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Pharmacokinetics and tissue distribution of *N*-(2-hydroxyphenyl)-2-propylpentanamide in Wistar Rats and its binding properties to human serum albumin

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ABSTRACT

N-(2-hydroxyphenyl)-2-propylpentanamide (HO-AAVPA) is a novel valproic acid derivative that has shown anti-proliferative activity against epitheloid cervix carcinoma (HeLa), rhabdomyosarcoma (A204), and several breast cancer cell lines. The aim of this research was to evaluate the pharmacokinetic profile and tissue distribution of HO-AAVPA in Wistar rats, as well as its human serum albumin binding potential by experimental and *in silico* methods. A single dose of HO-AAVPA was given to male rats by intravenous, intragastric or intraperitoneal routes at doses of 25, 100, and 100 mg/kg, respectively. Then, blood samples were drawn at predetermined intervals of time, and the HO-AAVPA concentration in the plasma was quantified with a validated HPLC method. The elimination half-life ($t_{1/2}$) was approximately 222 min, and the systemic clearance (CL) and apparent volume of distribution (Vd) were 2.20 mL/min/kg and 0.70 L/kg, respectively. The absolute oral bioavailability of HO-AAVPA was 33.8%, and the binding rate of HO-AAVPA with rat plasma proteins was between 66.2% and 83.0%. Additionally, *in silico*, UV and Raman spectroscopy data showed weak interactions between the test compound and human serum albumin. Thus, the results that were obtained demonstrated that despite its low oral bioavailability, the potential anticancer agent HO-AAVPA exhibits acceptable pharmacokinetic properties that would allow it to reach its site of action and exert its pharmacological effect in Wistar Rats, and it has a convenient profile for future assays to evaluate its human applications.

1. Introduction

Valproic acid (VPA) is a branched short-chain fatty acid (2-propyl pentanoic acid) and is considered one of the four first-line antiepileptic drugs that are used in the treatment of primary generalized, partial or myoclonic seizures [1–3]. VPA acts as a cytotoxic agent, and it has been evaluated as an anticancer agent either alone or in combination with other drugs in clinical trials (phases I and II) [4,5]. The mechanism by which VPA inhibits tumor growth is asso-

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ciated with its capacity to inhibit the catalytic activity of histone deacetylases (HDACs) [6,7] and to induce their degradation [8,9].

Unfortunately, several problems have been observed when VPA is administered to humans; one of the main disadvantages of VPA is its narrow therapeutic window and at concentrations higher than 125 mg/L toxic side effects can appear, including nausea, vomiting, diarrhea, tremor and thrombocytopenia, and concentrations higher than 175–200 mg/L can cause encephalopathy, bone marrow suppression, teratogenicity [10], and hepatotoxicity, and these effects appear to be the most severe in young children [11].

The second problem concerning the use of VPA in humans is its pharmacokinetic characteristics, as studies in humans have shown that VPA has a nonlinear and single-compartment pharmacokinetic profile, has a variable half-life with a range of 4–17 h and an average half-life of 10–12 h. VPA binds highly to plasma proteins (87–95%), and it is primarily metabolized in the liver through glucuronic conjugation, degradation by cytochrome P450 s and mitochondrial β-oxidation, and it has been shown that VPA inhibits several microsomal enzymes and the hepatic metabolism of other drugs, which leads to a high probability of various drug interactions [12].

Due to the limited efficacy and pharmacokinetic disadvantages of VPA, many derivatives have been developed to improve its anticonvulsant and anticancer efficacies as well as to reduce the toxic effects that are associated with VPA [13,14]. Our research group designed several VPA arylamide derivatives, obtaining a compound *N*-(2-hydroxyphenyl)-2-propylpentanamide (HO-AAVPA), which showed greater antiproliferative activity than VPA in cervical cancer (HeLa) cells, as well as in cancer cells of rhabdomyosarcoma (A204) and the breast (SKBR3, MCF-7, and MDA-MB-231) [15]. In addition, HO-AAVPA has not been shown to be hepatotoxic in Wistar rats [16].

Using rational drug design, we sought to generate a molecule with better pharmacokinetic properties than VPA, and thus we raised the hypothesis that the chemical modifications that were done on VPA to yield the HO-AAVPA molecule could favor either the binding or release from human serum albumin, increasing its half-life and tissue distribution, allowing for a more suitable oral bioavailability. Therefore, the present study aimed to evaluate the pharmacokinetic profile and tissue distribution of this VPA derivative by experimental assays in rats, as well as its binding properties with human serum albumin (HSA) using *in silico* and *in vitro* methods.

2. Materials and methods

2.1. Drugs

HO-AAVPA was synthesized in our laboratory. Its purity was determined using high-performance liquid chromatography (HPLC), and the compound was used as the reference standard due to its high purity (99.2%). The molecular structure of HO-AAVPA was characterized using Fourier Transform Infrared Spectroscopy (FT-IR), Nuclear Magnetic Resonance of ^{1}H and ^{13}C (NMR ^{1}H and ^{13}C), Mass Spectrometry (MS), and X-ray Diffraction (XRD) [15; patent request MX/a/2013/002766]. These analyses were conducted at the Centro de Nanociencias y Micro y Nanotecnología-IPN (www.nanocentro.ipn.mx).

2.2. Chemicals and reagents

HPLC-grade acetonitrile and HPLC-grade water were purchased from Tecsiquim, S.A. de C.V. (Toluca, México), and acetic acid (analytical grade), polysorbate 80 (tween) and propylene glycol were purchased from Sigma-Aldrich (St. Louis, MO, USA). The anesthetics ketamine (CLORKETAM®) and xylazine (PROCIN®) were for veterinary use and were acquired from Vétokinol (Lure Cedex, France) and PISA Agropecuaria, S.A. de C.V. (Hidalgo, México), respectively. Heparin (1000 UI/mL) and 0.9% sodium chloride were purchased from PISA Farmacéutica, S.A. de C.V. (Jalisco, México). For the HSA binding assay by dialysis, the propanone, sodium phosphate, sodium chloride and fatty acid free human serum albumin (HSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and the 20 KDa dialysis membrane was purchased from Millipore (Massachusetts, USA).

2.3. HO-AAVPA formulation

100 mg of HO-AAVPA were dissolved in 10 mL of a mixture consisting of 80% propylene glycol-polysorbate and 0.9% sodium chloride at a ratio of 9:1:90, (v/v/v), respectively. The formulation was a clear and slightly yellow solution. The solution was vortexed for 3 min and was sterilized by filtration before it was passed through a nylon syringe filter of 0.22 μm, after which the final concentration was 10 mg/mL. This solution was used for intragastric (i.g.), intravenous (i.v.), and intraperitoneal (i.p.) administrations.

2.4. Animals

Male Wistar rats with weights of 300 ± 20 g were obtained from the Bioterio of the Escuela Superior de Medicina-IPN. Rats were allowed to acclimatize one week before the experimental assays and were fed with Rat Chow 5012 (Purina) and water *ad libitum*. For the pharmacokinetics studies, the animals were randomly divided in three groups (n = 6). For the tissue distribution study, the rats were randomly distributed in three groups (n = 4). Rats were fasted for 8 h before dosing but allowed free access to water.

2.4.1. Ethics statement

The animal procedures were conducted in accordance with the Mexican Official Standard NOM-062-ZOO-1999, Technical Specifications for Production, and Care and Use of Laboratory Animals. The animal protocol was approved by the Research Committee for the Care and Use of Laboratory Animals (CICUAL) of the Escuela Superior de Medicina-IPN (Approval number: ESM.CICUAL-02/27-07-2015).

The experiments were finished once the experimental endpoint had been met. However, all animals were monitored for early indicators of a human-relevant endpoint [17]. After the collection of samples, the animals were sacrificed with 72 mg/kg of sodium pentobarbital *via* intraperitoneal injection.

2.5. Instrumentation and analytical conditions

The analyses were carried out in an HPLC device from Agilent 1260 Infinity Series (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump delivery system, robotic autosampler, column thermostat, and a multiwavelength UV detector. The results were analyzed using OpenLab CDS EZChrom. A Zorba x 5 μm SB-C18 column (4.6 mm x 150 mm, Agilent Technologies, Palo Alto, CA, USA) was used for separation; the column temperature was 25 °C and UV detection was measured at 242 nm, and the injection volume was 40 μL. The mobile phase was composed of a mixture of (A) 0.2% (v/v) acetic acid in water, pH 3.0 and (B) acetonitrile, at a ratio of 40% A and 60% B. The total analysis time of each sample was 16 min using an isocratic elution.

2.6. Preparation of calibration standards and quality control (QC) samples

Six stock solutions (1.0 mg/mL) were prepared by dissolving the appropriate weight of the compound in a mixture of acetonitrile-