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Concentration-dependent inhibitory effects of baicalin on the metabolism of dextromethorphan, a dual probe of CYP2D and CYP3A, in rats

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ABSTRACT

Baicalin has been shown to possess many pharmacological effects, including antiviral, antioxidant, anti-cancer and anti-inflammatory properties. In the current study, we reveal the inhibitory effects of baicalin on the metabolism of dextromethorphan (DXM), a dual probe substrate of CYP2D and CYP3A, in rats. Lineweaver–Burk plots demonstrated that baicalin inhibited the activities of CYP2D and CYP3A in a non-competitive manner in rat liver microsomes (RLMs). Concomitant administration of baicalin (0.90 g/kg, i.v.) and DXM (10 mg/kg, i.v.) increased the maximum drug concentration (C_{max}) (37%) and the area under concentration–time curve (AUC) (42%) and decreased the clearance (CL) (27%) of DXM in a randomised, crossover study in rats ($P < 0.01$). The change in the AUC of DXM was significantly correlated with the C_{max} and AUC of baicalin ($P < 0.05$). The inhibitory effects of multiple doses of baicalin (0.90 g/kg, i.v., 12 days) on the metabolism of DXM were similar to those observed following a single dose in rats. The activity of CYP3A in excised liver samples from rats following multiple baicalin treatment was significantly decreased compared to that of the control group ($P < 0.05$), whereas multiple doses of baicalin had no obvious effect on the activity of CYP2D. Taken together, these data demonstrate that baicalin inhibits the metabolism of DXM in a concentration-dependent manner in rats, possibly through inhibiting hepatic CYP2D and CYP3A activities.

1. Introduction

Scutellaria baicalensis (*S. baicalensis*), also known as Huangqin in Chinese, is the dried root of a *Labiatae* perennial herb, is one of the most widely used Chinese herbs in Eastern and Western medicine and has the potential for commercial production [1,2]. Baicalin (baicalein-7-glucuronide, BG) (Fig. 1) is the major active constituent of the isolated root of *S. baicalensis* and it has been found to possess a number of pharmacological effects, including antiviral [3], anti-inflammatory [4–6], anti-nociception [7] and immune regulatory [8] properties. *S. baicalensis* yield is about 34,300 tons each year in China, and the yield of baicalin from the root of *S. baicalensis* is about 280 tons [9]. Almost all the baicalin was used for the pharmaceutical purpose. According to the traditional Chinese medicine theory, *S. baicalensis* is usually combined with other herbs. For example, it was the major ingredient of the popular traditional Chinese medicine, Shuang-huang-lian injection, which is extracted from *S. baicalensis, Lonicerae japonicae flos, and Forsythiae fructus* [10]. In the Pharmacopoeia of People's Republic of China (PPRC), *S. baicalensis* has been used as the main constitute of about 100 kinds of traditional Chinese medicine preparations, which are widely applied in the treatment of inflammation, cardiovascular diseases, respiratory and gastrointestinal infections. It should be emphasized that, baicalin, has been regarded as mainly bioactive constituent and phytochemical marker for the quality control of those Chinese medicine [11]. Moreover, baicalin, a monomer, is also included commonly in a combined therapy to treat patients with hepatitis in China.

Drug–drug interactions is one of the most important reasons for terminating the development of promising new therapies, withdrawing drugs from the market, or placing severe restrictions on the utilisation of drugs [12]. It is known that the cytochrome P450 (CYP)-dependent monooxygenase system plays an important role in the elimination of a variety of xenobiotics and is often a major component of drug–drug and herb–drug

Abbreviations: S. baicalensis, *Scutellaria baicalensis*; CYP, cytochrome P450; CYP2D, cytochrome P450 2D; CYP3A, cytochrome P450 3A; DXM, dextromethorphan; BG, baicalin; RLMs, rat liver microsomes; K_m, substrate concentration at half-maximal product formation rate; K_i, inhibition constant; C_{max}, maximum drug concentration; AUC, area under concentration–time curve; CL, clearance; HPLC, high-performance liquid chromatography.

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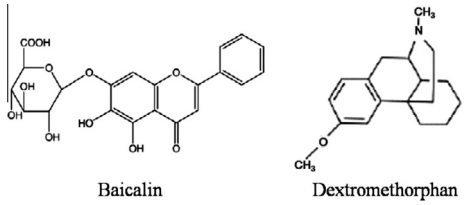

Fig. 1. Chemical structures of baicalin and dextromethorphan.

interactions [13,14]. Among the various CYP isoforms, CYP2D6 is a major oxidative isoenzyme that affects the metabolic fate of approximately 30% of medicines those are prescribed clinically, despite the fact that its level in the human liver is relatively low (approximately 2% of total CYP content) [15,16]. CYP3A is the most abundant human hepatic CYP isoform, catalysing the metabolism of approximately 60% of therapeutic agents [17]. Therefore, the interactions involving the inhibition of CYP2D6 and CYP3A are generally considered to be undesirable as they may be manifested as unwanted side effects for drugs with a narrow therapeutic window.

Due to the broad application of baicalin in drug therapy mentioned above, it would be expected that baicalin might be consumed with other synthetic drugs. As a result, the interaction between baicalin and other synthetic drugs warrants investigation [1]. Many reports have demonstrated that baicalin, baicalein, and other main components of *S. baicalensis* could modulate the activity of the CYP3A subfamily [18–21]. Co-administration of baicalin with cyclosporine has been reported to significantly increase the C_{max} and $AUC_{0-540\ min}$ of cyclosporine [21], but the effect of baicalin on CYP3A is unclear. A recent report [22] demonstrated that baicalin was mapped to the pharmacophore model of CYP2D6 inhibitors and could dock into the active site of CYP2D6. To our knowledge, the inhibitory effect of baicalin on the activity of CYP2D has not been reported in humans or animals. Therefore, well-designed studies should be carried out to explore the inhibitory effects of baicalin on CYP2D/CYP3A.

Dextromethorphan (DXM) (Fig. 1), a safe antitussive and analgesic drug, is widely used in daily clinical situations [23]. The DXM metabolic pathway is mediated by O-demethylation to generate dextrorphan, with CYP2D6 as a rate-limiting step, and N-demethylation to 3-methoxymorphinan via CYP3A in humans [24]. Dextrorphan and 3-methoxymorphinan undergo further N- and O-demethylation, respectively, through CYP3A and CYP2D6 to produce 3-hydroxymorphinan [23,25,26]. Inhibition studies using CYP2D inhibitors such as quinine, methadone, and propafenone have confirmed that the metabolism of DXM to dextrorphan is mostly via CYP2D in rats [27]. Therefore, based on these data, O-demethylation of DXM to dextrorphan appears to be a suitable marker for CYP2D in rats [28]. The alternative pathway for DXM metabolism to 3-methoxymorphinan through N-demethylation is believed to be mostly through CYP3A enzyme in rats and the formed 3-methoxymorphinan is rapidly converted to 3-hydroxymorphinan [27,28]. Therefore, DXM has been widely used as a probe drug to simultaneously assess CYP2D and CYP3A activities both in vivo and in vitro to monitor potential drug interactions in humans and in rats [23,24,28,29].

The present study aims to evaluate the inhibitory effects of baicalin towards CYP2D and CYP3A activities using DXM as a substrate and to explore the underlying mechanisms of action of baicalin. The possible involvement of CYP2D and CYP3A in this inhibition was examined by measuring enzymatic activity. These results are important for the investigation of CYP inhibition and herb-drug interactions associated with baicalin and other *S. baicalensis*-derived products.

2. Materials and methods

2.1. Reagents

DXM HBr (99.0%) was obtained from Labor Dr. Ehrenstorfer-Schafers (Augsburg, Germany). Dextrorphan tartrate was purchased from Cerilliant (Cerilliant Corp.). 3-methoxymorphinan HCl (Sigma–Aldrich; St. Louis, MO, USA) was kindly donated by Jing-Cheng Tang at Capital University of Medical Sciences, China. Injectable DXM HBr was donated Jiangsu Lianshui pharmaceutical Co., Ltd. NADPH was purchased from Roche Co., Ltd. Baicalin (purity $\geqslant$ 98.5%) was a kind gift of Henan Provincial Institute of Food and Drug Control. HPLC solvents and other chemicals were of high-performance liquid chromatography (HPLC) grade and were commercially available.

2.2. Animals

Male Sprague–Dawley rats (230–260 g), 6–8 weeks of age, were supplied by Laboratory Animals Center of Henan Province, China. The animals were housed in a temperature-controlled room with a 12 h light and dark cycle and had free access to standard laboratory chow and water. The experiments were initiated after acclimation to these conditions for at least 1 week. The rats were fasted overnight the day before the pharmacokinetic experiments. All experiments were performed with approval from the Animal Research Ethics Committee of Zhengzhou University.

2.3. Drug administration and sampling

Sixteen rats were randomly separated into control and baicalin treatment groups ($n = 8$). DXM and baicalin were injected via the tail vein. Saline was used as a vehicle control and was injected into the rats at the time of baicalin administration.

In the single dose studies, the baicalin treatment group was used to explore the impacts of baicalin on the metabolism of DXM in a randomised, crossover study. Bacalin (0.90 g/kg) was divided into three dosages (0.45, 0.225 and 0.225 g/kg) on the basis of intravenous infusion for routine clinical use and administrated to rats at 0, 1.25 and 2.5 h. DXM (10 mg/kg) was immediately injected to rats after the first injection of baicalin (0.45 g/kg). Blood samples (0.5 mL) were collected before baicalin dosing and at 0, 0.25, 0.75, 1.25, 2.5, 3.5, 5 and 7 h after DXM dosing by orbital bleeding into heparinised tubes. Plasma was obtained by centrifugation at 4000 rpm for 10 min at 4 °C and frozen at −80 °C prior to analysis.

After the single treatment study, the same eight rats were included in a multiple dosing investigation. The rats were treated with baicalin (0.90 g/kg) once a day for 12 days. On the last day, the procedure for drug administration and sampling were consistent with those described in the single treatment study.

Rats from both groups were sacrificed 24 h after the last dose by cervical dislocation to excise the liver. This time setting is considered to be suitable for investigating whether multiple baicalin treatments inhibited the activities of CYP2D and CYP3A, as the pharmacokinetics of baicalin showed that it has a relatively short $t_{1/2}$ in rats. Liver microsomes were prepared by calcium aggregation [30] and stored at −80 °C until determining. Protein contents were quantified using the Bio-Rad (Beyotime Institute of Biotechnology, China) protein assay kit, which is based on the Bradford method [31].

2.4. Determination of IC_{50} and K_i of baicalin against DXM metabolism in RLMs

O-demethylase (CYP2D) and N-demethylase (CYP3A) activities were assessed by the formation of dextrorphan and