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Research paper

Evaluation of the transport, *in vitro* metabolism and pharmacokinetics of Salvinorin A, a potent hallucinogen

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ABSTRACT

Salvinorin A is an unregulated potent hallucinogen isolated from the leaves of Salvia divinorum. It is the only known non-nitrogenous kappa-opioid selective agonist, and rivals synthetic lysergic acid diethylamide (LSD) in potency. The objective of this study was to characterize the *in vitro* transport, *in vitro* metabolism, and pharmacokinetic properties of Salvinorin A. The transport characteristics of Salvinorin A were assessed using MDCK-MDR1 cell monolayers. The P-glycoprotein (P-gp) affinity status was assessed by the P-gp ATPase assay. *In vitro* metabolism studies were performed with various specific human CYP450 isoforms and UGT2B7 to assess the metabolic characteristics of Salvinorin A. Cohorts ($n = 3$) of male Sprague Dawley rats were used to evaluate the pharmacokinetics and brain distribution of Salvinorin A (10 mg/kg, intraperitoneal (i.p.) over a 240-min period. A validated UV-HPLC and LC/MS/MS method was used to quantify the hallucinogen concentrations obtained from the *in vitro* and *in vivo* studies, respectively. Salvinorin A displayed a high secretory transport in the MDCK-MDR1 cells ($4.07 \pm 1.34 \times 10^{-5}$ cm/s). Salvinorin A also stimulated the P-gp ATPase activity in a concentration (5 and 10 μM)-dependent manner, suggesting that it may be a substrate of (P-gp). A significant decrease in Salvinorin A concentration ranging from 14.7 ± 0.80% to 31.1 ± 1.20% was observed after incubation with CYP2D6, CYP1A1, CYP2C18, and CYP2E1, respectively. A significant decrease was also observed after incubation with UGT2B7. These results suggest that Salvinorin A maybe a substrate of UGT2B7, CYP2D6, CYP1A1, CYP2E1, and CYP2C18. The *in vivo* pharmacokinetic study showed a relatively fast elimination with a half-life ($t_{1/2}$) of 75 min and a clearance (Cl/F) of 26 L/h/kg. The distribution was extensive (Vd of 47.1 L/kg); however, the brain to plasma ratio was 0.050. Accordingly, the brain half-life was relatively short, 36 min. Salvinorin A is rapidly eliminated after i.p. dosing, in accordance with its fast onset and short duration of action. Further, it appears to be a substrate for various oxidative enzymes and multi-drug resistant protein, P-gp.

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1. Introduction

Psychotropic natural products are widely available through a variety of commercial sources; however, they represent a class of agents that are understudied and possibly toxic and possess pharmacologic properties consistent with drug abuse liability. Salvinorin A (Fig. 1), active component of Salvia divinorum, is a potent hallucinogen whose use is associated with "altered consciousness", and its status in this country and abroad has been under review. This substance has been banned in five states (Delaware, Louisiana, Missouri, Oklahoma, and Tennessee), and two states (New Jersey and New York) are currently formulating legislature on Salvia divinorum. Other countries such as Australia, Denmark, Finland, Italy, and Sweden have recently classified Salvia divinorum as a controlled substance [1–5]. Reports have predicted that its use will most likely reach the levels associated with similar hallucinogenic agents such as 3,4-methylenedioxy methamphetamine (MDMA), phencyclidine (PCP), and lysergic acid diethylamide (LSD) in the next 5–10 years [6,7]. This is evidenced by a sharp increase in its consumption by college students and young adults over the last few years [3,6–10].

Salvia divinorum is typically consumed by smoking a quantity of dried leaves, although buccal absorption by chewing dried leaves or ingesting a tincture is also used. The onset of action is

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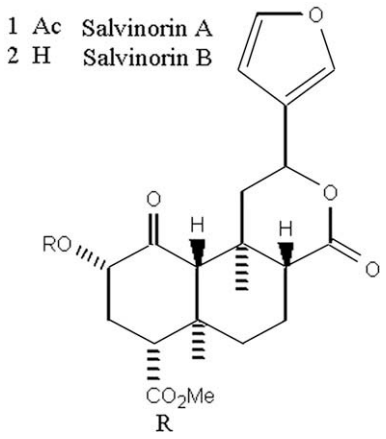

Fig. 1. Chemical structures of Salvinorin A and B.

relatively rapid, on the order of 30 s for smoking and 5–10 min for buccal absorption after ingestion [2,11]. Salvinorin A is an extremely potent naturally occurring hallucinogen, with an effective dose, when smoked, of 200–500 μg [2,12]. It induces an intense, short-lived hallucinogenic experience in humans appearing in less than 1 min and lasting for 15 min or less [13]. Its effect is reported to be qualitatively distinct from that induced by the classical hallucinogens such as LSD, psilocybin and mescaline. Salvinorin A is a neoclerodane diterpene (Fig. 1), and chemically, it lacks a basic nitrogen structure which is an uncommon feature associated with psychoactive hallucinogenic agents; however, it does have a number of carbon atoms that enhance its lipophilicity.

Pharmacologically, Salvinorin A does not act at the molecular target responsible for the actions of classic hallucinogens, the serotonin 5-HT2A receptor [14,15]. Salvinorin A is selective for the κ opioid receptor (KOR) [16,17], and produces KOR-like discriminative effects in rhesus monkeys [18], low dopamine levels in the mouse caudate putamen [19], and rat nucleus acumbens [20] through activation of KORs. Presently, limited research has been conducted to characterize Salvinorin A. It is suggested that Salvinorin B (Fig. 1) is an inactive metabolite of Salvinorin A [21,22] and that it possibly shares metabolic pathway(s) with cocaine, heroin, tetrahydrocannabinol (THC) and MDMA and is metabolized by esterase in the blood [23]. In addition, a pharmacokinetic study in rhesus monkeys found that its elimination half-life is rapid (56.6 ± 24.8 min), [24] which corresponds with its short duration of action. Recently, positron emission tomography (PET) studies performed in baboons indicated extremely rapid brain uptake. [^{11}C]-Salvinorin A was distributed throughout the brain with the highest concentration in the cerebellum and a notable concentration in the visual cortex perhaps accounting for its physiological effects when smoked [25].

To date, there are several investigations underway to evaluate Salvinorin A's pharmacologic properties; however, its blood–brain barrier (BBB) transport, metabolism, and pharmacokinetics have not been described well. As such, the rate and extent of its distribution across the BBB into the central nervous system (CNS) responsible for producing hallucinogenic effects are unknown as are those dispositional properties that mediate its duration of action. To investigate these, the following three objectives were pursued: (1) assessment of the *in vitro* transport mechanism of Salvinorin A across MDCK-MDR1 cell monolayers, (2) characterization of the *in vitro* metabolism of Salvinorin A using recombinant human CYP450 (InVitroSomes™) and UGT2B7 enzyme (Supersomes™ membrane fractions from insects cells expressing UDP-glucurosyltransferases (UGT) isoform), and (3) evaluation of the single-dose pharmacokinetics of Salvinorin A in male Sprague Dawley rats.

2. Materials and methods

2.1. Materials

Salvinorin A was provided by Dr. Thomas Prisinzano (Iowa University, Iowa). 4-Chlorobenzotropine (BZT) was synthesized and provided by Dr. Amy H. Newman (NIH, Baltimore, MD). The purities of Salvinorin A and BZT were >98%. All chemicals and solvents were of American Chemical Society analytical grade or HPLC grade. InVitroSomes™, human recombinant cytochrome P450 enzymes, were purchased from InVitro Technologies (Baltimore, MD). Human UGT2B7 Supersomes™ enzymes were purchased from BD Biosciences Discovery Labware (Woburn, MA). MDCK-MDR1 cells were provided by Dr. Peter W. Swaan (University of Maryland). DMEM, phosphate buffered saline, non-essential amino acid, fetal bovine serum (FBS), L-glutamine, penicillin G-streptomycin sulfate antibiotic mixture and trypsin (0.25%)–EDTA (1 mM) were purchased from Invitrogen Laboratories (Carlsbad, CA). Polymyxin, amphotericin, heparin, and dextran were purchased from the Sigma Chemical Co. (St. Louis, MO). Twelve-well transport plates (cell culture treated) were purchased from Corning Costar (Cambridge, MA).

2.2. Salvinorin A-stimulated P-gp ATPase activity

In order to assess whether Salvinorin A was a P-gp substrate, we determined its ability to stimulate ATPase activity. Salvinorin A-stimulated P-gp ATPase activity was estimated by Pgp-GIO assay system (Promega, Madison, WI). This method relies on the ATP dependence of the light-generating reaction of firefly luciferase where ATP consumption is detected as a decrease in luminescence. In a 96-well plate, recombinant human P-gp was incubated with P-gp-GIO assay buffer™ (20 μL), verapamil (200 μM), sodium orthovanadate (100 μM), and Salvinorin A (2.5–100 μM). Each compound was loaded into four individual wells. Verapamil served as a positive control, while sodium orthovanadate was used as a P-gp ATPase inhibitor. In the presence of sodium orthovanadate, ATP consumption by P-gp is negligible, and without sodium orthovanadate, P-gp consumes ATP to a greater or lesser extent than the control, which is dependent on the effect of the test compounds. The reaction was initiated by the addition of MgATP (10 mM), stopped 40 min later by the addition of 50 μL of firefly luciferase reaction mixture (ATP detection reagent) that initiated an ATP-dependent luminescence reaction. Signals were measured 100 min later by Lmax® luminometer (Molecular Devices Corporation, Sunnyvale, CA), and were converted to ATP concentrations by interpolation from a luminescent ATP standard curve. The rate of ATP consumption (pmol/min/μg protein) was determined as the difference between the amount of ATP in the absence and presence of sodium orthovanadate. Salvinorin A-stimulated P-gp ATPase activity was reported also as fold-stimulation relative to the basal P-gp ATPase activity in the absence of the compound (control).

2.3. MDCK-MDR1 cells

MDCK-MDR1 cells were cultured in Dulbecco's modified eagle serum (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/mL of penicillin and streptomycin. The cells were plated onto 12-well Costar Transwell® inserts (0.4 μM pore size, 1 cm^2 surface area) at a density of 425,000 cells/cm^2. The cells were cultured and maintained in DMEM supplemented with 10% FBS, 2% L-glutamine, 1% non-essential amino acid, 1% penicillin-streptomycin under standard conditions of 5% CO_2, 37 ± 0.5 °C and 95% humidity until confluence was reached on day four. The medium was changed every day after seeding, and confluent monolayers were used for transport studies outlined below. Monolayer integrity was checked by measuring the transepithelial resistance (TEER), and