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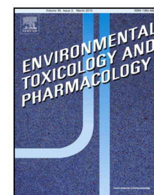

High fat diet aggravates the nephrotoxicity of berberrubine by influencing on its pharmacokinetic profile

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ABSTRACT

Berberrubine (BRB), the active metabolite of berberine (BBR), possesses various pharmacological activities. In this study, we found BRB showed not only a stronger lipid-lowering effect than berberine but also a specific nephrotoxicity in mice fed with high fat diet (HFD). To explore the underlying mechanism, the pharmacokinetics of BRB were evaluated. There was a greater *in vivo* exposure of BRB in C57BL/6J mice fed with HFD than with routine chows, in terms of Cmax, $AUC_{0\text{-}t}$, levels of BRB in kidney and urinary excretion. Moreover, *in vitro* assessment clearly showed BRB had a toxic effect on renal cell lines, while the primary metabolite, berberrubine-9-*O*-β-D-glucuronide (BRBG), did not show any obvious toxicity. These results suggested HFD aggravated BRB-induced nephrotoxicity by promoting the *in vivo* exposure of BRB especially in urine and kidney. Although our previous study indicated BRB could be metabolized into BRBG, BRBG did not show any obvious toxicity *in vitro*.

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1. Introduction

Drug-induced physical toxicity has obtained extensive attention for many years, since safety is the core concern in drug therapy process. Numerous diseases could be effectively treated with drugs, while some drugs may exert severe body injury and other side-effects(Begriche et al., 2011). Anti-tuberculosis drugs such as isoniazide, rifampicin and pyrazinamide are widely used for treatment of tuberculosis, but these treatments are all associated with a high risk of hepatotoxicity(Prince et al., 2002; Su et al., 2014; Younossian et al., 2005). Typically, antineoplastic agent, cisplatin(Bajwa et al., 2015), and aminoglycoside antibiotics have been confirmed to have a serious nephrotoxicity(Morales et al., 2001). Moreover, many other effective agents may have some organ specific toxicity, immune injury or metabolic disturbance, *etc.*

Numerous signaling pathways are involved in drug-induced toxicity, since our body is an extremely complex metabolic system which contains countless small molecular substances and biomacromolecules. Generally, the injury is caused by the interaction of drug with its target molecule or the change of endogenous substances in process of drug metabolism and disposition, which results in the dysfunction of body cells. The drug-induced toxic effect contains allergic reaction which is not dose dependent, such as penicillin allergy(Weltzien and Padovan, 1998) and acute allergic interstitial pneumonitis induced by hydrochlorothiazide(Biron et al., 1991). But in most cases the toxicity is in a dose- and time-depend manner, and toxic effect could be aggravated though drug accumulation. Hence, toxicokinetics studies are extraordinary important to interpret the drug-induced toxicity.

As previous studies reported, pharmacokinetic characteristics of drugs are related with disease status, circadian rhythm, nutritional condition and many other factors. For instance, the pharmacokinetics of diazepam was significantly influenced in patients with liver cirrhosis(Avallone et al., 1998). Circadian rhythm shows a

Abbreviations: BRB, berberrubine; BBR, berberine; BRBG, berberrubine-9-*O*-β-d-glucuronide; THB, tetrahydroberberine; HFD, high fat diet; CD, control diet; TC, total cholesterol; TG, total triglyceride; BUN, clood urea nitrogen; Cr, creatinine; UA, uric acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AKP, alkaline phosphatase; AUC, area under the curve.

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critical role in the metabolism of erlotinib(Liu et al., 2015). Nutritional condition and diet have a complex effect on drug metabolic enzymes and transporters(Harris et al., 2003; Sugatani et al., 2010). Therefore, the changes of pharmacokinetic characteristics caused by these factors may exert an uncertainty of drug-induced toxicity.

In this study, we found BRB, the primary metabolite of BBR, possessed an effective lipid-lowering effect. Surprisingly, it also produced a specific nephrotoxicity especially in mice fed with HFD. To interpret the aggravation effect on nephrotoxicity caused by BRB on HFD, we focus on the pharmacokinetics difference of BRB between control diet (CD) and HFD. We found HFD could significantly affect the pharmacokinetic behavior of BRB in mice, *i.e.*, HFD could promote BRB urinary excretion, increase the average AUC and Cmax values associated with accelerated plasma elimination rate, and enhance kidney distribution of BRB. Since urinary excretion is the main way of drug elimination and kidney possesses large amounts of blood delivery, the changes of drug concentration in urine and plasma are of great important for drug-induced nephrotoxicity. Moreover, our pilot study showed that BRB had a potential toxicity on renal cell lines. Although our previous study proved BRB could be largely metabolized into BRBG, BRBG did not show any significant toxicity *in vitro* assessment. In conclusion, we hypothesized that the increased kidney distribution and urinary excretion of BRB in C57BL/6J mice on HFD would be highly responsible for the aggravated renal injury.

2. Materials and methods

2.1. Reagents

BRB (purity >95%) was synthetized by Chemzam Pharmtech Co (Nanjing,China), BBR (purity >98%) was purchased from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China), tetrahydroberberine (THB, purity >99%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). BRBG was prepared and identified by Key Lab of Drug Metabolism and Pharmacokinetics (China Pharmaceutical University, Nanjing, China, unpublished data). Total cholesterol (TC), triglyceride (TG), blood urea nitrogen (BUN), creatinine (Cr), uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AKP) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). IL-6 and TNFα ELISA kits were purchased from ExCell Biology Inc (Shanghai, China). Acetonitrile, methanol, formic acid (chromatography grade) were purchased from Merch (Darmstadt, Germany).

2.2. Anti-obesity and lipid-lowering effect of BRB

C57BL/6J mice (Male; Six weeks old; weighing around 22 g were purchased from college of animal science and technology (Yangzhou University, China) and were housed under a 12-h light-dark cycle at a temperature of 22 °C to 24 °C and given free access to water. All animal studies were performed with the ethical guidelines of the Animal Ethics committee of China Pharmaceutical University. Mice were randomly divided into the control diet group (CD, AIN-93M,Trophic Animal Feed High-Tech Co., Ltd, Nanjing, China) or high fat diet group (HFD, 60% calories from fat and 1% cholesterol, Trophic Animal Feed High-Tech Co., Ltd, Nanjing, China). Mice fed with HFD were intragastrically administered with vehicle CMC—Na, BBR (150 mg/kg) or BRB (high dose group: 50 mg/kg; low dose group: 25 mg/kg), respectively (n = 6). Weights of mice were recorded weekly. After six weeks of consecutive treatment, all mice were euthanized. At the time of euthanization, plasma, liver, kidney were collected and then stored at −80 °C for further analysis. Livers were stained with hematoxylin/eosin (H&E) and Oil Red O with a standard protocol(Kong et al., 2009).Partial liver was weighed accurately and homogenized with ethanol (1 g: 9 mL) and centrifuged at 2500 rpm for 10 min, the supernatant was collected. TC and TG were determined by the enzymatic-colorimetric assay kits.

Table 1
Primer sequences for real-time PCR.

Name	Sequence	Orientation
β-actin	5'ATGGAGGGGAATACAGCCC3′	Sense
	5'TTCTTTGCAGCTCCTTCGTT3′	Antisense
Nephrin	5'GTTCCAAGCCAAAGGATGCC3′	Sense
	5'CTGCAACCTGCAACCCAAAA3′	Antisense
Synaptopodin	5'CTCGGAGGGCCAGAGAAAAG3′	Sense
	5'TCTCCGTGATCCCCCTCATT3′	Antisense
IL-6	5'TGGCTAAGGACCAAGACCATCCAA3′	Sense
	5'AACGCACTAGGTTTGCCGAGTAGA3′	Antisense
IL-β	5'ACTCAACTGTGAAATGCCACCTT3′	Sense
	5'TGCTGCTGCGAGATTTGAAG3′	Antisense
TNF-α	5'ACAAGGCTGCCCCGACTAC3′	Sense
	5'TTTCTCCTGGTATGAGATAGCAAATC3′	Antisense

2.3. High fat diet aggravate BRB's nephrotoxicity in C57BL/6J mice

Another batch of C57BL/6J mice was randomly divided into four groups: C (CD with vehicle CMC-Na), CB (CD with 50 mg/kg of BRB), H (HFD with vehicle CMC-Na and HB (HFD with 50 mg/kg of BRB). After six weeks of consecutive treatment, all mice were euthanized. At the time of euthanization, plasma, liver, kidney were collected. Renal function was characterized by H&E staining and determination of BUN, Cr and UA in the serum and IL-6, TNFα in the kidney was performed by the specific commercial kits. Liver function was also investigated by H&E staining and the activities of serum transaminases (ALT, AST) and AKP. Total RNA of liver and kidney was isolated using TRIzol method(Mannhalter et al., 2000) and reverse-transcribed into cDNA according to previous protocol (Takara). Quantitative real-time PCR was used to measure the relative mRNA levels of two important proteins necessary for the function of the renal filtration barrier, nephrin and synaptopodin. Primer sequences are shown in Table 1.

2.4. Pharmacokinetics studies on BRB after oral administration in C57BL/6J mice

C57BL/6J mice (Male; Six weeks old; weighing 18–22 g) were randomly divided into three groups: CSB (control diet with single dose of BRB), CMB (control diet with multiple dose of BRB), HMB (high fat diet with multiple dose of BRB). The mice in CMB and HMB were intragastrically administered 50 mg/kg BRB for consecutive six weeks, and mice in CSB were gavaged with vehicle CMC-Na as control. Urine samples were collected in metabolic cages for 12 h after administration. All mice were fasted overnight and given free access to water before euthanization. At the time of euthanization, all mice were p.o. administrated with 50 mg/kg BRB. Blood samples were collected into heparinized 1.5 mL Eppendorf tubes at 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8 h after administration (n = 4 ~ 6 at each time interval) and then immediately centrifuged at 8000 rpm for 10 min to separate the plasma. Tissues including liver, kidney, intestine and gall bladder (contains bile) were dissected at 0.083, 0.25 and 1.5 h after p.o. administration of BRB. All samples were stored at −80 °C for further analysis.

2.5. Sample preparation and LC–MS/MS conditions

The plasma samples were thawed at room temperature before analysis. An aliquot of 50 μL of plasma was added with 200 μL

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