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Permeability through the Caco-2 cell monolayer of 42 bioactive compounds in the TCM formula Gegen-Qinlian Decoction by liquid chromatography tandem mass spectrometry analysis

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ABSTRACT

Caco-2 cell monolayer model was used to evaluate the intestinal permeability of 42 bioactive compounds in the famous traditional Chinese medicine (TCM) formula Gegen-Qinlian Decoction (GQD). These compounds include alkaloids, flavonoids and glycosides, triterpenoid saponins, and coumarins. Their transportations across the cell monolayers in the forms of herb extract and formula extract were monitored by liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) analysis. Most alkaloids from Huang-Lian; flavonoid *C*-glycosides from Ge-Gen and Huang-Qin; *O*-glycosides from Ge-Gen, Huang-Qin and Gan-Cao; *O*-glucuronides from Huang-Qin; and coumarins from Gan-Cao exhibited favorable permeability. Their P_{AB} values were > 1.05×10^{-5} cm/s, and efflux ratios (ER, P_{BA}/P_{AB}) were ≤ 1.0. In contrast, triterpenoid saponins showed poor permeability ($P_{AB} \leq 1.50 \times 10^{-6}$ cm/s, ER ≤ 1.5), indicating a paracellular diffusion mechanism. Furthermore, GQD could remarkably improve the intestinal transport of alkaloids in Huang-Lian, flavonoid *C*-glycosides in Ge-Gen, as well as coumarins and flavonoid *O*-glycosides in Gan-Cao. These results indicate herb–herb interactions in GQD.

1. Introduction

Traditional Chinese medicines (TCM) are usually used in the form of multi-herb compound formulas. Intestinal transportation is a key factor that affects drug absorption [1,2]. To clarify the effective compounds and mechanism of action of TCM formulas, it is important to understand their intestinal absorption process [3]. Caco-2 cell monolayer model could simulate drug absorption in humans [4,5]. It has been used to study the transport mechanisms of a number of natural products [6–8]. However, few reports are available on the transport of TCM formulas due to their complex chemical composition [7,9].

Gegen-Qinlian Decoction (GQD) is a popular TCM formula. It contains four herbs, namely Puerariae Lobatae Radix (P), Scutellariae Radix (S), Coptidis Rhizoma (C), and Glycyrrhizae Radix et Rhizoma (G), in the weight ratio of 8:3:3:2 [10,11]. GQD exhibits anti-diarrhea, anti-bacterial, antiviral, and anti-diabetic activities [12,13]. Our recent report reveals it contains at least 125 compounds [14]. We have also studied the *in vivo* metabolism of GQD. A total of 131 metabolites comprising 46 original phytochemicals and 85 biotransformed products were detected in rats after oral administration of GQD [15,16].

In this work, the dynamic absorption process of GQD was studied by simultaneously monitoring 42 major bioactive compounds using the Caco-2 cell monolayer model. The impact of herbal combination on the transport behaviors was also investigated.

2. Materials and methods

2.1. Chemicals and reagents

Daidzin (**1**), 3′-methoxypuerarin (**2**), daidzein (**3**), genistin (**4**), formononetin 8-*C*-apiofuranosyl-(1,6)-glucoside (**5**), genistein 8-*C*-apiofuranosyl-(1,6)-glucoside (**6**), puerarin (**7**), 3′-methoxymirificin (**37**), ononin (**39**), and (4*S*)-puerol B 2″-*O*-glucopyranoside (**41**) were isolated from P. Wogonin (**8**), oroxylin A (**9**), chrysin 6-*C*-α-L-arabinoside-8-*C*-*β*-D-glucoside (**10**), chrysin 6-*C*-*β*-D-glucoside-8-*C*-*β*-L-arabinoside (**11**), baicalein (**12**), wogonin 5-*O*-glucoside (**13**), norwogonin 7-*O*-glucuronide (**14**), oroxylin A 7-*O*-glucuronide (**15**), wogonoside (**16**), baicalin (**17**),

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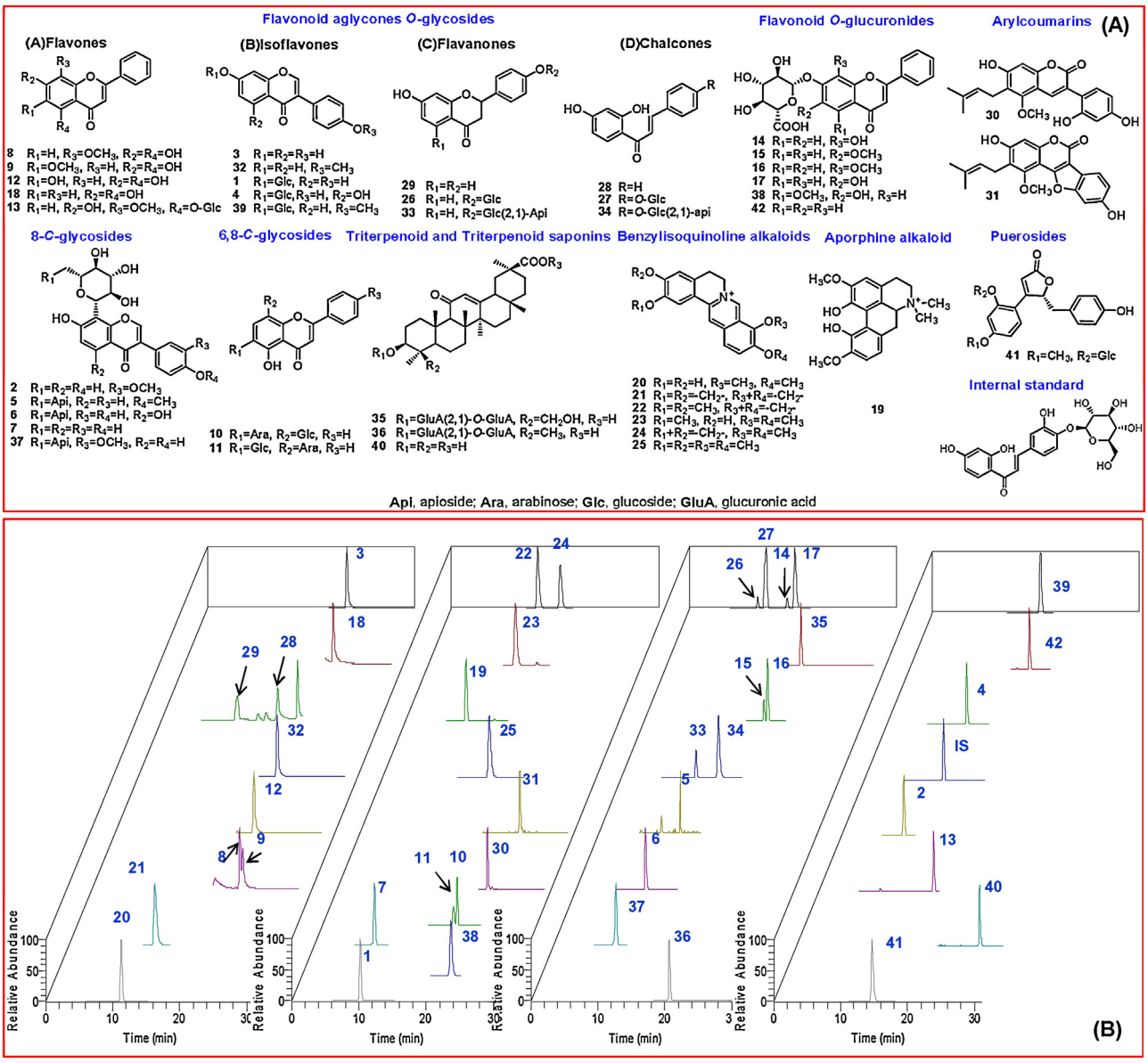

Fig. 1. Chemical structures of **1-42** (A). Detection of **1-42** by LC/MS/MS (SRM chromatograms) at the basolateral side of the Caco-2 cell culture at 150 min after GQD treatment (B).

chrysin (**18**), lateriflorein 7-*O*-glucuronide (**38**), and chrysin 7-*O*-glucuronide (**42**) were isolated from S. Liquiritin (**26**), isoliquiritin (**27**), isoliquiritigenin (**28**), liquiritigenin (**29**), glycycoumarin (**30**), glycyrol (**31**), formononetin (**32**), liquiritin apioside (**33**), isoliquiritin apioside (**34**), licorice-saponin G2 (**35**), glycyrrhizic acid (**36**), and glycyrrhetinic acid (**40**) were from G. Compounds **1**–**18**, **26**–**42**, and butein 4-*O*-glucoside (IS) were isolated by the authors, as described in our previous reports [16–18]. Structures for all the above compounds were characterized by NMR spectroscopic analyses, and the purities were above 98%. Demethyleneberberine (**20**) was purchased from Feiyu Fine Chemical (Jiangsu, China). Magnoflorine (**19**), coptisine (**21**), epiberberine (**22**), jatrorrhizine (**23**), berberine (**24**), and palmatine (**25**) were from Mansite Bio-Technology Co., Ltd. (Chengdu, China). Structures of **1-42** and IS are given in Fig. 1A. Paclitaxel was from Beijing SL Pharmaceutical Co., Ltd (Beijing, China). Methanol, acetonitrile, and formic acid were of HPLC grade (Mallinckrodt Baker, NJ, USA). De-ionized water was prepared with a Milli-Q water purification system (Millipore, Billerica, MA, USA).

2.2. Herbal materials and drug preparation

P, S, C, and G were the same batches of herbs as we had previously reported [14]. They were identified as the roots of *Pueraria lobata* (Willd.) Ohwi, roots of *Scutellaria baicalensis* Georgi, rhizomes of *Coptis chinensis* Franch., and roots and rhizomes of *Glycyrrhiza uralensis* Fisch., respectively [10]. The GQD extract was prepared by extracting the four component herbs (P 25.04 g, S 9.43 g, C 9.43 g, G 6.31 g) in 400 mL water for three times (1.5 h, 1.5 h, 0.5 h), following a pre-extraction of P using 200 mL water for 0.5 h. The decoctions were combined, filtered, and then concentrated in vacuum to 50 mL at 50 °C. GQD_{-GC} contains only P, S and C with the same weight ratio of GQD to obtain the water extract (equivalent to 1 g crude drug per mL). The water extracts of four component herbs were separately prepared by decocting in 8-fold volume of water for 1.5 h, 1.5 h, and 0.5 h, respectively, and the final concentration was 0.5 g crude drug per mL.

The above extracts were respectively freeze-dried to obtain powder, and suspended in DMSO and then diluted with HBSS