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Rui Filipe Libânio Osório Marta

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REVIEW ARTICLE

Metabolism of lysergic acid diethylamide (LSD): an update

Rui Filipe Libânio Osório Marta[a,b]

[a]Department of Sciences, INFACTS – Institute of Research and Advanced Training in Health Sciences and Technologies, University Institute of Health Sciences (IUCS), CESPU, CRL, Gandra, Portugal; [b]Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, UCIBIO, REQUIMTE, University of Porto, Porto, Portugal

ABSTRACT

Lysergic acid diethylamide (LSD) is the most potent hallucinogen known and its pharmacological effect results from stimulation of central serotonin receptors (5-HT_2). Since LSD is seen as physiologically safe compound with low toxicity, its use in therapeutics has been renewed during the last few years. This review aims to discuss LSD metabolism, by presenting all metabolites as well as clinical and toxicological relevance. LSD is rapidly and extensively metabolized into inactive metabolites; whose detection window is higher than parent compound. The metabolite 2-oxo-3-hydroxy LSD is the major human metabolite, which detection and quantification is important for clinical and forensic toxicology. Indeed, information about LSD pharmacokinetics in humans is limited and for this reason, more research studies are needed.

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Introduction

Hallucinogens are psychoactive substances with the ability to induce states of altered perception, thought, and feeling at low doses, without causing marked psychomotor stimulation or depression (Nichols 2004). They cause hallucinations, or sensations that seem real even though they are not. Additionally, hallucinogens users may feel out of control or disconnected from their body and environment (Nichols 2004, 2016). These compounds are also known as psychotomimetic substances (i.e. psychosis mimicking) (Hoffer 1967) or psychedelic agents (i.e. they gave a mind-manifesting capability) (Osmond 1957). In fact, these compounds are also further referred to as entheogenic substances since they can provoke mystical experiences and evoke feelings of spiritual significance (Ruck et al. 1979; Nichols 2004).

According to its chemical structure, hallucinogens can be divided into two main chemical classes: (a) phenethylamines and (b) indolamines or tryptamines (subclassified into simple tryptamines and ergolines). The group of phenethylamines includes compounds like mescaline or 2,5-dimetoxi-4-metilamfetamina (DOM), while psilocybin (a prodrug for psilocin) and *N,N*-dimethyltryptamine (DMT) are classified as simple tryptamines. Lysergic acid diethylamide (LSD) is the classic example of the ergolines group (Nichols 2004; Araújo et al. 2015). Hallucinogens can also be categorized into naturally occurring substances such as mescaline (Dinis-Oliveira et al. 2018) and psilocybin (Dinis-Oliveira 2017) or synthetic compounds like LSD-25 (Hofmann 1979).

Their main mechanism of action is based on an agonist (or partial agonist) action at serotonin (5-$HT)_{2A}$ receptors (Rickli et al. 2016), which explains the unique and powerful ability of hallucinogens to affect the human psyche (Halberstadt 2015; Preller et al. 2017). The production of altered states of consciousness are the most important clinical effects, although their consumption is marked by a series of somatic symptoms (dizziness, weakness, tremors, nausea, drowsiness, paresthesia, and blurred vision), perceptual symptoms (altered shapes and colors, difficulty in focusing objects, sharpened sense of hearing, and rarely synesthesia) and psychic symptoms (alterations in mood, tension, distorted time sense, difficulty in expressing thoughts, depersonalization, dreamlike feelings, and visual hallucinations) (Hollister 1984; Nichols 2004). The use of hallucinogenic substances can cause two similar but distinct phenomena. People can experience flashbacks, i.e., hallucinations that occur weeks, months or even years after the drug was last taken (Nichols 2004) or a Hallucinogen Persisting Perception Disorder (HPPD) characterized by a continual presence of sensory disturbances (Halpern and Pope 2003). The main difference is

CONTACT Rui Filipe Libânio Osório Marta ruifilipemarta@gmail.com Department of Legal Medicine and Forensic Sciences, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

that the flashbacks are transient, while HPPD is persistent. At times, the users can also experience a 'bad trip,' which is a state of frightening and disturbing hallucinations, characterized by panic and unpredictable behavior, leading to fatal accidents (e.g. run across a road or try to fly) (Nichols 2004).

Unlike other abused drugs, hallucinogens are physiologically safer compounds with low toxicity. Moreover, the dependence potential of psychoactive drugs such as LSD, psilocybin or mescaline is very low, and they are not considered to be reinforcing substances (O'Brien 2001; Nichols 2004; Johnson et al. 2018).

LSD is a semi-synthetic compound derived from lysergic acid as found in fungus ergot *Claviceps purpurea*, which grows on rye. It is the most potent hallucinogen known with the ability to alter the states of consciousness and perception (Abraham et al. 1996; Fantegrossi et al. 2008). Its chemical structure consists of an indole system with a tetracyclic ring ($C_{20}H_{25}ON_3$) (Passie et al. 2008). It is a colorless, odorless, and flavorless liquid. Usually, LSD is dissolved in water and applied to paper, sugar cubes, gelatin cubes, or other substances for ingestion (Hovda et al. 2016) and it is also known as '*Acid*,' '*Battery Acid*,' '*Blotter*,' '*California sunshine*,' '*Heavenly Blue*,' and other colorful names. It was synthetized by Albert Hofmann in 1938, although the accidental discovery of its psychic effects (Hofmann 1979) only happened 5 years later, in 1943. Over the course of the 1960s and 1970s, it was very popular as a recreational and spiritual drug due to its psychedelic effects, which are the result stimulation of the serotonin (5-HT) receptors (LSD acts as agonist at 5-HT_{2A} receptors found in cortex) (Nichols 2016). In general, LSD is considered physiologically safe, therefore, exhibit low toxicity (Schmid et al. 2015; Nichols and Grob 2018). Most probably, the effects of LSD intake (a 'trip') vary according to the person, i.e. some people have dilated pupils, increased blood pressure, and elevated body temperature, while others may also feel dizzy, sweat, have blurred vision and feel tingling in their hands and feet, although the main effects are visual (Nichols 2016).

Recent studies reintroduced the belief that psychedelics can be applied to the treatment of a variety of disorders such as alcoholism and other addictions, anxiety and depression, schizophrenia, and even autism, obsessive-compulsive disorder, cluster headaches, and others (Bogenschutz 2013; Bogenschutz and Johnson 2016; Liechti 2017). Indeed, the use of LSD as a medicine has been a subject of many studies, as it happened between the 50s and 80s. Increased ability of patients to deal with trauma, depression, and other disorders, as well as the relief of emotional strife in patients with terminal illnesses (Gasser et al. 2014) are some examples of the use of LSD as adjunct to psychotherapy. The pharmacology of LSD is complex, and its mechanisms of action are still not completely understood. The focus of this manuscript is to provide all the available data regarding LSD focusing on its metabolism and discussing its relevance.

Methodology

An English extensive literature research was carried out in PubMed (U.S. National Library of Medicine) without a limiting period to identify relevant articles on LSD and its metabolism. Electronic copies of the full papers were obtained from the retrieved journal articles as well as books on LSD, and then further reviewed to find additional publications related to human and non-human studies.

Absorption and distribution

There is a limited amount of studies concerning pharmacokinetics of oral LSD in humans, which would be of interest for clinical and forensic toxicology due to widespread consumption of this substance and the current use in therapeutics. LSD is mainly administered *via per os* (p.o.), but can be smoked, injected, or snorting. Ingested orally, it is absorbed rapidly and completely in gastrointestinal tract, presenting an oral bioavailability of approximately 71% (Dolder, Liechti, et al. 2015; Dolder, Schmid, et al. 2015). Absorption occurs within 1 h and effects last 6–12 h depending on dose. Since food in the stomach slows absorption when ingested, the absorption of LSD depends on the size of meal, the pH values of stomach and duodenum as well as the gastric evacuation rate as showed by Upshall and Wailling (1972).

Aghajanian and Bing (1964) performed a small pharmacokinetic study (single i.v. doses of 2 ug/kg in five healthy male human subjects) whose plasma concentrations were 6 to 7 ng/mL 30 min after i.v. administration, 4–6 ng/mL at 30–120 min, and approximately 1 ng/mL at 8 h. They estimated a plasma elimination half-life of 175 min (Aghajanian and Bing 1964). In another study (single oral doses of 160 μg in 13 male human subjects), plasma levels peaked 40–130 min after oral administration, and peaks ranged from 1.8 to 8.8 ng/mL (Upshall and Wailling 1972). The onset of symptoms varies according to the route of administration (Hoch 1956).

Recently, two separate dose studies (200 and 100 μg) were conducted to determine the pharmacokinetic profile of LSD (Dolder, Schmid, et al. 2015; Dolder et al. 2017). In the first one, 16 male and female subjects

ingested a single oral dose of 200 μg LSD. The concentrations were maximal after 1.5 h (median) and gradually declined to very low levels by 12 h, with a half-life of 3.6 h during the first 12 h (Dolder, Schmid, et al. 2015). In the most recent study, a 100-μg LSD dose was administered orally by 24 healthy subjects. It was found a mean peak plasma concentration of 1.3 ng/mL, 1.4 h after administration; lower than 3.1 ng/mL after 1.5 h after administration of the 200-μg dose. After new analysis, both studied doses presented a predicted mean half-life of 2.6 h. The mean duration of the subjective effects of LSD was 8 and 11 h after administration of the 100- and 200-μg doses, respectively (Dolder et al. 2017). It was found a close relationship between the plasma concentrations of LSD over time and physiologic response, with no evidence of acute tolerance (Dolder, Schmid, et al. 2015; Dolder et al. 2017).

Pharmacokinetic studies in animals showed that ^{14}C-labeled LSD is well absorbed and there is a quick distribution from plasma into tissues, being larger in the liver (where it undergoes metabolization), followed by kidney, spleen, brain, muscle and fat tissue (Boyd et al. 1955; Lanz et al. 1955; Axelrod et al. 1957; Boyd 1959). LSD distribution was studied in the mouse (Stoll et al. 1955; Haley and Rutschmann 1957), guinea pig, rhesus monkey, rat (Siddik et al. 1979) and cat (Axelrod et al. 1957). In rat, after 3 h, LSD was in a higher percentage in gut contents and liver (Boyd et al. 1955), while in the cat, after 90 min, bile, plasma, lung, and liver presented the highest percentages (Axelrod et al. 1957).

As the drug penetrates the central nervous system (CNS), LSD brain concentration is expected. The concentration on visual brain areas and the limbic and reticular activating systems are correlated with perceived effects (Levine and Jenkins 2003). Considerable LSD amounts were detected in the brain and cerebrospinal fluid of mice, rats, cats, and monkeys (Lanz et al. 1955; Axelrod et al. 1957; Boyd 1959; Freedman and Coquet 1965; Passie et al. 2008), which points to an easy passage through the blood-brain barrier. *In vitro* study on guinea pigs showed that LSD binds extensively to plasma proteins (65–90%), at plasma concentrations of 0.1 and 20 mg/L (Axelrod et al. 1957).

There is some controversy about the relationship between LSD effects and LSD tissue concentration. It remains to be clarified if there is a linear or logarithmic-linear relation or neither, although several attempts have been made based on two compartment-model (Wagner et al. 1968; Levy 1969; Metzler 1969). Dolder et al. (2017) found no correlations between LSD concentrations and its effects across subjects after oral administration of 100 and 200 μg of LSD. Recently, Holze et al. (2019) determined that plasma concentrations of LSD were not associated with the subjective effects of LSD when analyzed across subjects after the use of a 100 μg dose (Holze et al. 2019).

The volume of distribution is reported to be low at 0.28 L/kg (Karch 2006).

Metabolism

There is still no fully defined LSD metabolism. The metabolism of LSD is presented in Figure 1. Most studies were performed in animals, especially in rats. It is assumed that LSD metabolic rate varies from species to species as well as the nature and number of metabolites formed (Passie et al. 2008). After an oral administration, LSD is extensively metabolized, which explains why only 1% of the dose is excreted unchanged in the urine (Lim et al. 1988; Canezin et al. 2001).

LSD is predominantly metabolized in liver tissue to structurally similar and inactive metabolites after N-dealkylation and/or oxidation processes. In humans, LSD undergoes metabolic *N*-demethylation at position 6 to form *N*-demethyl-LSD (*Nor*-LSD), although it is a minor metabolic pathway. The half-life of *Nor*-LSD has been reported to be approximately 10 h, higher than LSD (Hoja et al. 1997; Steuer et al. 2017). Nor-LSD was firstly identified as human metabolite of LSD in urine (Lim et al. 1988) and, more recently, it was detected for the first time in human plasma (Steuer et al. 2017).

Furthermore, it seems to occur an aromatic hydroxylation of LSD at positions 13 and 14 to form 13-hydroxy-LSD and 14-hydroxy-LSD, respectively (Lim et al. 1988). The urinary excretion of 13- and 14-hydroxy-LSD glucuronides from LSD users point to a probable presence of 13- and 14-hydroxy-LSD (Canezin et al. 2001).

However, before Steuer et al. (2017) investigation, the chemical structure of 13- or 14-hydroxy LSD metabolites in humans was not completely defined. The authors identified a single peak corresponding to a hydroxy metabolite (its glucuronide was also identified) in human plasma and advanced with some explanations, such as: only one hydroxy metabolite is formed in humans, or the method failed to detect due to low abundance or the chromatographic technique was unable to differentiate hydroxy metabolites (Steuer et al. 2017).

The presence of potential LSD glucuronides seems clearly to be an important detoxification step, which is the most common and most important phase II reaction in humans.

Both LSD metabolites, Nor-LSD and hydroxy-LSD, showed to have a half-life higher than LSD (Steuer et al. 2017).

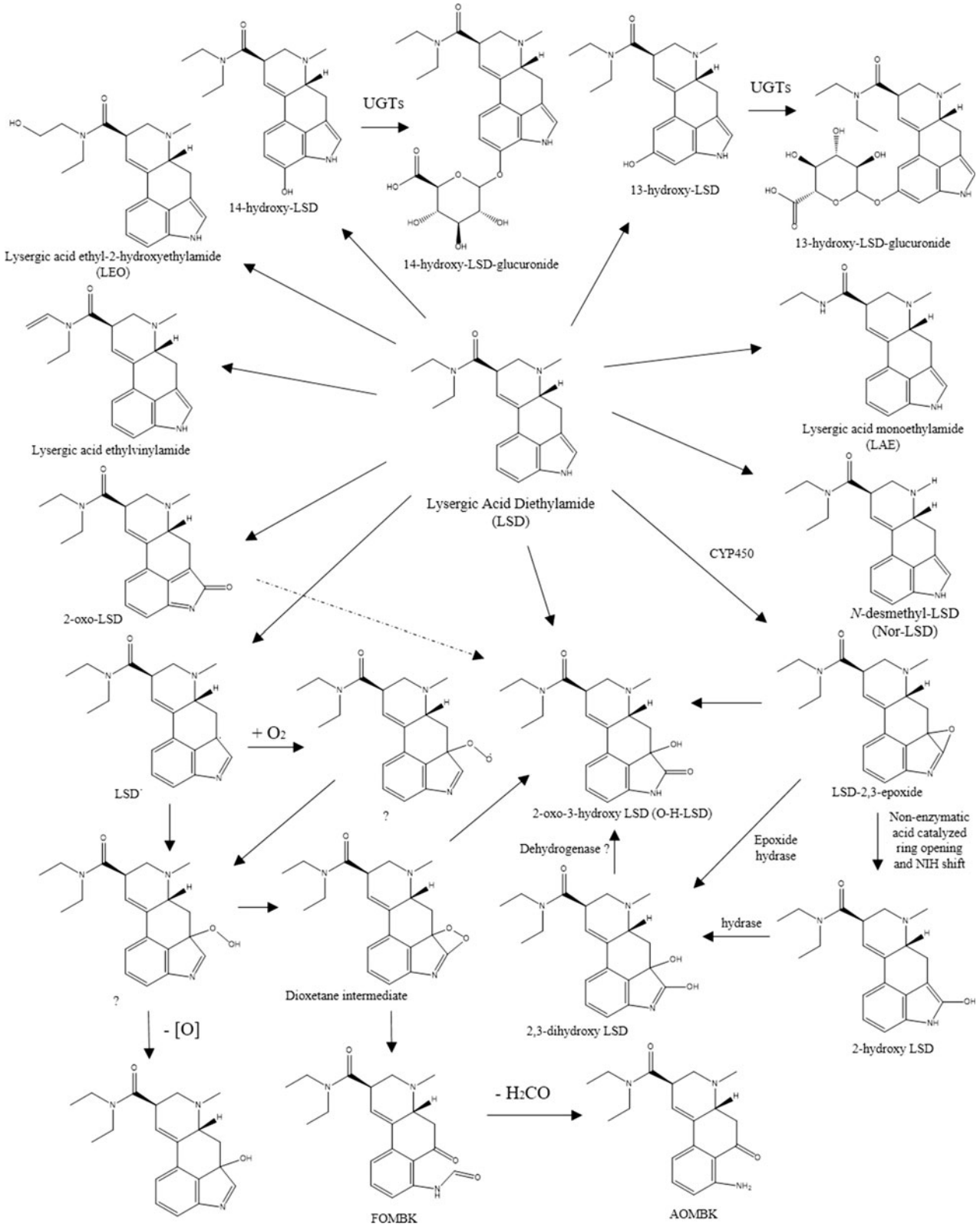

Figure 1. Metabolism of lysergic acid diethylamide (LSD). All represented metabolites were identified in human or animal studies.

After LSD human liver microsomal incubation was identified two new metabolites known as lysergic acid ethylamide (LAE), after an dealkylation reaction, and 2-oxo-LSD, after a oxidation reaction, as well as other *in vitro* metabolites suggested to be mono- and trioxylated metabolites of LSD (Cai and Henion 1996). The presence of LAE and mono-oxylated LSD metabolites was also positively detected in human urine (Cai and

Henion 1996). The authors also indicated deethylation as the major metabolic route of LSD by human liver microsomes.

2-Oxo-3-hydroxy-LSD (O-H-LSD) seems to be the major human metabolite of LSD (Foltz 1995; Poch et al. 1999). The higher concentrations (16–43 times greater than LSD) and longer detection window of O-H-LSD, possibly days/hours, make it the main marker for identification of LSD use (Poch et al. 1999; Reuschel, Percey, et al. 1999; Canezin et al. 2001; Johansen and Jensen 2005). *In vitro* studies using liver microsomes and human cryopreserved hepatocytes showed that O-H-LSD is a product of LSD biotransformation whose amount increases in a time-dependent manner. In the same study was detected another metabolite in a time-dependent manner upon incubation, the dihydroxy-LSD (Klette et al. 2000). Therefore, LSD is oxidized to 2-oxo-LSD, which undergoes subsequent hydroxylation to O-H-LSD. Although the formation of O-H-LSD may occur *via* intermediate metabolite dihydroxy-LSD (Figure 1) (Klette et al. 2002). In addition to the LSD metabolites already identified, Canezin et al. (2001) described the potential presence of trioxylated-LSD and lysergic acid ethyl-2-hydroxy-ethylamide (LEO) in human urine after an oxidation reaction and a dealkylation reaction, respectively, although no further reliable MS/MS data was used for identification.

Recently, Wagmann et al. (2019) identified several metabolites from LSD and LSD-based new psychoactive substances after *in vitro* studies with pooled human liver S9 fraction (pS9). In relation to LSD, the authors were able to identify several compounds such as *N*-deethyl LSD (LAE) and N^6-demethyl LSD (nor-LSD), hydroxy LSD isomer 2 and its glucuronide, dihydroxy LSD (2-oxo-3-hydroxy LSD) and LSD hydroxy isomer 1. Hydroxy LSD isomer (LEO) and trihydroxy LSD were not detected in the *in vitro* conditions, whose explanation is related to the low concentrations (below the detection limit) or the wrong choice of incubation time (Wagmann et al. 2019). Wagmann et al. (2019) also studied the importance of monooxygenases to hepatic clearance. CYP3A4, CYP1A2, and CYP2C19 play an important role in the metabolism of LSD. The use of CYP1A2 inhibitor alpha-naphtoflavone and CYP3A4 inhibitor ketoconazole confirmed the importance of both enzymes, after a significant reduction of metabolite formation (Wagmann et al. 2019). CYP2D6, CYP2E1, and CYP3A4 are significantly involved in metabolism of LSD to Nor-LSD, whereas CYP1A2, CYP2C9, CYP2E1, and CYP3A4 contribute significantly to the formation of O-H-LSD (Luethi et al. 2019).

The involvement of several CYPs raises serious questions about the influence of genetic polymorphisms and drug interactions on LSD pharmacokinetics and pharmacodynamics. Thus, drug-drug interaction and pharmacogenomic studies in humans are extremely important from the clinical and forensic point of view.

Moreover, the consumption of LSD derivatives in recreational doses created new challenges for forensic and clinical toxicology. Metabolically, LSD-based compounds have some metabolites in common (based on comparable fragmentation patterns), which makes it difficult to identify and differentiate the compounds consumed. 1-Propionyl-LSD (1P-LSP) is theorized to act as a prodrug for LSD (Wagmann et al. 2019). A positive result for LSD should be thoroughly analyzed, as it may not be a result of LSD consumption.

Literature data reveal the presence of iso-LSD in the urine and other body fluids from LSD users at higher concentrations than LSD (Cai and Henion 1996). However, iso-LSD is not an LSD metabolite but as a major contaminant in many illicit preparations, which is used as an additional marker for LSD consumption (Wagmann et al. 2019). It is an inactive diastereoisomer formed during the synthesis from lysergic acid (Reuschel, Eades, et al. 1999). Iso-LSD showed an elimination half-life greater than LSD, median 12 and 4.2 h, respectively (Steuer et al. 2017).

Recently, iso-LSD, O-H-LSD, Nor-LSD, LAE, LEO, 2-oxo-LSD, 13 and 14-hydroxy-LSD were positively detected in human plasma samples after a controlled clinical trial, but were too low for quantification (Dolder et al. 2018).

Gomes et al. (2012) proposed a new metabolic pathway for LSD, which consists of the oxidation of LSD by peroxidases. The authors studied the oxidation of LSD by HRP/H_2O_2 (Horseradish Peroxidase/hydrogen peroxide) system and by activated human neutrophils, which contains myeloperoxidase (MPO). They concluded that both peroxidases were capable of metabolizing LSD to the same compounds that have been observed *in vivo*, O-H-LSD and Nor-LSD and, additionally, *N*,*N*-diethyl-7-formamido-4-methyl-6-oxo-2,3,4,4a,5,6-hexahydrobenzo[*f*]quinoline-2-carboxamide (FOMBK) and 7-amino-*N*,*N*-diethyl-4-methyl-6-oxo-2,3,4,4a,5,6-hexahydrobenzo[*f*]quinoline-2-carboxamide (AOMBK) formed from the dioxetane intermediate. AOMBK is a product of FOMBK deformylation reaction (Gomes et al. 2012). The action of peroxidase system on LSD metabolism is supported by the fact that MPO is present in the brain, particularly in neurons and microglia (monocyte-derived cells) (Malle et al. 2007; Gomes et al. 2012).

The limited information about LSD human metabolism and pharmacokinetics is outweighed by *in vitro* and *in vivo* studies performed in animals such as rats (Boyd et al. 1955; Rothlin 1956; Slaytor and Wright 1962; Szara 1963; Niwaguchi et al. 1974; Siddik et al. 1975; Back and Singh 1976; Siddik et al. 1979; Inoue et al. 1980b, 1980a; Parker et al. 1980), mice (Stoll et al. 1955; Haley and Rutschmann 1957; Idanpaan-Heikkila and Schoolar 1969), cat and guinea pigs (Axelrod et al. 1957), and rhesus monkeys (Axelrod et al. 1957; Sullivan et al. 1978). In animals, LSD undergoes N-demethylation, N-deethylation, aromatic hydroxylation, and oxidation at position 2, depending on the species. The data obtained by Inoue et al. (1980a) suggest that LSD biotransformation is performed by at least three enzyme systems, in which cytochrome P450 plays an important role. Briefly, the diethylamide group at the 8-position, the *N*-methyl group at the 6-position and the aromatic proton at the 13-position undergo changes, respectively (Inoue et al. 1980a, 1980b).

Excretion

Only 1% of the dose is excreted in urine as unchanged LSD (Dolder, Schmid, et al. 2015; Richeval et al. 2017), whose concentrations range from 1.5 to 55 ng/mL as evidenced the analysis of urine specimens from eight subjects after ingesting 200 and 400 μg (Taunton-Rigby et al. 1973). The study of urine specimens from LSD users revealed higher concentrations of 2-oxo-3-hydroxy-LSD relative to parent compound (Poch et al. 1999), which could be detected in urine for up to 4 d (Foltz and Reuschel 1998). After glucuronidation, the water-soluble conjugates of hydroxylated metabolites are easily excreted in the urine (Levine and Jenkins 2003). According to Faed and McLeod (1973) study, the rate of excretion of LSD reaches a maximum approximately 4–6 h after an oral administration of 200 μg in humans.

In rats, LSD glucuronides are mainly excreted in the bile (Back and Singh 1976; Parker et al. 1980), nearly 80% of a dose (Parker et al. 1980). There is a limited enterohepatic circulation, since hydroxylated metabolites released by intestinal hydrolysis of their glucuronides have a relatively low lipid-solubility. Therefore, these compounds are poorly absorbed and are mainly excreted in the feces (Parker et al. 1980).

Dolder, Schmid, et al. (2015) established an LSD renal clearance value of 1.32 ± 0.6 mL/min (or approximately 1.6% of the apparent total clearance after oral administration).

Considerations about applied analytical strategies for metabolite identification

The analysis of LSD and its metabolites in body fluids is challenging since the doses involved are very small and there is an extensive metabolism (Peel and Boynton 1980). Additionally, drug's volatility, its thermal instability, and its tendency to undergo adsorptive losses during gas chromatographic also make the LSD analysis difficult (Lim et al. 1988) and, therefore, it is impossible to quantify or even identify new metabolites (Dolder et al. 2018). Therefore, successful efforts have been undertaken to the development of a sensitive method for measurement of LSD and its metabolite (Chung et al. 2009; Steuer et al. 2017; Dolder et al. 2018).

Gas chromatographic (GC)-mass spectrometry (MS) (Clarkson et al. 1998; Sklerov et al. 1999) and liquid chromatography (LC)-MS(/MS) (Sklerov et al. 2000; Canezin et al. 2001; Johansen and Jensen 2005; Favretto et al. 2007; Chung et al. 2009; Martin et al. 2013; Dolder, Liechti, et al. 2015; Steuer et al. 2017; Holze et al. 2019) techniques have been routinely applied to confirmatory analysis on LSD detection and quantification. All methods developed aim to overcome the issues of the low concentrations of LSD and its metabolites. Steuer et al. (2017) developed and validated an ultra-fast and sensitive Microflow LC-MS/MS method to quantify LSD and its metabolites in human plasma samples, although there is a lack of ruggedness in this technique.

The application of different sample workup procedures led to positive results, such as liquid–liquid extraction (LLE) (Canezin et al. 2001; Johansen and Jensen 2005; Favretto et al. 2007; Chung et al. 2009), solid phase extraction (SPE) (Martin et al. 2013), online extraction (Dolder, Liechti, et al. 2015), and protein precipitation (Dolder et al. 2018). Steuer et al. (2017) tested different LLE procedures using different organic solvents (butyl acetate; butyl acetate/ethyl acetate (1:1); diethyl ether/ethyl acetate (1:1) and 2-propanol/dichloromethane/ethyl acetate (1:1:1)). However, the desired low concentration range was only achieved with a SPE with cation exchange sorbents like HCX where all analytes were detected (Steuer et al. 2017).

Usually, the dose involving in LSD trials is in the range 40–800 μg (Dolder, Schmid, et al. 2015; Dolder et al. 2016; Mardal et al. 2017). Brain tissue is a very suitable biological specimen since LSD acts on the CNS. The analysis of postmortem brain samples allowed to quantify LSD at concentrations from 0.34 to 10.8 μg/kg, higher than in peripheral blood. Iso-LSD and O-H-LSD were also quantified, although in smaller amounts. Moreover, the drug-to-metabolite ratio in brain tissue

was higher than in blood and urine samples, which can be explained by the fact that O-H-LSD being more hydrophilic than LSD (Mardal et al. 2017).

LSD is unstable under prolonged exposure to heat, alkaline, natural sunlight, and UV light irradiation (Twitchett et al. 1978; Peel and Boynton 1980; Francom et al. 1988; Li et al. 1998). Therefore, urine samples stored under a close fluorescent light; or at elevated temperatures; or under alkaline and acidic conditions; or with traces amounts of metal ions cause LSD decomposition (Li et al. 1998). Indeed, the concentrations of LSD, O-H-LSD, and nor-LSD were stable after freezing the urine samples (at −20 °C), however, at a higher temperature, there is a decrease in analytes concentrations (Peel and Boynton 1980; Francom et al. 1988; Reuschel, Eades, et al. 1999; Skopp et al. 2002).

Under refrigerated or frozen conditions, LSD, O-H-LSD, and nor-LSD showed a long-term stability (6 months) (Klette et al. 2002; Martin et al. 2013). Steuer et al. (2017) used plasma samples frozen at −80 °C for a maximum of 18 months, although long-term stability experiments were not performed during the method validation process. However, there was no relevant conversion of LSD to iso-LSD during the sample analysis (Steuer et al. 2017). Holze et al. (2019) verified that conversion of LSD to iso-LSD occurred in higher proportions in vials that were stored at room temperature (0.1–3.6% after 4–24 months) comparatively the solution that was stored at 4 °C (0–9.5% after 2–24 months).

The concentrations of the processed samples decreased up to −60% within 24 h at ambient temperature (20–23 °C), while the samples that were stored within the closed autosampler at 4 °C were stable up to 24 h (Dolder et al. 2018).

To close, the higher concentrations of LSD metabolites and their greater stability improve the detection of parent compound, increasing detection time. Therefore, it is preferable the simultaneous analysis of LSD and its metabolites (Skopp et al. 2002).

Conclusion and future perspectives

The popularity of hallucinogens has not been constant in the drug community over time. Indeed, these compounds remain a target for those looking for psychedelic experiences, mostly young people. Nowadays, LSD and other hallucinogens have been applied to treatment of various psychic disorders as therapeutic aids. However, the persistent use of psychedelic substances can lead to serious psychologic consequences, although these compounds have not been directly responsible for causing death (Nichols 2016).

In this work, the metabolism of LSD was reviewed. LSD is extensively metabolized into inactive metabolites and only very little of unchanged LSD is excreted (Passie et al. 2008; Dolder et al. 2017; Steuer et al. 2017; Dolder et al. 2018). Urine samples from LSD users indicated that O-H-LSD seems to be the major metabolite in urine and can be detected a few days after LSD ingestion. Since blood concentrations are relatively low, LSD analysis is often performed in urine, although, recent studies have validated methods in plasma samples (Steuer et al. 2017). The identification of new metabolites can be helpful to prove consumption in a wider detection window. For the first time, LSD and its metabolite O-H-LSD were detected in vitreous humor, which can be an advantage in forensic determination of *postmortem* LSD levels (Favretto et al. 2007). LSD was also detected in human hair from drug abusers (Nakahara et al. 1996; Rohrich et al. 2000; Jang et al. 2015). Therefore, the analysis of new biological specimens is a step forward.

There is an obvious need to increase the sensitivity of method employed in order to overcome the low concentrations of the analytes as well as an improved understanding of LSD metabolism.

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No writing assistance was utilized in the production of this manuscript.

ORCID

Rui Filipe Libânio Osório Marta http://orcid.org/0000-0002-1263-2393

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