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Ginseng polysaccharides enhanced ginsenoside Rb1 and microbial metabolites exposure through enhancing intestinal absorption and affecting gut microbial metabolism

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ABSTRACT

Ethnopharmacological relevance: Polysaccharides and small molecules commonly co-exist in decoctions of traditional Chinese medicines (TCMs). Our previous study outlined that ginseng polysaccharides (GP) could interact with co-existing ginsenosides to produce synergistic effect in an over-fatigue and acute cold stress model via gut microbiota involved mechanisms.

Aim of the study: This study aimed to verify the interactions by examining the impact of GP on oral pharmacokinetics of ginsenoside Rb1 (Rb1), the dominant protopanoxadiol (PPD)-type ginsenoside in Ginseng, on a dextran sulphate sodium (DSS) induced experimental colitis model which was characterized by gut dysbiosis, and to delineate the underlying mechanisms *in vitro*.

Materials and Methods: Rats received drinking water (normal group), 5% DSS (UC group), or 5% DSS plus daily oral administration of GP (GP group) for 7 days and fecal samples were collected on day −3, 0 and 6. On day 7 all animals received an oral dosage of Rb1 and blood samples were withdrawn for pharmacokinetic study. The *in vitro* metabolism study of Rb1 in gut microbiota from normal and UC rats and the transport study of Rb1 across Caco-2 cell monolayer were carried out in presence/absence of GP. Rb1 and its bacterial metabolites ginsenoside Rd (Rd), ginsenoside F2 (F2), Compound K (CK) and PPD were determined using LC-MS/MS. Total and target bacteria in fecal samples were determined by using 16S rRNA-based RT-PCR. β-Glucosidase activity was determined by measuring 4-nitrophenol formed from 4-nitrophenyl-β-D-glucopyranoside hydrolysis.

Results: DSS induction did not alter AUC_{0-t} and C_{max} of Rb1, which, however, were doubled together with elevated AUC_{0-t} of the metabolites, in particular Rd and CK, in GP group. GP influenced the microbial composition and showed a prebiotic-like effect. Accordingly, GP treatment could partially restore the β-glucosidase activity which was reduced by DSS induction. The presence of GP resulted in quicker microbial metabolism of Rb1 and higher Rd formation in first 8 h of incubation, while the impact on F2 and CK formation/conversion became obvious after 8 h. More interestingly, GP slightly stimulated Caco-2 cell growth and facilitated Rb1 transport across the Caco-2 monolayer in both directions, increasing the P_{app} of Rb1 from 10^{-7} cm/s to 10^{-6} cm/s. *Conclusions:* GP alleviated DSS-induced colitis-like symptoms and enhanced the systemic exposure of Rb1 through enhancing microbial deglycosylation and intestinal epithelial absorption of Rb1. These findings further demonstrated the important role of gut microbiota in the multifaceted action of polysaccharides in the holistic actions of traditional decoction of TCMs.

Abbreviations: DSS, dextran sulphate sodium; UC, ulcerative colitis; GP, Ginseng polysaccharides; Rb1, ginsenoside Rb1; PPD, protopanaxadiol; CK, Compound K; PNPG, 4-nitrophenyl-β-D-glucopyranoside; DAI, disease activity index; MPO, myeloperoxidase; IL, interleukin; TNF-α, tumor necrosis factor-α; AUC, area under the plasma concentration-time curve; LC-MS/MS, liquid chromatography coupled tandem mass spectrometry; TCMs, traditional Chinese medicines; DMSO, dimethyl sulfoxide; DMEM, Dulbecco's Modified Eagle's medium; FBS, fetal bovine serum; MTT, 3-(4, 5-dimethylthiazole-2-ly)-2, 5-diphenyl tetrazolium bromide; BHI, Brain Heart Infusion

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1. Introduction

Traditional Chinese medicines (TCMs) constitute an important part of the public health system in China and in long-term medicinal practice, TCMs have demonstrated advantages in prevention and alleviation of some chronic diseases. Oral decoction is the conventional form to apply TCMs in practice. The chemical complexity of decoction undoubtedly is the basis of the multi-target action mode of TCMs which makes them attractive, in particularly, in an era when more diseases are found to be multifactorial and demand combination drug therapy (Lin et al., 2017), while on the other hand, it hampers the mechanistic understanding on their therapeutic benefits. As two important and dominant chemical types in TCMs decoctions, small molecules and polysaccharides are usually investigated separately with the latter usually being ignored or underestimated due to their poor absorption and ambiguous mechanisms of actions. Recently, we demonstrated that the bio-macromolecules could enhance the exposure levels of co-existing small molecules in a case study of Du-Shen-Tang (DST), the decoction of Ginseng containing both polysaccharides and ginsenosides, in an over-fatigue and acute cold stress model, which we speculated an altered microbial metabolism and intestinal absorption of ginsenosides through comparing the exposure levels (C_{max} and AUC values) in blood and excreted amounts of ginsenosides in feces (Zhou et al., 2016).

Microbial metabolism and intestinal absorption are two determinant steps for the absorption and exposure of many small molecular components, in particular glycosides, in orally administrated TCMs decoctions. Previous studies have revealed that many bacterial species possess particular enzymes to catalyze deglycosylation of glycosides, while they exhibit different substrate selectivity (Braune and Blaut, 2016). For example, *Bifidobacterium breve* K-110, *B. longum* H-1 and *Lachnospiraceae* strain CG19-1 (Braune and Blaut, 2011; Hyun et al., 2012; Jung et al., 2012) catalyze deglycosylation of multiple glycosides of saponins, flavones and terpenoids. As dominant bacterial groups in human feces, most of the *Bifidobacterium* spp. and *Bacteroides thetaiotaomicron* carry β-glucosidase activity (Dabek et al., 2008). So far, many diseases, such as diabetes, obesity, inflammatory bowel disease, have been demonstrated to be associated with altered microbial compositions. Strategies to intervene these diseases include restoration of the microbial balance using probiotics, prebiotics, antibiotics as well as many natural products including TCMs. Some polysaccharides and oligosaccharides, such as arabinoxylans (Sommer and Baeckhed, 2013), arabinogalactans (Mendis et al., 2016), inulin (Dion et al., 2016), galacto-oligosaccharides (Rakoff-Nahoum et al., 2016) and fructo-oligosaccharides (Akbari et al., 2015), could stabilize the intestinal microbial ecosystem and maintain intestinal homeostasis (Xu et al., 2013). Inevitably, the disease progression and interventions are accompanied with changes of microbial metabolism, including deglycosylation by β-glucosidases. For examples, high fat diet in atherosclerotic rats significantly increased the activity of β-glucosidase. Dextran sulphate sodium (DSS) induced chronic experimental colitis in rats resulted in decreased β-glucosidase activity (Wu et al., 2017). Daily application of probiotics alone and in combination with antibiotic increased β-glucosidase activity (Hijova et al., 2017). However, how botanical polysaccharides affect microbial β-glucosidase activity and related drug disposition has not been addressed.

Recently, a few studies have reported the absorption-enhancing effects of polysaccharides of plant origins on drugs in concomitant use (Schepetkin and Quinn, 2006). Mucoadhesive polysaccharides from *Bletilla striata* or tamarind seeds facilitated delivery of antibiotics to eyes of rabbits (Ghelardi et al., 2000; Wu et al., 2010). Polysaccharides of Gastrodiae Rhizoma enhanced the absorption of co-existing gastrodin in the herbal extract (Felt et al., 2001). *Aloe vera* whole leaf extract or polysaccharides could improve the absorption of vitamin C and E in humans and decrease the transepithelial electrical resistance (TEER) of Caco-2 cell monolayers (Vinson et al., 2005). *A. vera* gel polysaccharides significantly reduced the TEER of excised rat intestinal tissue and enhanced the transport of atenolol, a small molecular β-blocker, across this tissue (Beneke et al., 2012). The absorption-enhancing effects of these botanical polysaccharides might be attributed partly to their polymeric nature and surface charge properties (Wu et al., 2012). In addition, the *Aloe* polysaccharide reduced efflux of cimetidine, a substrate of P-glycoprotein (Carien et al., 2013; Chen et al., 2009), and *Astragalus* polysaccharides down-regulated the expression of P-glycoprotein in H22 tumor-bearing mice (Tian et al., 2012), suggesting impact of these botanical polysaccharides on P-glycoprotein-mediated transcellular efflux. These observations highlight the potential of botanical polysaccharides as absorption-enhancers via both paracellular and transcelluar pathways. In our study with DST, ginseng polysaccharides (GP) treatment led to enhanced exposure of ginsenosides in the over-fatigue and acute cold stress model, while it is unclear whether this can be attributed to an absorption-enhancing effect of ginseng polysaccharides.

Therefore, in this study, we hypothesized that the polysaccharides in DST enhance the systemic exposure of co-existing ginsenosides in two manners: facilitate microbial deglycosylation and enhance intestinal absorption. To test the hypothesis, we chose ginsenoside Rb1 (Rb1), the major protopanaxadiol (PPD) type ginsenoside in Ginseng, and studied the effects of GP on Rb1 pharmacokinetics and exposure of its microbial metabolites in DSS-induced experimental acute colitis rat model which is characterized with gut dysbiosis. The dosages of GP and Rb1 were determined based on the daily dosage of Panax ginseng herb recommended for human in China Pharmacopoeia at a ratio of the two components found in the ginseng decoction we prepared (Zhou et al., 2016). Further, the impact of GP on microbial deglycosylation activity and intestinal permeability were investigated on an *in vitro* anaerobic microbial incubation system and the Caco-2 cell monolayer model.

2. Materials and methods

2.1. Materials and reagents

Ginsenoside Rb1, ginsenoside Rd (Rd), ginsenoside F2 (F2), Compound K (CK), PPD, ginsenoside Rg1 (Rg1) and digoxin were supplied by Chengdu Must Bio-technology co., LTD. (Chengdu, China). The purity was > 98%. GP was extracted by the Natural Chemical Laboratory, Jiangsu Province Academy of Traditional Chinese Medicine (Nanjing, China) and the sugar composition and molecular mass distribution were described in a recent publication (Zhou et al., 2016). DSS (MW: 36000-50000) was purchased from MP Biomedicals Co. (Santa Ana, CA, US). 4-Nitrophenyl-β-D-glucopyranoside (PNPG), 4-nitrophenol, dimethyl sulfoxide (DMSO), hemin bovine, vitamin K1 and lucifer yellow were supplied by Sigma-Aldrich (St. Louis, MO, US). Dulbecco's Modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin and nonessential amino acids were obtained from Gibco BRL Life & Technologies (Grand Island, NY, US). 3-(4, 5-dimethylthiazole-2-ly)-2, 5-diphenyl tetrazolium bromide (MTT) and L-cystine was obtained from USB Co. (Cleveland, OH, US). Bacterial extraction reagent was supplied by Sangon Biotech (Shanghai, China). Pierce BCA protein assay kit was from Thermo Fisher Scientific (Waltham, MA, US). Brain Heart Infusion (BHI) medium, GasPak™ EZ anaerobe container system with indicator and GasPak™ EZ large incubation container were purchased from Becton Dickinson (Franklin Lakes, NJ, US). Methanol, 1-butanol, formic acid and acetonitrile were HPLC-grade from Merck (Darmstadt, Germany). Dulbecco's phosphate buffered saline (PBS) was provided by Life Technologies (Carlsbad, CA, US). Deionized water was in-house prepared by a Milli-Q purification system (Millipore, Bedford, MA, US). Transwell® plates (12-well, 0.4-μm pore size, 1.12 cm^2, polycarbonate membrane) were purchased from Corning Costar Co. (Cambridge, MA, US). Caco-2 cells were obtained from the American Type Culture Collection (Rockville, MD, US).