Fitoterapia 86 (2013) 193–201

Contents lists available at SciVerse ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Evaluation of the pharmacodynamics and pharmacokinetics of brucine following transdermal administration

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ARTICLE INFO

Article history:
Received 31 December 2012
Accepted in revised form 23 February 2013
Available online 15 March 2013

Keywords:
Brucine
Transdermal
Pharmacodynamics
Pharmacokinetics
Tissue distribution

ABSTRACT

Before the design of brucine-containing transdermal formulations, the pharmacodynamics and pharmacokinetics of brucine following transdermal administration should be evaluated. In this study, the effect of addition of ethanol on solubility of bruicne was investigated and 20% ethanol was added into PBS to obtain 10 mg/mL brucine solution. Then three transdermal doses (10, 20 and 40 mg/kg) were administered to mice to evaluate pharmacological activity. It had been demonstrated that brucine possessed analgesic and anti-inflammatory activity in a dose-dependent manner. Cytotoxicities of brucine against various tumor cells including skin tumor cell were also compared *in vitro*. Brucine was found to possess antitumor activity in a concentration and time-dependent manner and gastrointestinal tumor cells seemed to be more sensitive to brucine. Then *in vitro* skin permeation behavior and *in vivo* pharmacokinetics following transdermal administration were further investigated. The cumulative amounts of brucine across mouse skin *in vitro* were found to be higher than 90%. The absolute bioavailability of brucine was determined to be 40.83%. And compared with intravenous administration, MRT and $T_{1/2}$ values were increased about 8 ~ 12-fold by transdermal route. Moreover, fluctuations of drug levels were found to be significantly decreased in tissues, especially in brain. Finally, no dermal toxicity of brucine was observed. The results of this study indicated that transdermal administration might be beneficial for the sustained efficacy and reduced toxicity of brucine.

1. Introduction

Strychnos nux-vomica L. (Loganiaceae) is a deciduous tree that grows in tropical areas and is distributed throughout India and Southeast Asia. The dried seed of this plant, *Semen Strychni* (previous known as *Nux Vomcia*), has been applied clinically in Chinese medicine for hundreds of years. As a major ingredient, *Semen Strychni* has been frequently prescribed in many prescriptions of traditional Chinese medicine. In addition, more than 60 formulations containing *Semen Strychni* have been reported in the literatures of the Indian system of medicine [1].

The main bioactive constituents of *Semen Strychni* are known to be alkaloids, responsible for both the pharmacological and toxic properties possessed by the seed. In our

Abbreviations: AUC, the area under the plasma concentration-time curve; C_{max}, peak plasma concentration; Cl, the total body clearance; DDH, diclofenac diethylamine hydrogel; HE, hematoxylin-eosin; IS, internal standard; LLOQ, the lower limit of quantification; MRT, the mean residence time; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide; NS, Normal saline; PBS, phosphate buffer solution; PIE, percentage of the inhibition of edema; PP, the percentage protection; RE, relative error; RSD, relative standard deviation; QC, quality control; $T_{1/2}$, the terminal elimination half-life; T_{max}, the corresponding time of peak plasma concentration; V_{ss}, the apparent volume of distribution at steady state.

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http://dx.doi.org/10.1016/j.fitote.2013.03.007

previous study, a total of 16 alkaloids have been separated and identified from *Semen Strychni* [2], among them strychnine and brucine (Fig. 1.) take up about 70%.

Strychnine, the most abundant alkaloid of *Semen Strychni*, is highly toxic to humans and most domestic animals. Israeli authorities have prohibited its use, as have Britain and the European Union [3]. Moreover, strychnine had been proved to possess little therapeutic value with respect to analgesic and anti-inflammatory activity. And the antitumor activity of strychnine was also significantly lower than that of brucine [4].

Brucine, originally isolated from *Semen Strychni* in 1819, is a white, odorless, crystalline solid with a molecular weight of 394.45. In contrast with strychnine, brucine, the second abundant alkaloid constituent of *Semen Strychni*, is much less toxic. Following i.p. administration, the LD_{50} values of strychnine and brucine were determined to be 1.10 and 50.10 mg/kg, respectively [5]. Brucine had been proved to be mainly responsible for the analgesic and anti-inflammatory effect produced by *Semen Strychni* [6].

Brucine was also indentified as the main component responsible for the anti-tumor effect of *Semen Strychni*. It had been demonstrated that brucine exhibited the strongest inhibitory effect on human hepatoma cell proliferation compared with other alkaloids from *Semen Strychni* [4]. Further study revealed that Ca^{2+} and Bcl-2 mediated mitochondrial pathway were involved in brucine-induced tumor cell apoptosis [7]. However, the comparison of cytotoxicities among different tumor cells should be further investigated.

Skin, the largest organ of human body, can offer many advantages as a route for drug administration. For transdermal drug delivery of systemically acting drugs, the advantages include avoidance of hepatic first-pass metabolism, decrease of fluctuations in drug plasma levels and good patient compliance. Different formulations have been prepared for the transdermal delivery of brucine [8,9]. However, the pharmacodynamics and pharmacokinetics of brucine following transdermal administration should be intensively evaluated before the design of drug delivery system.

2. Materials and methods

2.1. Chemicals and reagents

Reference substances of brucine and the internal standard (IS) strychnine were supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Brucine was obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). HPLC grade acetonitrile was purchased from Tedia (Fairfield, OH, USA). Distilled water, prepared from demineralized water, was used throughout the experiment. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide (MTT) was also purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA); All the other chemicals were purchased from Nanjing Chemical Reagent Corporation (Nanjing, China) and of analytical grade. The diclofenac diethylamine hydrogel (DDH) was used as the positive drug and obtained from Wuhan Mayinglong Pharmaceutical Group Co., Ltd. (Wuhan, China).

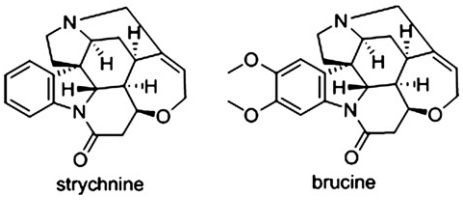

Fig. 1. Chemical structure of strychnine (A) and brucine (B).

2.2. HPLC analysis

All analytical procedures were performed using an Agilent 1100 HPLC-UV system (Agilent Company, USA) consisting of a G1312A pump, G1313A autosampler and a G1315A DAD detector. Chromatographic separation was achieved on a Kromasil C_{18} analytical column (4.6 mm × 250 mm, 5 μm, Kromasil, Sweden) coupled with a C_{18} guard cartridge (4.6 mm × 10 mm, 5 μm, Hanbang, China), maintained at 35 °C. The mobile phase consisted of 21% acetonitrile and 79% buffer (isometric mixture of 0.01 mol/L sodium heptane sulfonate and 0.02 mol/L potassium dihydrogen phosphate, the pH value was adjusted to 2.8 with 10% phosphoric acid), which was selected according to the Pharmacopoeia of the People's Republic of China. The prepared mobile phase was filtered using a vacuum filter system equipped with 0.45 μm filter and was delivered at a flow rate of 1.0 mL/min. The detection wavelength was set at 264 nm.

2.3. Animals

ICR mice (18 ~ 22 g, male and female) and Hartley guinea pigs (250 ~ 280 g, male) were obtained from the Slac Experiment Animal Co, Ltd (Shanghai, China). They were housed in plexiglass cages at 22 ± 2 °C, relative humidity 55 ± 5% with 12 h light/12 h dark cycle and provided with standard pellet diet with tap water ad libitum. The animal experiments were performed in accordance to the Principles of Laboratory Animal Care and Use in Research (Ministry of Health, Beijing, China). The protocols of animal experiments were approved by the Animals Ethics Committee of Nanjing University of Chinese Medicine. They were fasted overnight before experiments.

2.4. Preparation of brucine solution

A solubility test of brucine was performed in various solvents with different ethanol concentration. Briefly, 2 mL of solvent was taken in cap tubes containing excess brucine powder. The samples were mixed, shaken and incubated in a water bath at 37 °C for 48 h. After centrifugation and dilution with methanol, the concentration of brucine was quantified by the above-mentioned HPLC analysis method.

2.5. Pharmacodynamic evaluation

2.5.1. Acetic acid-induced writhing response

Briefly, 50 mice were randomly divided into the control group (20% ethanol in PBS), the positive group (DDH, 24 mg/kg), the high dose group (brucine solution, 80 mg/kg), the medium dose group (brucine solution, 40 mg/kg) and the low dose group (brucine solution, 20 mg/kg). The abdomens of mice