type of delivery (spray, drop, powder or gel), manufacturer preference and costs, study sponsor and many more reasons. As long as there is no “best” technique there will be no “best” device.

Prior to our study, drops vs. spray investigations did not had the ability to overcome the head position difference^{5,39,73}. As device novelty, the introduction of a unit dose device for topical delivery (studies) seems an improvement. This spray can be combined with a head position and seems to have advantages in overcoming gravity in the first seconds of administration. Further research using unit dose sprays is needed.

2.6 Future drug deposition research

Drug deposition research is indicated to improve the treatment of nasal polyposis and chronic rhinosinusitis. It seems less relevant in other aims of nasal drug delivery (allergy, systemic treatment).

When conducting a nasal drug deposition study it has to be clear that nasal drug delivery is multifactorial. In addition, in the investigational set up it is hard to control the many factors influencing deposition, like: the type of drug formulation, drug volume, particle size and various delivery devices and delivery techniques^{General Introduction & 17,35,51,57}. Furthermore, there is a great variety of research methods and selection criteria to choose from as mentioned above^{1,5,8,38,76}.

Since several issues are important in the set up of future deposition studies, we would like to give the following advices:

- The aim of the study should be clear (middle meatus deposition, distribution/clearance over time).
- Take time to select the patients as they have to be their own controls (intra-individual comparison) and come in on several days.
- Include patients with nasal polyps or chronic rhinosinusitis; select and test on morphological differences (e.g. pre- & post surgery, mild and severe polyps)
- Include healthy volunteers and investigate the influence of their specific anatomical morphology on the deposition.

- The method should be either endoscopic or with nuclear scanning. Endoscopic videos provide the best information of the middle meatus (as compared to pictures).
- Formulation, volume and concentration should not be changed between subjects or techniques.
- A single unit dose device should be included in future studies.
- Decongestants and analgesics should not be used.
- The search for one ‘overall’ best technique should *not* be aimed for.
- An efficacy crossover study in patients using different techniques could clarify the influence of the administration technique.

2.7 Conclusions

- It is unlikely that there is ‘one best technique’ of topical nasal drug delivery.
- The best nasal drug delivery technique is most likely ‘personal’ and depends on individual anatomical differences.
- LHL and LHB head position have the potential of being most successful and comfortable in topical drug delivery
- Patients with frequent rhinosinusitis or nasal polyposis should be included in nasal drug deposition research projects.

3. Are nasal drugs potentially harmful to the cilia?

Impairment of the mucociliary system causes longer contact times of the airway mucosa with bacteria, viruses, irritants or even toxic substances, which could lead to infection or damage of the respiratory tract^{General Introduction}. This is the main reason why the influence of drugs, excipients and nasal drug formulations on the ciliary activity has been studied in the past three decades by many research groups. Most of these studies have been using *in vitro* methods, like CBF (ciliary beat frequency) measurements, which are very sensitive. In chapter 5, we have classified the *in vitro* effects of drugs, excipients and drug products in relative terms, by comparing the negative effects on ciliary movement of individual compounds⁵⁵. One advantage of our approach is the fact that we measured reversibility of the inhibition of the ciliary movement. The reversibility of the cilio-inhibiting effects was tested after 15 minutes (normal nasal residence time of a nasal drug product) and the recovery of the ciliary beat frequency was measured during the following 45 minutes. The classification of the effect in three categories (cilio-friendly, cilio-inhibiting, ciliotoxic) enables us to assess the negative effects of some drugs

and excipients in the nasal drug products. For instance it confirmed that some additives and in particular preservatives contribute substantially to the ciliostatic potential of whole drug formulations.

3.1 Preservatives and clinical relevance

Various formulation excipients such as preservatives^{7,15,24,68} and absorption enhancing compounds^{54,68} have been tested in the past. We confirmed, in chapter 5, that benzalkonium chloride (BAC), and other preservatives often used in nasal formulations, have an *in vitro* inhibiting effect on ciliary movement⁵⁵. However, does this mean that preservatives like BAC have to be banned in nasal formulations? What is the clinical relevance of *in vitro* cilio-inhibiting effects?

The *in vitro* cells are cut from their supplies, placed in a new environment and completely surrounded by a test formulation, which makes the outcome of the test probably much more sensitive than the *in vivo* effect. In 1982 van der Donk et al, as one of the first, wrote about the strong correlation between the *in vitro* CBF tests and the *in vivo* mucociliary clearance, advocating a clinical inhibiting effect of some preservatives, especially the preservatives used at that time^{29,30}. In the past 15 years BAC became the most popular preservative used in nasal formulations. As the use of nasal formulations increased so did the use of BAC, reflecting in mild² to strong believe^{9,33,34} in a clinical noticeable negative effect of BAC and other preservatives on the nasal mucosa. In contrast, the review of Marple et al. in 2004 states that the intranasal products using BAC as preservative appear to be safe *in vivo* and well tolerated for both long- and short-term use⁵². We agree that BAC is probably safe to use, but still caution should be taken in the development of new nasal drugs. If BAC will inhibit the ciliary activity and effect patients with already vulnerable mucosa or decreased ciliary clearance is not known and not tested. It seems better for chronic treatment to use preservative-free formulations. In our opinion, the study of Naclerio et al⁵⁹ reflects the effects of BAC in a right manner: corticosteroid users *without* BAC were compared to corticosteroid users *with* BAC to assess the clinical relevance of the cilio-inhibiting effects of BAC. The *in vivo* clearance in the BAC group was diminished after two weeks use, supporting the *in vitro* results, but the complaints of the two groups were not significantly different⁵⁹.

Section III and the *in vitro* and *in vivo* literature on the ciliary activity of drugs, preservatives and additives, support the following

Conclusions:

- *In vitro* results predict in a too sensitive way the outcome of *in vivo* tests, creating an *in vitro* fine tuned measurement tool with slight clinical value.
- Ciliary beat frequency measurements are useful to classify nasal drugs and drug compounds in the evaluation of one aspect of their safety and during the development of nasal drugs.
- The effect on ciliary movement of most nasal drugs is due to the preservatives and/or additives, and not to the drug itself.

Future research should focus on patients with a decreased mucociliary transport, as they are prone to have increasing complaints due to cilio-inhibiting nasal formulations. Meanwhile, physicians have the option of recommending preservative-free formulations, as preservatives are not needed in modern sterile nasal devices.

3.2 Saline and Locke Ringer solution

We used in our *in vitro* experiments Locke-Ringer (LR) instead of physiological saline as control solution, because LR does not influence ciliary activity for at least 60 minutes^{16,55}. It is an interesting question whether this LR solution is also clinically a better rinsing solution than the NaCl 0.9% solution widely used now?

LR is safe, inexpensive and easy to produce, hence a valuable alternative. In addition, NaCl 0.9% solution has a more, but minor, *in vitro* inhibiting effect on ciliary beating, but clinical differences between LR and NaCl 0.9% are not reported.

4. Do nasal drugs have a direct route to the cerebrospinal fluid?

Diseases of the central nervous system (CNS) like Parkinson's disease, epilepsy and Alzheimer's disease are prone to benefit from nasal drug delivery if a direct transport of the drug via the 'nose to CSF/brain' route is confirmed. The question is whether this new route of drug delivery to the brain is a real treatment option or merely a scientific hype, mainly based on animal experiments. We conducted several studies in man and in rats to answer the question if intranasally administered drugs reach the CSF directly via the olfactory region, without the drugs being absorbed into the systemic circulation and passing the blood-brain barrier (BBB)?

4.1 Proof of 'nose to brain/CSF' pathway

The great amount of studies on this topic is mainly carried out in animals (Section I; paragraph 1.6.3)^{10,25,42,53}. In animals, investigations with several dyes and metal ions and also histological studies with viruses and bacteria suggest a pathway for those compounds to travel from the nose to the CSF and brain via the olfactory neurons, but intravenous comparison was almost always lacking and presence of the substance solely in the CSF/brain does not give proof of a direct nose-brain pathway. In some studies with drugs like hormones, anesthetics, chemicals, CNS-, HIV- and antibacterial-compounds the intranasal and intravenous route have been compared, but clear evidence of a direct pathway could not be found^{21,26,40}. Some of these investigations used the AUC_{CSF}/AUC_{plasma} ratio, in which concentration over time is measured and compared between the two compartments, CSF and plasma. This ratio was comparable to the ratios found in our studies described in chapter 7-9^{56,11,14}. None of these ratios exceeded 1, demonstrating no extra (direct) transport from the nasal cavity into the CSF/brain^{21,26,40}. Hormones like estradiol and progesterone, which have been suggested to be able to enter the CSF by a direct pathway^{3,27}, have been proven not to be transported via a direct route but via uptake in the systemic circulation and transport via the BBB^{10,12}.

Recently, several pharmacodynamic studies of one research group claim the nose to brain transport of several peptide drugs^{General Introduction,28,48,65,66,71}. Although it looks that these investigations are a strong support for direct nose to brain transport, it is easily possible that small amounts of these peptides are absorbed nasally and transported to the brain via the BBB. No convincing pharmacokinetic proof is given. Also other investigators did not find any support for the nose to brain transport hypothesis, for instance: intravenous and intranasal cocaine gave a similar 'high' sensation and similar dopamine transport blockage with comparable plasma levels⁷⁵.

Human pharmacokinetic studies investigating the direct transport of drugs from nose to CSF have only been published once before. In 2002 Born et al., published data after administering neuropeptides intranasally and detecting an uptake of minor amounts of those peptides in the CSF¹⁸. The results suggest that very small amounts of peptide molecules travel to the CSF via the olfactory region, but unfortunately these authors did not carry out control experiments with intravenous administration of those peptides in similar amounts as can be expected after intranasal administration. In our experiments with melatonin and hydroxocobalamin both in man and in rats we were able to show good uptake of both compounds in the CSF after

intranasal administration, but more importantly we found a similar uptake after intravenous administration and thus the results were rejecting the direct nose to CSF/brain hypothesis ⁵⁶.

4.2 Research methods in nose to CSF/brain transport

In the discussion of most animal studies it is suggested that probably nose to CSF/brain transport is also feasible in humans. However, between man and animals (like rats and mice), there are huge differences: firstly a much larger olfactory area, secondly a smaller CSF volume and thirdly, a different CSF turnover rate in animals ^{43,74}. Furthermore, many formulations used in the animal studies contained mucosa-damaging permeation enhancers (e.g. organic solvents) ^{3,4} and some nasal formulations were administered in a relatively aggressive way (continuous perfusion of the nasal cavity for hours, insufflation of the formulation by force with an atomizer) ^{3,4,69}. Some researchers even tied the esophagus off, hampering natural clearance of the drug to the stomach ^{21,22,23}. Such a treatment would be unrealistic in the human situation. Therefore one should be careful in the interpretation of animal results, especially in translating them to the human situation.

A good comparison of our human and rat data ^{Section IV, 11,14,56} was possible. We used similar methods ^{13,70}, similar formulations, similar sampling and at the end we obtained analogous results (for instance Section IV, CSF ratio melatonin human: 0.71; rat 0.76).

Some remarks have to be made. Because of the detection limits of the HPLC assay, the administered melatonin dose in rats had to be increased. The same dose in rats as used in humans would not have been detectable. In the study with the neurosurgery patients (chapter 7) we had difficulties including all patients and also with the analytical methods due to several reasons⁵⁶. Including neurosurgery patients who had been admitted and operated for a subarachnoid heamorrhage, appeared to be not an easy task, according to the strict inclusion criteria we used. They had to be tested in two days once they were fully conscious and cooperative, but before the CSF drain was removed. During the two testing days there had to be no change in medical condition, therapy plan or logistics (like increased headache or arterial canule failure). In total 24 patients met the including criteria but only 8 resulted in a complete set of data. Furthermore, some patients had a cisternal drain and others a ventricular drain, which have different distance to the olfactory region and a different length of drain. Length and volume of the drain were measured upon removal, but did not change the outcomes of our study when taken into account. A difficulty in the analytical methods used was the fact that the

concentrations of hydroxocobalamin in the first hydroxocobalamin patients included in the study, were too low to detect by standard radio-immuno assay and the CSF samples too small to reanalyze. Concentration of the CSF samples by evaporation had to be done before CSF concentrations of hydroxocobalamin could be detected properly. For the low melatonin level in the CSF we developed a new HPLC method as described in chapter 6 ⁷⁰.

4.3 Nose-brain/CSF transport in perspective.

We found no evidence of direct transport of drugs from the nose to the CSF in animal and human investigations using two model drugs melatonin and hydroxocobalamin. The human and animal experiments have undoubtedly shown that an intranasal administration gives an good absorption in the blood and subsequently a good transport via the BBB, followed by an uptake in the CSF, but not differently than after intravenous administration of a comparable amount of the drug. We consider these data as convincing because of the intravenous control and the identical results found in the animal and human study. We believe that the experimental set up chosen in this thesis could be an example for further studies with other substances that are thought to have direct access to the CSF/brain. This knowledge is needed to solve the controversy of the nose to brain research and stop the debate between scientists and a further separation between ‘believers and non-believers’. Researchers who still believe in the nose to brain/CSF pathway should, if possible, prove this in humans using an intravenous comparison. One believer of the nose to brain pathway has recently criticized our human data ⁴¹, but Van den Berg has demonstrated in her thesis ¹⁰ that the criticism was incorrect due to a miscalculation of our hydroxocobalamin human data and misinterpretation of the rat data. In this respect it is good to realize that the difficult gathering of human data in nose-CSF/brain research makes animal studies, like the studies carried by Van den Berg et al ¹⁰⁻¹⁴, still valuable as long as they are performed in a realistic manner.

The impact of the presented results is more than just data rejecting a hypothesis. The United States Food and Drug Administration (FDA), the European Medicines Evaluation Agency (EMA) and the pharmaceutical industry can be relieved, because everyone would be very concerned in case there would be scientific proof of a direct access to the CSF or brain of drugs administered via a ‘simple’ nose drops or a nasal spray.

4.4 Future research of nose to CSF/brain drug transport.

Convincing publications in favor of an existing nose to brain/CSF pathway are lacking. Since scientists like to explore new strategies to circumvent the BBB, drugs with different pharmacologic properties than used in our studies, are likely to be tested in future. It seems plausible that larger molecules, like the peptides used in the study of Born et al, are investigated with an intravenous control¹⁸. A comparable animal study to confirm the data would be advisable.

Furthermore, it is hard to advise on the set up of a clinical study, as the inclusion of neurosurgery patients with an ventricular or cisternal CSF drain right after an operated subarachnoid hemorrhage is difficult. On the other hand a temporary spinal tap in healthy volunteers is medical ethical not an easy set up. Again, if a human study would look into the nose to brain/CSF pathway, support of a comparable animal study would increase the significance of the human results. Overall, there is still room for more convincing human data in the discussion about direct nose to brain/CSF pathway. Whether new data will lead to evidence of such a new drug route, seems doubtful on the basis of our results.

Addendum 1

Nasal drug deposition studies, divided in three categories: Endoscopy, Patty count and Nuclear scanning. In the methods is noted what the *aim* of the study was. The background *target* of the study (improve systemic, topical or middle meatus drug delivery) is described when mentioned. Most remarkable results and conclusions are given.

Endoscopy	Method	Result/Conclusion
Dowley et al. (2001)	Endoscopic photography after nasal delivery of a methylene blue dyed aqueous formulation via an azelastine spray device. Target: middle turbinate. Deposition and peak inspiratory nasal flow (PINF) were measured with congestion (exercise) and decongestion (oxymetazoline). Aim: to investigate the influence of congestion on topical nasal drug delivery to the middle meatus.	Congestion/decongestion manoeuvres altered PINF significantly. Delivery to middle meatus is influenced significantly by congestion/decongestion.
Homer & Raine (1998)	Endoscopic photography after nasal delivery of a methylene blue dyed aqueous formulation via an azelastine spray device. Target: middle turbinate. Aim: The effect of vigorously inhaling whilst spraying was studied.	No significant difference in amount of formulation delivered to the middle turbinate, with or without vigorous inhalation.
Kayarkar et al (2002)	Endoscopy photography to assess (colored pixels) middle meatal penetration of fluorescein-dyed betamethasone drops of three head positions. Also a visual analogue scale to scale the (dis)comfort of the head positions: Lying head back (LHB); head down and forward (HDF) and head back (HB).	Distribution: LHB 55.5%; HDF 31.55%; HB 6.87%. Discomfort: HB least, HDF most. Recommended: LHB position.
Kubba (1999)	Method & aim: Visual analogue scale to scale the (dis)comfort of the head positions: Lying head back (LHB); head down and forward (HDF) and head back (HB).	HDF most uncomfortable. Recommended: LHB position.
Kubba et al. (2000)	Endoscopy 30 sec and 30 min after administration of betamethasone dyed with methylene blue drops. No decongestants used, three head positions tested (HB, HDF, LHB) Target: middle meatus. Aim: to evaluate distribution of nasal drops.	HB: drops mainly in nasal floor and nasopharynx. LHB and HDF: drops were in middle meatus and on middle and inferior turbinates. Recommended: LHB as more comfortable than HDF.
Weber et al (1999)	Analysis after 1% fluorescein via Pulmicort Topinasal® metered pump administration in patients and in a nasal model. Videoendoscopy of the patients (8 healthy volunteers and 10 adults after sinus surgery) and a nasal model to analyze. Descriptive study; no quantitative data before and after decongestant spray. Aim: to describe the effect on deposition to the middle meatus of decongestion, sinus surgery and anatomy and angle of spraying.	“Large majority of solution deposited in anterior, non-ciliated portion of nose, before and after decongestion. Only a small fraction reaches the middle meatus”. Anterior, but not posterior, septal spurs diminished penetration: dye reached middle meatus slightly better in non-operated than operated patients and in decongested than in a congested situation. “Breathing in deeply while spraying appears to have a positive effect.”

Patty count	Method	Result/Conclusion
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Homer et al (2002)	Randomized prospective crossover study. Absorption of Tc^{99m} -radio-labelled saline onto patty in middle meatus after drops LHB or spray HUR application (Nasacort® device, 45-degree angulation). Premedication with co-phenylcaine. Intra-individual comparison. Aim: to quantify the deposition (on a patty) in the middle meatus.	No significant differences among techniques but wide variability of patty uptake as percentage of administered drug (0.03% to 39.5% of the drug formulation). Premedicated with topical decongestant/analgesic. Optimal technique per individual rather than for the whole group.
Karagama et al. (2001)	Comparison of HDF, LHB, LHL and HB positions via dyed saline drops on neurosurgical patty in middle meatus after decongestant/analgesic spray. 10-point visual analogue scales for patty saturation and position comfort. Aim: investigate the 'best' head position technique.	LHL and LHB superior to HDF and HB position for patty saturation; HDF least comfortable.
Tsikoudas and Homer (2001)	Randomized prospective crossover study. Absorption of saline dyed with 0.1% methylene blue onto patty in middle meatus after drops LHB or spray HUR application (Nasacort® device, 45-degree angulation). Premedication with decongestant and topical analgesic. Intra-individual comparison. Aim: to quantify the deposition (on a patty) in the middle meatus.	No significant difference in delivery techniques. Small study (5 patients). Relevance of decongested volunteers questionable.

Nuclear scanning	Method	Result/Conclusion
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Aoki and Crowell (1976)	Distribution of technetium Tc^{99m} -labeled human serum albumin in nasal passages after nasal drops (pipette, patient supine) or spray ('injector device', patient sitting with head tilted lateral to have a chin-to-external auditory canal horizontal plane). Aim of study: to investigate drug distribution and the time-course of drug removal using radiolabelled formulation, gamma counter and nuclear medicine head scans. Target: to optimize antiviral distribution on the nasal epithelium.	Drop method had a significant higher proportion of good distributions. Volume and concentration variation did not alter distribution.
Hardy et al (1985)	Distribution and clearance of technetium Tc^{99m} -labeled human serum albumin in the nasal cavity after administration of nasal drops (1 or 3 drops and Mygind procedure [Mygind 1979]) or spray (HUR position). Gamma scintigraphy evaluation. Target: optimize local but also systemic nasal drug delivery.	Deposition with the spray mainly anteriorly (non-ciliated) and slow clearance, 1 drop increased spread and faster clearance. Three drops best spread and fast clearance.
Newman et al (1987a)	Scintigraphy to evaluate distribution of Tc^{99m} -labelled Teflon particles into nasal passage from pump sprays. Aim study: to assess the distribution of aerosols released from a pump spray in one position vs. two positions. Target: optimize local but also systemic nasal drug delivery.	Neither the quantity of aerosol reaching the nasal cavity, nor its initial distribution pattern within the nose are depending on the position of the metered dose spray (either one or two positions). More than half of the dose failed to reach the turbinates.
Newman et al (1987b & 1987c)	Scintigraphy to evaluate distribution and clearance of Tc^{99m} -labelled Teflon particles into nasal passage from pump sprays. Aim study: to assess the distribution and clearance of aerosols released from a pump spray. Target: optimize local but also systemic nasal drug delivery.	Drug particles released from nasal pump sprays are distributed to both ciliated and non-ciliated zones. Volume of twice 50µl retained better than once 100µl in the nasal cavity. Spray cone angle influences distribution to nasal mucosa. 56% of the dose was retained at initial nasal deposition site, 44% cleared to the nasopharynx.
Morén et al (1988)	Tc^{99m} -labeled human serum albumin distribution after nasal drops via "turning the head to five positions" (tilting back for drops, then turning to right for 30 s, then to left, then back to original position, then tilting forward) vs. "rapid nasal application" (tilting back with two strong sniffs) after drop application. Target: optimize local but also systemic nasal drug delivery.	Retention of 50% in both techniques (remained in vestibule). Turning the head had 15% better distribution after 30 min. Two minute procedures unrealistic for patients.

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Chapter 11

Summary

Summary	182
Samenvatting	187

Summary

I. General introduction

Nasal drug delivery is used for local treatment and increasingly during the past two decades for systemic drug absorption. It is a field of constant development and many topics concerning nasal drug administration are currently investigated. In **chapter 1** some basic knowledge about nasal drug delivery and several key issues of research are discussed. In **chapter 2** three questions of current scientific interest and aims of this thesis are presented:

- (How) do nasal drugs reach the middle meatus?
- Are nasal drugs potentially harmful to the cilia and is it possible to compare ciliostatic effects of drugs, preservatives and other excipients with each other?
- Do intranasally administered drugs reach the CSF directly via the olfactory region, without being absorbed first into the systemic circulation and without passing the blood-brain barrier. In other words: do nasal drugs have a direct route to the cerebrospinal fluid?

These three topics are the “current aspects of nasal drug delivery” and they divide the core of this thesis in three sections.

II. Nasal drug administration to the middle meatus

The middle meatus is known as the best location for corticosteroid nasal drug treatment in the treatment of chronic rhinositis and nasal polyposis. The best way to deposit the drugs in this region remains unknown due to several reasons. Firstly, there is no consensus about the technique, the formulation or device. Secondly, research methods used so far into this issue differ, which makes comparison between studies difficult. Thirdly, the role of the individual anatomical differences has not been established (*General introduction*). Topical nasal drug delivery can be achieved by multidose container spray, like almost all corticosteroid sprays, by one unit dose spray and by drops, like in nasules. Nasal drops can be administered in several head positions (figure 2, *General introduction*). Although all administration techniques are extensively investigated, consensus about a single superior technique is lacking. In our studies we used an endoscopic observational single blind dyed drug deposition method to compare administration techniques (chapter 3) and the influence of anatomy and head position (chapter 4).

In **chapter 3** we compared 7 techniques of nasal administration and concluded that there is no such thing as ‘one best technique’ of topical nasal drug delivery. A trend towards better middle meatus deposition was seen with a spray, also in different head positions, but no statistically significant differences were established. Head position seems to be an independent factor in topical nasal drug delivery, as outcomes of different head positions were unrelated to the device used. A single unit nose spray, not used for the treatment of nasal disease before, was very helpful to combine head position and spray use. It could have advantages over drop deposition in similar head positions, but the real value of this device still needs to be established.

In **chapter 4** we found that a minor septal deviation, a hypertrophic inferior turbinate or a narrow nasal valve could alter the expected drug delivery. When correlating the anatomy to the drug delivery, a more obstructed nostril will result in less drug delivery in the more cranial regions (like the middle meatus). The more lateral head positions, like LHL and LHB, seemed to be more successful in bypassing these obstructions. Due to the influence of individual anatomy, a more ‘personal’ approach would be more appropriate to optimize middle meatus drug delivery.

In the *General discussion and conclusions* we pointed out the vast use of nasal corticosteroid drugs and the use of drops for nasal polyposis treatment. We discussed the various research methods, the influence of head position and device and the fact that deposition studies are performed with healthy volunteers. The advice is given to include chronic sinusitis- or nasal polyposis patients, individual anatomy and an unit dose device in future research.

III. Effects of nasal drugs and nasal drug formulations on nasal ciliary activity

The respiratory epithelium is the major lining of the human nasal cavity and is essential in the clearance of the nasal mucosa by the mucociliary system. It is the defense mechanism of the nose capturing and removing harmful particles or substances. It is obvious that during chronic intranasal drug application, the drug itself and the formulation excipients should not disturb the nasal mucociliary clearance.

The influence of nasally administered drugs and the various formulation excipients on the ciliary beat frequency (CBF), measured in *in vitro* experiments, is a valid method to establish their safety (*General introduction*).

In **chapter 5** the effect on the respiratory cilia of various formulations, preservatives and other drug excipients was tested and classified with regard to the 'natural' residence time of a nasal formulation (about15 minutes). CBF was measured by a photoelectric registration method. Excised ciliated chicken trachea tissue was incubated for 15 minutes in the formulation, CBF measured at regular time intervals, followed by a reversibility test of 45 minutes. According to the CBF after 60 minutes every drug or excipient was classified as follows: *Ciliofriendly*: after 60 minutes the CBF has regained 75% or more of its initial frequency. *Cilio-inhibiting*: after 60 minutes the CBF has regained between 25 and 75% of its initial frequency. *Ciliostatic*: after 60 minutes the CBF has regained 25% or less of its initial frequency.

Most formulations tested are ciliofriendly or cilio-inhibiting and only some are ciliostatic. Our study shows that preservatives, like BAC and chlorobutanol, play a major role in the cilio-inhibiting effect of drugs. In addition, additives like benzylalcohol, propylene glycol and phosphate buffer, contribute to the toxicity profile of nasal drug formulations. In conclusion, this section shows that the effect on ciliary movement of most drug formulations is due to the preservatives and/or additives and mostly not to the drug itself. In *General discussion and conclusions* several results obtained have been put in perspective. The effects of drugs and excipients, as measured in this study, are only *indicative* for the effects of nasal drugs on cilia activity *in vivo*. Clinical relevance of the ciliary inhibiting effect of drugs should still be established in patients with diminished ciliary activity or nasal pathology. Practical consequences of the less inhibiting effect on the cilia of Locke Ringer solution compared to saline solution (NaCl 0.9%) remains to be seen. The main conclusion of this section is that the presented classification can be helpful in the design and development of new nasal drug formulations.

IV. Nasal drug delivery and transport to the CSF and brain

For more than 30 years, a large number of studies, mainly in animals, have described the direct transport of a variety of compounds directly from the nose to the CSF after intranasal administration. Diseases of the central nervous system (CNS) like Parkinson's, epilepsy and Alzheimer's are prone to benefit from nasal drug delivery if this 'nose to brain' route is confirmed. Still, in humans the question remains if it is possible to circumvent the BBB and achieve a direct access to the CSF or brain by administering drugs intranasally? In 2002, a human study suggests that "sniffing neuropeptides" may lead to an accumulation of these peptides in the CSF within 80 minutes.

The authors did not compare intranasal with intravenous administration and admit that their data cannot establish that intranasal administration results in greater uptake in the CSF than does intravenous administration (*General introduction*).

In order to deal with this question we decided to perform a study in neurosurgery patients with two endogenous model compounds, melatonin and hydroxocobalamin. First a new method to analyze low levels of melatonin in plasma and CSF was developed (**chapter 6**). In **chapter 7** we compared the uptake of these two model drugs in CSF and plasma after intranasal *and* intravenous drug administration. Eight neurosurgery patients with a CSF drain received either melatonin or hydroxocobalamin intranasally and on the following day the same drug intravenously in a dose comparable to the intranasal dose. On both days the plasma and CSF concentrations were measured up to 3 hours after drug delivery.

We found *no* additional melatonin transport to the CSF when comparing the CSF uptake of melatonin after intranasal administration in relation to the concentrations after intravenous administration. The uptake of hydroxocobalamin into the CSF followed exactly the same pattern as the uptake in blood after intranasal and intravenous administration, with a time lag of about 30 minutes. It seems plausible to suggest that this time is needed to pass the BBB.

In this human study we found *no* evidence of direct access of the drugs from the nose to the CSF. In comparable studies in rats, we found the same results after hydroxocobalamin administration (**chapter 8**) and melatonin administration (**chapter 9**), confirming the human data. In the *General discussion and conclusions* emphasis is put on the ‘suggested proof’ of a nose-brain/CSF pathway in previous studies and the sometimes misleading methods used. Difficulties experienced during our neurosurgery patient study are highlighted and the importance of an intravenous comparison is underlined.

Thesis conclusions and closing remarks

After an overview of the literature on nasal drug delivery and the aims of this thesis in **Section I**, local deposition was investigated to improve topical nasal drug delivery to the middle meatus in **Section II**. We concluded:

- It is unlikely that there is ‘one best technique’ of topical nasal drug delivery.
- The best nasal drug delivery technique is ‘personal’ and depends on individual anatomical differences.
- Patients with frequent rhinosinusitis or nasal polyposis should be included in nasal drug deposition research projects.

The aim of **Section III** was to classify the in vitro effects of drugs, excipients and drug products in relative terms, by comparing the negative or even toxic effects on ciliary movement of individual compounds. We concluded:

- CBF measurements are a valuable tool to classify the inhibiting effects of nasal drugs and their compounds in a comprehensive scale.
- CBF measurements are very sensitive and useful in the design and development of nasal drugs.
- The effect on ciliary movement of most nasal drugs is due to the preservatives and/or additives, and not to the drug itself.

Section IV describes investigations in humans and rats into the possibility of drugs to circumvent the blood-brain barrier by a direct route from the olfactory region to the cerebrospinal fluid. After developing a new detection method of low levels of one of our model drugs melatonin, we conducted the first controlled human study in which the plasma and CSF levels were compared after intranasal and after intravenous application. Secondly we carried out two rat studies with the same model drugs and concluded:

- We found no direct drug transport from the nose to the cerebrospinal fluid.
- Animal studies can have a predictive value for human ‘nose to CSF’ studies, but caution should be taken to translate animal results directly to humans.
- Intravenous comparison is needed to prove direct transport from the nose to the cerebrospinal fluid without being absorbed first into the systemic circulation.

Samenvatting

I. Inleiding

In toenemende mate wordt de toediening van geneesmiddelen via de neus (nasaal) onderzocht. In de *General Introduction (hoofdstuk 1, pagina 11)* worden verscheidene aspecten betreffende toediening van nasale geneesmiddelen besproken en de huidige wetenschappelijke stand van zaken wordt aangestipt. Uit deze verschillende aspecten zijn er drie nader onderzocht:

De vraagstellingen van dit proefschrift (hoofdstuk 2, pagina 51)

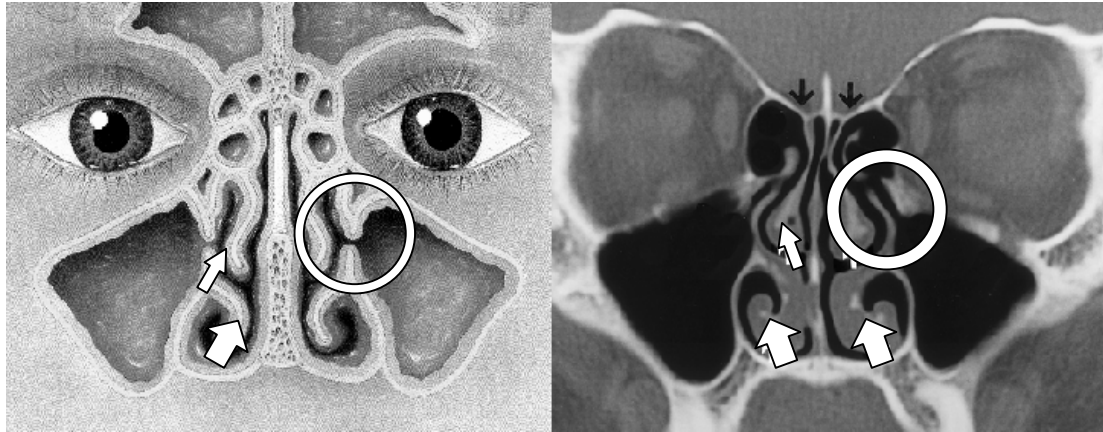
- Met welke nasale toedieningswijze bereikt een geneesmiddel het beste de middelste neusgang?
- Zijn nasale geneesmiddelen potentieel schadelijk voor de neustrilharen? Is het mogelijk om de schadelijke effecten van nasale geneesmiddelen en hun bestanddelen te vergelijken en in te delen op basis van hun schadelijke effecten?
- Kunnen nasale geneesmiddelen direct het hersenvocht bereiken via het reukslijmvlies boven in de neus, zonder eerst opgenomen te worden in de bloedbaan en zonder de bloed-hersen barrière te passeren? Kortom: is er een directe route voor geneesmiddelen van de neus naar het hersenvocht?

Deze vraagstellingen hebben betrekking op drie hedendaagse aspecten van de toediening van nasale geneesmiddelen (“Current aspects of nasal drug delivery”) en ze verdelen dit proefschrift in drie delen (Sectie II-IV).

II. Geneesmiddelen toediening naar de middelste neusgang

Algemene bouw neus. Ieder neusgat heeft, na een nauwe doorgang, een holte met aan de zijkant 3 neusschelpen. De holte wordt naar boven toe steeds smaller en helemaal bovenin zit het reukorgaan (olfactory region).

Neusgangen, neusschelpen en bijholten. De onderste, middelste en bovenste neusschelpen vormen onder hun schelpvorm een buisvormige neusgang (zie figuur 1). Door de onderste twee gangen wordt voornamelijk geademd, de moeilijker toegankelijke bovenste neusgang is voornamelijk voor de reuk. De middelste neusgang is belangrijk als er sprake is van frequente bijholte-infecties of neuspoliepen. In deze middelste neusgang zijn namelijk de uitgangen gelegen van bijna alle bijholten. ‘Op’ en ‘hoe’ geneesmiddelen deze middelste neusgang bereiken is onderzocht in hoofdstuk 3 en 4.



Figuur 1. Anatomie van de neus in een vooraanzicht tekening (links) en een röntgen CT scan (rechts) Onderste en middelste neusschelpen (respectievelijk brede en smalle witte pijlen) delen de neusholte in meerdere gangen. Aan weerszijden van de neusholte zijn de kaakholttes zichtbaar, die hun uitgang in de middelste neusgang hebben (cirkel). De zwarte pijlen geven de regio aan waar de reukzenuw zich bevindt.

Onderzoeksopzet. Bij 10 gezonde vrijwilligers (20 neusgaten) is op verschillende dagen via een spray of druppelmethode in combinatie met verschillende houdingen van het hoofd, een gekleurd geneesmiddel nasaal toegediend. Aansluitend is met een kleine camera gekeken waar in de neus zich kleurstof bevond en of deze gelokaliseerd was rondom de middelste neusgang. Bij iedere vrijwilliger zijn 7 methoden van toediening vergeleken (pagina 61-62) om uit te maken of er één methode het beste was en of de individuele anatomie invloed zou hebben op de verspreiding van het geneesmiddel.

Resultaten. Een groot deel van het toegediende geneesmiddel was zichtbaar in het begin van de neus en kwam dus niet bij de beoogde middelste neusgang. Met betrekking tot de lokalisatie rondom de middelste neusgang waren er geen statistisch significante verschillen tussen de verschillende methodes of hoofdhoudingen. Slechts enige verbetering was waarneembaar bij het gebruik van een spray t.o.v. druppels. De hoofdhouding had ook enige invloed. Bij verdere analyse had de bouw van de neus per individu invloed op de verspreiding van het nasaal toegediend geneesmiddel. Dit zou kunnen verklaren waarom er niet één methode beter is in de totale groep.

Conclusies.

- Het is onwaarschijnlijk dat er één enkele methode als beste in staat is om geneesmiddelen bij elke willekeurige patiënt in de middelste neusgang te brengen.
- De beste methode om geneesmiddelen in de middelste neusgang te brengen is persoonsgebonden en afhankelijk van individuele anatomie.

III. Het effect op de neustrilhaar functie van geneesmiddelen en hun bestanddelen.

Neusslijmvlies en trilharen. De huid voor in de neus verandert geleidelijk in slijmvlies dat verder ook in de luchtweg en longen zit. Dit slijmvlies bevat meerdere soorten cellen en een groot deel van deze cellen heeft boven op hele kleine trilhaartjes, *cilia* genoemd (zie ook illustratie pagina 14). Deze cilia zorgen ervoor dat de slijmlaag op de neuscellen voortbewogen wordt naar de keel. Zo worden, als afweermechanisme van de neus, bacteriën en stofdeeltjes weggevangen en naar de keel verplaatst. Deze cilia zijn dus onderdeel van een belangrijk mechanisme en mogen niet zomaar stil komen te liggen. In hoofdstuk 5 wordt beschreven of nasale geneesmiddelen invloed hebben op de frequentie van de trilhaarslag.

Onderzoeksopzet. Door trilharen in een badje met een geneesmiddel te brengen kun je, via metingen door een microscoop, zien of het geneesmiddel invloed heeft op de trilhaarslag-frequentie. De trilharen zouden minder snel kunnen gaan trillen of zelfs stoppen met bewegen.

Meerdere geneesmiddelen en ook hun bestanddelen zijn getest door trilharen 15 minuten in het geneesmiddel te leggen, te spoelen en 45 minuten in een neutrale vloeistof te leggen (reversibiliteits-test). Na 60 minuten werd gekeken hoe goed de trilharen nog trilden (0-100%). De stoffen werden, naar gelang hun invloed op de trilhaarbeweging, ingedeeld in drie categorieën: ‘trilhaar-vriendelijk’, ‘trilhaar-vertragend’ of ‘trilhaar-blokkerend’ (zie ook illustratie pagina 95).

Resultaten. Het was goed mogelijk om geneesmiddelen en hun bestanddelen te testen op hun trilhaar-remmende effecten en om ze aansluitend eenvoudig in te delen. De meeste geneesmiddelen en hun bestanddelen waren trilhaar-vriendelijk of –vertragend, slechts enkele trilhaar-blokkerend. Conserveermiddelen en geneesmiddel-toevoegingen hadden het grootste aandeel in de trilhaar-remmende effecten.

Conclusie.

- Het trilhaar-remmende effect van de meeste geneesmiddelen komt door het conserveermiddel of andere bestanddelen en niet door het geneesmiddel zelf.

IV. Transport van geneesmiddelen van de neus direct naar de hersenen/ hersenvocht.

Geneesmiddel opname en bloed-hersen barrière. In de neus bevindt zich een goede bloedsomloop. Dat is nodig om de ingeademde lucht te verwarmen, te bevochtigen en de luchtweerstand te reguleren. Vanwege deze goede doorbloeding is het ook gemakkelijk om nasale geneesmiddelen in de bloedbaan te laten komen. Voorwaarde is natuurlijk dat geneesmiddelen worden opgenomen door het neusslijmvlies (absorption) (paragraaf 1.6, pagina 31-35).

Een nieuw idee van sommige wetenschappers is dat geneesmiddelen misschien niet alleen vanuit de neus in de bloedbaan kunnen worden opgenomen, maar ook via het reukorgaan kunnen worden opgenomen in het hersenvocht of hersenen. De toediening van geneesmiddelen naar de hersenen zou hierdoor gemakkelijker worden aangezien de hersenen onder normale omstandigheden goed beschermd zijn tegen het binnendringen van allerlei stoffen vanuit de bloedbaan. Geneesmiddelen gaan normaal via de bloedbaan naar de hersenen. Een filter, de bloed-hersen barrière genoemd, voorkomt echter gemakkelijke doorgang van deze geneesmiddelen. Of er een directe route van geneesmiddelen via de neus naar het hersenvocht bestaat wordt behandeld in hoofdstuk 6 t/m 9.

Onderzoeksopzet. Allereerst wordt er in hoofdstuk 6 een nieuwe meetmethode beschreven om één van de modelstoffen, het hormoon melatonine, in lage concentraties te kunnen bepalen in het bloed en het hersenvocht.

Patiënten die door de hersenchirurg zijn behandeld, hebben soms een slang (drain) voor de afvoer van hersenvocht. Via deze drain valt hersenvocht af te tappen. Patiënten met zo'n drain zijn benaderd voor dit onderzoek, waarbij ze twee dagen onderzocht werden. Op de eerste dag kregen ze de modelstof (hormoon melatonine of vitamine B₁₂) via een neusspray toegediend en op dag twee een vergelijkbare hoeveelheid modelstof direct in de bloedbaan toegediend. Na het toedienen werden er in enkele uren verscheidene hersenvocht-, en bloedmonsters afgenomen om daarin de concentratie van de modelstof te bepalen. Er werd gewerkt met een onschadelijke modelstof zodat de behandeling van de patiënt niet beïnvloed werd. De ene modelstof was vetoplosbaar (melatonine) de ander wateroplosbaar (vitamine B₁₂).

Indien na toediening via de neus(spray) meer modelstof in het hersenvocht zou komen dan na toediening via de bloedbaan, dan pleit dat voor een direct transport van de neus naar het hersenvocht.

Na de proeven in patiënten, zijn dezelfde proeven nogmaals herhaald in een zelfde opstelling bij ratten (hoofdstuk 8 en 9).

Resultaten. Hoewel het moeilijk was geschikte patiënten te vinden werden bij 8 patiënten de concentratie-reeksen gemeten, zowel na toediening via de neus als na toediening via de bloedbaan. De concentraties in het bloed lieten zien dat de stof goed in het bloed kwam na het toedienen via de neus. Bij het vergelijken van de concentraties in het hersenvocht bleek dat het toedienen via de neus niet tot hogere concentraties leidde dan na toediening via de bloedbaan. Er werd zelfs een zelfde opnamepatroon gevonden. In de onderzoeken bij ratten werd hetzelfde gevonden voor beide modelstoffen en werd dus bewezen dat voor deze stoffen in deze opstelling de bloed-hersen barrière *niet* te omzeilen valt.

Conclusies:

- Geneesmiddelen die in de neus worden toegediend worden in het bloed opgenomen. Direct transport vanuit de neus via het reukorgaan naar het hersenvocht werd door ons *niet* gevonden.
- Om extra transport van de neus naar de hersenen te onderzoeken is het nodig om de ‘gebruikelijke route’, namelijk via de bloedbaan, ook te onderzoeken.

Appendix

Dankwoord	194
Sponsors	199

Dankwoord

Het was een lang traject, waarbij het niet altijd duidelijk was dat mijn wetenschappelijk werk zou resulteren in een promotie. Maar het is mede dankzij velen dat het uiteindelijk tot dit resultaat heeft geleid.

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Yannick, zonder jou zou papa nu nog bezig zijn.



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