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Human microsomal cyttrochrome P450-mediated reduction of oxysophocarpine, an active and highly toxic constituent derived from *Sophora flavescens* species, and its intestinal absorption and metabolism in rat

Lili Wu [a,1], Wanping Zhong [a,1], Junjin Liu [a], Weichao Han [a], Shilong Zhong [b], Qiang Wei [c], Shuwen Liu [a,c,*], Lan Tang [a,c,*]

[a] Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China
[b] Medical Research Center of Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong 510080, China
[c] State Key Laboratory of Organ Failure Research, Guangdong Provincial Institute of Nephrology, Southern Medical University, Guangzhou, China

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ABSTRACT

Oxysophocarpine (OSC), an active and toxic quinolizidine alkaloid, is highly valued in *Sophora flavescens* Ait. and *Subprostrate sophora* Root. OSC is used to treat inflammation and hepatitis for thousands of years in China. This study aims to investigate the CYP450-mediated reduction responsible for metabolizing OSC and to evaluate the absorption and metabolism of OSC in rat in situ. Four metabolites were identified, with sophocarpine (SC) as the major metabolite. SC formation was rapid in human and rat liver microsomes (HLMs and RLMs, respectively). The reduction rates in the liver are two fold higher than in the intestine, both in humans and rats. In HLMs, inhibitors of CYP2C9, 3A4/5, 2D6, and 2B6 had strong inhibitory effects on SC formation. Meanwhile, inhibitors of CYP3A and CYP2D6 had significant inhibition on SC formation in RLMs. Human recombinant CYP3A4/5, 2B6, 2D6, and 2C9 contributed significantly to SC production. The permeability in rat intestine and the excretion rates of metabolites were highest in the duodenum ($p < 0.05$), and the absorbed amount of OSC in duodenum and jejunum was concentration-dependent. The metabolism could be significantly decreased by CYP3A inhibitor ketoconazole. In conclusion, the liver was the main organ responsible for OSC metabolism. First-pass metabolism via CYP3A4/5, 2B6, 2D6, and 2C9 may be the main reason for the poor OSC bioavailability.

1. Introduction

Oxysophocarpine (OSC) and sophocarpine (SC), which are major active and toxic quinolizidine alkaloids, are highly valued and are important in traditional Chinese medicine (TCM). These TCMs include *Sophora flavescentis* radix (Kushen) and *Subprostrate sophora* Root (Shandougen), which are widely distributed in Asia, Oceanica, and the Pacific islands. These medicines are commonly used for the treatment of inflammation, hepatitis, and cardiac diseases for thousands of years in China. In recent years, they have held impressed attraction in the scientific field because of their neuroprotective, anti-viral, anti-hepatitis B virus, antioxidant, cardioprotective, and anti-tumor activities [33]. "Kushen" injection and capsule, derived from the TCM and mainly composed of Kushen, had been clinically used in China for the treatment of chronic hepatitis or oligoleukocythemia caused by liver cancer. In addition, Kushen was used to improve liver function and ameliorated the degree of liver injury.

Quinolizidine alkaloids, which have powerful and wide pharmacological activities, have been proven to be the main bioactive components in *S. flavescens*. OSC showed significant anti-HBV activity with inhibitory potency against HBsAg secretion [4] and has significant neuroprotective effects that can be attributed to the inhibition of endoplasmic reticulum stress-induced apoptosis [33]. OSC administration is also suggested to have anti-nociceptive effects on the central and peripheral nervous systems [27]. SC is an effective agent for treating colonic inflammation; SC can significantly decrease myeloperoxidase activity and ameliorate dextran sulfate sodium induced colitis by regulating the production of anti-inflammatory cytokines IL-6 and IL-1 [26].

Our research has demonstrated that the absolute bioavailability of oxymatrine, having a similar structure with OSC, was only 6.79 ± 2.52%. About 50% of OSC was converted to its active metabolite SC in vivo; hence, the absolute bioavailability of OSC was speculated to

* Corresponding authors at: Department of Pharmaceutics, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China.
E-mail addresses: liusw@smu.edu.cn (S. Liu), tl405@smu.edu.cn (L. Tang).
[1] Lili Wu and Wanpin Zhong contribute equally to this paper.

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be poor as well [24]. Understanding the disposition of OSC is the first step toward solving a major challenge associated with OSC development. The fast biotransformation of OSC in vivo caused us to hypothesize that first-pass metabolism was the main reason why only 50% OSC was quantifiable in rat plasma in vivo, with substantial amount of SC found in the plasma. Despite that the basic information on bio-efficacy of OSC and SC is readily accessible; the available information on the characterization of the metabolism mechanism is poorly understood. Researchers have recently reported that the formation of SC was rapid. The AUC value of SC was about the same as that of OSC, thus suggesting that SC could also play an important role in the pharmacological action of orally administered OSC [24,29].

In another study, oxymatrine was metabolized into six metabolites as a result of a phase I reaction, including OSC and SC in rats [2]. The available information appears to indicate that OSC may also undergo phase I metabolism, the likely major pathway for its elimination, which is mediated mainly by cytochrome P450 (CYP) enzymes. CYP450 enzymes are significant phase-I drug-metabolizing enzymes used in xenobiotic metabolism and detoxification. They are also involved in the metabolism of more than 90% of all currently available drugs. Up to 90% of human drug metabolism may be attributed to six main enzymes, namely, CYP1A2, 2C8, 2C19, 2D6, 2E1, and 3A4/5. The increased polarity of drugs via CYP metabolism results in their increased water solubility and rapid elimination from the body. However, CYP, which forms a part of the microsomal mixed-function oxidase system, has also been shown to participate in the reduction of several drugs. Particularly, under anaerobic conditions, CYP has been shown to function in a reductive rather than in an oxidative manner. Examples of substrate reduction include halogenated alkanes, triarylmethanes, nitro compounds, azodyes, and NAPQIs. Preliminary evidence has also suggested the involvement of CYP in the reductive bioactivation of quinones, such as danthron and 1-pipenidinoanthraquinone; CYP2B1 was involved in the one-electron reduction of adriamycin, and nitrofurantoin is metabolized through the CYP450 system [7].

The liver is considered a major site of metabolism. Thus, the focus of our study was on liver metabolism, along with some disposition studies in the rat intestine. In the current study, the phase I metabolism of OSC in rat liver and human liver microsomes was investigated as well as in recombinant CYP450 enzymes. Selective chemical inhibitors were used, along with liver and intestine microsomes from human and rat, to determine the CYP isoforms that contributed to OSC metabolism. Moreover, the absorption of OSC should be considered, and the possible mechanism should be clarified because of poor bioavailability and OSC disposition, which is very important and urgent to its clinical application. A rat intestinal perfusion model was used for the investigation of absorption and metabolism.

Taken together, this investigation aims to identify the reasons for OSC's poor bioavailability, to represent a detailed and systematic study of the cytochrome P450-mediated reduction of OSC, and to characterize the evaluation of absorption and metabolism in situ and in vitro. The current investigation will light up a safe and clinical application of OSC.

2. Materials and methods

2.1. Materials

OSC and sophocarpine (≥98%, HPLC grade) were purchased from Chengdu Wheat Kashi Chemical Company (Chengdu, China). HEPES, sodium bicarbonate, sodium chloride, glucose, formic acid and Hanks' Balanced Salts were purchased from Sigma-Aldrich (St Louis, MO, USA). NADPH regenerating System-Solution A, NADPH regenerating System-Solution B, male human liver microsomes (HLM), male human intestinal microsomes (HIM) and nine types of supersomes isoforms (Human CYP3A4 + reductase + b5, CYP2E1 + reductase + b5, CYP2C9 + reductase + b5, CYP2C8 + reductase + b5, CYP2B6 + reductase + b5, CYP2C19 + reductase + b5, CYP3A5 + reductase + b5, CYP1A2 + reductase + b5, CYP2D6 + reductase + b5) were purchased from BD Bioscience (Woburn MA). Male rat liver and intestinal microsomes were prepared at Southern Medical University (Guangzhou, China). Testosterones, urethane, α-naphthoflavone, gemfibrozil, aimodarone hydrochloride, omeprazole, quinidine, ketoconazale, chlorpromazine hydrochloride, and saquinavir were purchased from Aladdin Industrial Corporation (Shanghai, China). Acetonitrile and methanol were typically analytical grade or better, and were used as received.

2.2. Animal surgery and rat liver/intestinal microsome preparation

The animal experiments were permitted by Ethics Committee of Southern Medical University. Male Sprague–Dawley rats weighing between 180 and 220 g were obtained from laboratory animal center of Southern Medical University. The rats were fasted overnight with free access to water before the date of the experiment.

The preparation of rat liver and intestinal microsome was conducted from a previously published method [20]. Microsomes are stored at −80 °C until use.

The rats were anesthetized with an i.p. injection of 2.8 mL/kg urethane (50%, w/v). During the surgery, the body temperature was maintained at 37 °C by a heating lamp or an electric blanket. The intestinal surgical procedures were modified from those described previously [12,13]. We perfused four segments of intestine, and each segment was 10 cm long. The blood circulation to the liver and intestine was not disrupted in this model. The inlet cannulate was insulated and flushed with warm OSC in HBSS, which was kept warm at 37 °C by a circulating water bath.

2.3. Perfusion experiments

Four segments of rat intestine, duodenum, upper jejunum, terminal ileum, and colon were perfused simultaneously with a perfusate containing OSC (25, 50 and 100 μM) using an infusion pump (model PHD2000; Harvard Apparatus, Cambridge, MA) at a flow rate of 10 mL/h. OSC at concentrations of 100 μM was chosen because when it was perfused into rat intestine for 2.5 h, the total amount of OSC for 4 segments was about 2.4 mg, which was less than the amount used (15 mg/kg) in the literature [24]. After 30 min washout period, four samples were collected from the four outlets cannulate every 30 min. We measured the intestinal length after perfusion as described [12,13].

2.4. Metabolism of OSC in phase I reaction systems in HLMs and RLMs

The incubation procedures for the CYP reaction using human liver microsomes or recombinant CYP were the same as those published previously [11] under anaerobic conditions (the reaction system was full of nitrogen before started). All incubations were performed in triplicate. For kinetic profiling of OSC with HLM and RLM, substrate concentrations in the range of 5–800 μM were used.

2.5. CYP450 inhibition experiments in RLMs and HLMs

The effects of specific chemical inhibitors for individual CYP enzymes on OSC metabolism were investigated in RLMs and HLMs. These inhibitors of specific CYP enzymes were α-naphthoflavone (CYP1A2) [6,16], gemfibrozil (CYP2C8) [6,16], amiodarone hydrochloride (CYP2C9) [1,6], omeprazole (CYP2C19) [6,16], quinidine (CYP2D6) [16], cimetidine (CYP2C6), clopidogrel (CYP2B6), chlorpromazine (CYP2A), DDC (CYP2E1), saquinavir (CYP3A4) and ketoconazole (CYP3A) [16] which were pre-incubated for 5 min with microsomes