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Research Paper

Pharmacokinetic mechanism of enhancement by Radix Pueraria flavonoids on the hyperglycemic effects of Cortex Mori extract in rats

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ABSTRACT

Ethnopharmacological relevance: Diabetes mellitus, characterized by abnormal blood glucose evaluation, is a serious chronic disease. In the treatment of the disease, α-glycosidase inhibitors play an important role for controlling the postprandial blood glucose level. Cortex Mori, a traditional Chinese herbal medicine, has a long history of use for the treatment of headaches, cough, edema and diabetes. Modern pharmacological studies have shown that the herb has beneficial effects on the suppression of postprandial blood glucose levels by inhibiting α-glycosidase activity in the small intestine. 1-Deoxynojirimycin (DNJ), the main active ingredient of this herb, is recognized as a potent α-glycosidase inhibitor. Our previous studies have shown that the hypoglycemic effect of Cortex Mori extract (CME) was significantly improved when giving CME in combination with Radix Pueraria flavonoids (RPF). In the present study, the pharmacokinetics and intestinal permeability of DNJ were comparatively investigated in rats after being given orally or by intestinal perfusion with CME alone or in CME–RPF pairs, to explore the mechanism of this synergistic effect.

Materials and methods: The role of RPF on the plasma and urine concentrations of DNJ from CME orally administered was investigated. Four groups of rats received a single oral dose of either CME or CME–RPF, at DNJ equivalent doses of 20 and 40 mg/kg, respectively. After dosing, plasma and urine were collected and assayed by LC/MS/MS. In addition, another two groups of rats were used for small intestinal perfusion with CME or CME–RPF at DNJ concentration of 10 μM.

Results: Compared to the data when dosing with CME alone, the C_{max} of DNJ were decreased from 5.78 to 2.94 μg/ml ($p < 0.05$) and 10.66 to 5.35 μg/ml ($p < 0.01$); T_{max} were delayed from 0.40 to 0.55 h and 0.35 to 0.50 h ($p < 0.05$); and MRT were significantly prolonged from 1.14 to 1.72 h ($p < 0.05$) and 0.95 to 1.62 h ($p < 0.01$), after dosing with CME–RPF at DNJ doses of 20 and 40 mg/kg, respectively. In addition, the urinary recovery of DNJ over the first 4 h after dosing significantly decreased from 48.76% to 33.86%. Effective permeability (P_{eff}) of DNJ was decreased from 7.53×10^{-3} to 3.09×10^{-3} cm/s ($p < 0.05$) when RPF was added to CME, when it was evaluated using the rat intestinal perfusion model.

Conclusions: All the above results demonstrate that RPF was able to suspend and delay the absorption of DNJ, but did not affect the total amount of DNJ in the body. The resulting higher concentration of DNJ in the small intestine produced a relatively stronger effect of depressing the elevation of the postprandial blood glucose level. These findings support the important role of RPF in the application of CME on blood glucose control.

1. Introduction

Carbohydrate digestion by α-glycosidase and subsequent glucose uptake at the intestinal brush border are critical for postprandial blood glucose control. The inhibition of α-glycosidase is an important therapeutic approach for decreasing postprandial hyperglycemia. Along with the applications of an array of chemical drugs, such as acarbose, miglitol and voglibose (Hughes and Rudge, 1994; Yoshikuni, 1988), scientists have paid much more attention to naturally occurring α-glycosidase inhibitors for the development of safe and efficient drugs. The traditional Chinese herbal medicine Cortex Mori, the root bark of *Morus alba* L, which is recorded as "sang bai pi" in the first Chinese dispensatory *Shen Nong Ben Cao Jing*, has a long history of use for the treatment of headaches, cough, edema and diabetes (Chen et al., 1995; Lee et al., 2012). According to ancient prescriptions, the aqueous decotion of the two Chinese medicines, Cortex Mori (12 g) and Fructus lycii (the fruit of *Lycium barbarum* L.) (15 g), is often applied to control

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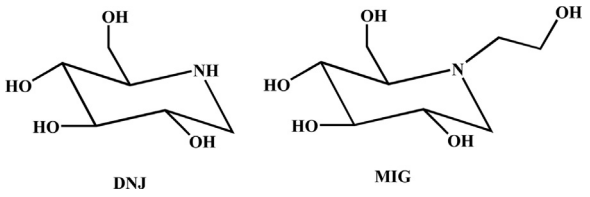

Fig. 1. Chemical structures of 1-deoxynojirimycin (DNJ) and miglitol (MIG) used as internal standard.

the blood glucose level for diabetes. Furthermore, recent pharmacological studies also suggested that the extracts and isolated compounds from the herb are beneficial for the suppression of abnormally postprandial blood glucose levels *in vivo* and *in vitro* (Kimura et al., 2007; Oku et al., 2006; Singab et al., 2005; Zhang et al., 2009). 1-Deoxynojirimycin (DNJ) (Fig. 1), a type of azasugar, was isolated from Cortex Mori (Yagi et al., 1976) as a main active component with a high content around 0.5% in the herb. Cortex Mori was demonstrated to perform significant activity for inhibition of intestinal α-glucosidase and postprandial hyperglycemia (Kong et al., 2008; Yoshikuni, 1988).

Radix Puerariae (Chinese name "ge gen"), the root of *Pueraria lobata* (Wild) Ohwi, was first documented as a herbal medicine in *Shen Nong Ben Cao Jing* for the relief of fever, diarrhea and emesis (Yan et al., 2004). This herb is now suggested for the treatment of fever, acute dysentery, diarrhea, thirst, diabetes and hypertension, with a recommended dose of 10–15 g (PPRC, 2005). The pharmacological studies have been reported that the herb possess various biological activities including antioxidant, anti-bone loss, antipyretic and analgesic, muscle relaxing, antidepressant and estrogen-like effects, as well as hypoglycemic activity (Shen and Xie et al., 1985; Zhang et al., 2010). Isoflavones and their glycosides are believed to be its major bioactive components (Rong et al., 2002). Radix Puerariae has been used for the management of diabetes for thousands of years in China, and accumulating evidence has supported the fact that the isoflavonoid compounds, especially puerarin and daidzin, display anti-diabetic effects in animal and cell models (Wonga et al., 2011).

Our previous study found that the hypoglycemic effect of Cortex Mori extract (CME) prepared from the root bark of *Morus alba* was significantly improved when co-administrated with the Radix Pueraria flavonoids (RPF) extracted from the root of *Pueraria lobatae*. This pharmacodynamic synergistic reaction of CME in the compatible application with RPF may have been caused by their pharmacokinetic interactions, including intestinal absorption. If the absorption of DNJ into the circulation system is inhibited, the relatively higher concentration of DNJ in the small intestine would produce a relatively stronger effect of depressing the elevation of the postprandial blood glucose level. Our hypothesis is that the RPF is capable of inhibiting the absorption of DNJ in the small intestine, so as to improve the hypoglycemic effect of DNJ. Despite the numerous beneficial effects of DNJ, there have been few reports on the absorption and metabolism of DNJ. Nakagawa et al. (2007) reported that orally administered mulberry DNJ was absorbed in the intact form from the intestine and then quickly excreted from the body. Kim et al. (2012) found that DNJ absorption from the mulberry extract seemed to be inhibited when compared to that of the purified DNJ compound, and there has been no published research referring to DNJ absorption after being given with Cortex Mori or Cortex Mori combined with other herbs. Therefore, in the present study, the oral absorption and pharmacokinetics of DNJ were determined in rats when given CME alone or in CME–RPF pairs at DNJ equivalent doses, then confirmed by in situ rat small intestinal perfusion experiments, in order to explore the effects of RPF on the oral absorption, pharmacokinetics and intestinal permeability of DNJ.

2. Materials and methods

2.1. Chemicals and reagents

The authentic standard 1-deoxynojirimycin (DNJ, 98%) was purchased from the Shanghai Winherb Medical Technology Company (ShangHai, China). Miglitol (MIG, 97%), used as internal standard, was kindly provided by the Zhejiang Medicine Co. Ltd. Xinchang Pharmaceutical Factory. Hank's Balanced Salt Solutions were purchased from Beijing Solarbio Science & Technology Co. Ltd. (Beijing, China). Acetonitrile with HPLC grade was purchased from Fisher Co. Ltd. (Emerson, USA). Ammonium formate, sodium chloride, sodium hydroxide and other reagents were all analytical grade and obtained from Beijing Chemical Works (Beijing, China). Milli-Q (Milford, MA, USA) water was used throughout the study.

2.2. Preparation of herb extracts

2.2.1. CME

The dried root bark of *Morus alba* was soaked with water three times. The pooled extract solution was loaded onto a column packed with strongly acidic cation exchange resin with polystyrene groups (Type 732, Chemical Plant of Nankai University, Tianjin, China). The resin was washed with de-ionized water until the eluent was neutralized, followed by elution with 0.5 M aqueous ammonia solution. The eluent was collected and concentrated by a rotary evaluator in vacuo to exhaustively remove the ammonia. The concentrated extract was then successively run through the two cascade columns, which were packed with strongly basic anion exchange resin with polystyrene groups (Type 711, Chemical Plant of Nankai University, Tianjin, China) and weakly acidic cation exchange resin with macroporous polycyclic acrylic structure (Type D151, Chemical Plant of Nankai University, Tianjin, China), respectively. After loading, the two cascade columns were eluted with de-ionized water until the final eluents became neutral. The eluents successively running from the two columns were collected and concentrated to dryness in vacuo to produce a brown powder, namely Cortex Mori extract (CME). The DNJ content in CME was 58.0% (w/w), determined by a reverse phase HPLC with fluorescence detection after derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) (Kim et al., 2003).

2.2.2. RPF

The root of *Puraria labata* was soaked with water three times. The extracted solutions were combined and loaded onto an absorption macroporous resin column (Type AB-8, Chemical Plant of Nankai University, Tianjin, China). After loading, the column was washed with de-ionized water, then eluted with 70% ethanol. The 70% ethanol eluents were concentrated to dryness in vacuo to produce a brown powder, named Radix Puerariae flavonoids (RPF). The content of the main active compound puerarin in RPF was 27.9% (w/w), determined by a reverse phase HPLC with UV detection (Chi and Zhang, 2006).

2.3. Animal experiments

2.3.1. Animals

The animal experiments were approved by the Animal Ethics Committee at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences. Male Sprague-Dawley rats (210 ± 20 g) supplied by Beijing Vital Laboratory Animal Technology (Beijing, China), were housed in a room with a controlled temperature (23 ± 1 °C) and free access to distilled water and food before the experiments.