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Intestinal absorption of hawthorn flavonoids – in vitro, in situ and in vivo correlations

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Abstract

Our previous studies identified hyperoside (HP), isoquercitrin (IQ) and epicatechin (EC) to be the major active flavonoid components of the hawthorn phenolic extract from hawthorn fruits demonstrating inhibitory effect on in vitro Cu^{+2}-mediated low density lipoproteins oxidation. Among these three hawthorn flavonoids, EC was the only one detectable in plasma after the oral administration of hawthorn phenolic extract to rats. The present study aims to investigate the intestinal absorption mechanisms of these three hawthorn flavonoids by in vitro Caco-2 monolayer model, rat in situ intestinal perfusion model and in vivo pharmacokinetics studies in rats. In addition, in order to investigate the effect of the co-occurring components in hawthorn phenolic extract on the intestinal absorption of these three major hawthorn flavonoids, intestinal absorption transport profiles of HP, IQ and EC in forms of individual pure compound, mixture of pure compounds and hawthorn phenolic extract were studied and compared. The observations from in vitro Caco-2 monolayer model and in situ intestinal perfusion model indicated that all three studied hawthorn flavonoids have quite limited permeabilities. EC and IQ demonstrated more extensive metabolism in the rat in situ intestinal perfusion model and in vivo study than in Caco-2 monolayer model. Moreover, results from the Caco-2 monolayer model, rat in situ intestinal perfusion model as well as the in vivo pharmacokinetics studies in rats consistently showed that the co-occurring components in hawthorn phenolic extract might not have significant effect on the intestinal absorption of the three major hawthorn flavonoids studied.

Keywords: Hawthorn flavonoids; Absorption; Hyperoside; Isoquercitrin; Epicatechin; Caco-2 monolayer model; Rat in situ intestinal perfusion model

Introduction

Hawthorn (*Crataegus*) has been used as traditional Chinese medicine for a long time (Gao and Feng, 1994). In China, hawthorn fruit was used to improve digestion and relieve food stasis (Chen et al., 1995). In Europe, the fruit, leaf and flower of hawthorn are used for astringent, diuretic, and cardiovascular disease (Cupp, 2000). The chemical components of hawthorn consist of sterols, triterpene acids, flavonoids, oligomeric pro-anthocyanidins, organic acids and cardioactive amines etc. (Chang et al., 2002). The pharmacological effects were believed to be mainly due to the hawthorn flavonoids, which possess a number of beneficial effects such as cardiotonic (Awang and Fugh-Berman, 2002), antiarrhythmic (Mueller et al., 1996), hypolipidemic (Zhang et al., 2002), antihypertensive (Chen et al., 1998) and antioxidative activities (Zhang et al., 2001). Hawthorn flavonoids are mainly composed of hyperoside (HP), quercetin, quercitrin, isoquercitrin (IQ), rutin, vitexin, isovitexin and epicatechin (EC) (Chang et al., 2002). Among them, EC, IQ, and HP (Fig. 1) are identified to be the major active flavonoids in the hawthorn phenolic extract (HPE) from hawthorn fruits that have demonstrated inhibitory effects on the in vitro Cu^{+2}-mediated low density lipoproteins (LDL) oxidation (Zhang et al., 2001). The content of EC, IQ and HP in HPE was determined to be 15.8%, 2.0% and 2.7%, respectively (Chang et al., 2001).

Although the pharmacological effects of hawthorn flavonoids have been substantially reported, their in vivo pharmacokinetics and intestinal absorption mechanisms have not been clearly defined. The in vivo data of EC were mainly generated from the studies after coadministration with other green tea catechins (Chen et al., 1997; Chow et al., 2001). The absorption of HP is uncertain from the published literature. It was suggested that the intestinal uptake of IQ in rats might involve its hydrolysis by

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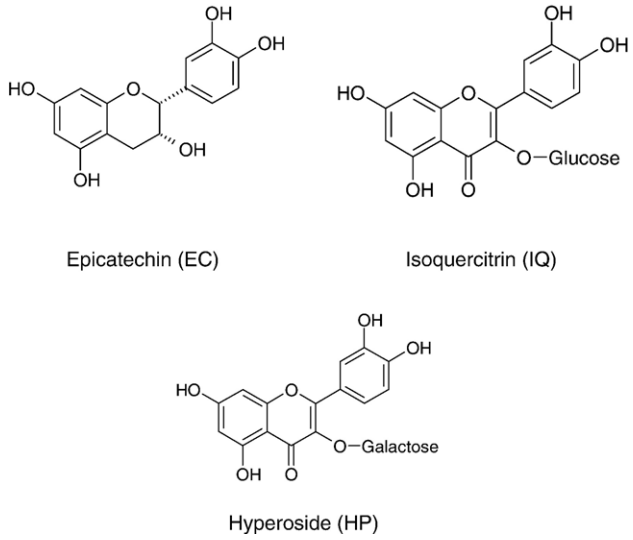

Fig. 1. Chemical structures of the studied hawthorn flavonoids.

lactase phlorizin hydrolase (LPH) to quercetin (Sesink et al., 2003). However, absorption of quercetin-4′-glucoside, one of IQ's analogue, was found to be actively transported by the intestinal Na^+/glucose cotransporter 1 (SGLT1) (Walgren et al., 2000). In summary, the absorption of flavonoid glycosides has been generally proposed to involve hydrolysis of the glucosides to their related aglycone in the small intestine before entry into systemic circulation (Walle et al., 2000). However, the active transport by SGLT1 has also been suggested for the intestinal absorption of flavonoid glycosides (Hollman et al., 1995).

During the past few years, our research group has conducted a series of preliminary pharmacokinetic studies on hawthorn flavonoids. Our study on oral administration of IQ and HP, the two structurally similar flavonoid glycosides, in rats found that IQ is rapidly absorbed and biotransformed to quercetin glucuronides, whereas HP could not be detected in plasma (Chang et al., 2005a). However, the detailed intestinal absorption and disposition mechanisms of IQ and HP have not been investigated yet. Moreover, our preliminary pharmacokinetics observation following oral administration of hawthorn extract found that only EC was absorbed in its parent form and its pharmacokinetics were generally not significantly different from that obtained from the oral administration of pure compound of EC (Chang et al., 2005b). But whether the co-occurring components in hawthorn phenolic extract would affect the corresponding metabolites of the hawthorn flavonoids formed after oral administration still needs further clarification. In order to answer these questions raised from our previous preliminary pharmacokinetics studies, a more detailed mechanistic investigation is essential.

Therefore, the present study was proposed aiming on the further investigation on the intestinal absorption mechanism of the three major active hawthorn flavonoids, namely EC, IQ and HP, using Caco-2 monolayer model, in situ intestinal perfusion model and in vivo animal pharmacokinetic studies. In addition, in order to further identify the effect of the co-occurring components on the intestinal absorption of the studied hawthorn flavonoids, the intestinal absorption transport profiles of these hawthorn flavonoids in forms of individual pure compound, mixture of pure compounds and extract are studied and compared for each model.

Materials

Chemicals

IQ and HP were purchased from Carl Roth, Germany. Epicatechin, rutin, naringin, β-glucuronidase/sulphatase and phosphate buffer tablets were from Sigma Chemical Co., USA. HPLC grade methanol and acetonitrile were from Labscan Asia Co. Ltd., Thailand. Other chemical reagents used were at least of analytical grade. Hawthorn phenolic extract (HPE) was prepared as described previously (Chang et al., 2005b).

Materials for cell culture

DMEM, FBS, 0.05% Trypsin–EDTA, Penicillin–Streptomycin, and non-essential amino acids were obtained from GibcoBRL, Life & Technologies, USA. Collagen type I and sodium pyruvate were purchased from Sigma Chemical Co., USA. Caco-2 cells were obtained from the ATCC. Six-well Transwell® plates (0.4 μm pore size, 4.71 cm^2, polycarbonate filter) were purchased from Corning Costar Co., NY, USA.

Methods

Caco-2 cell monolayer model study

Cell culture and cytotoxicity test

The culture condition of Caco-2 cell line was similar to our previous study (Zhang et al., 2004). Hawthorn phenolic extract (HPE) was dissolved in the transport buffer that was used for Caco-2 model transport studies (PBS^+: phosphate buffer saline supplemented with 0.45 M calcium chloride and 0.4 M potassium chloride and adjusted to pH 6.8) at concentrations of 1.5, 0.75, 0.38, 0.19, 0.094, 0.047 and 0.023 mg/ml. The cytotoxicities of HPE at the above concentrations were examined as described in our previous study and calculated as the percentage of the absorbance relative to control (Zhang et al., 2006).

Preparation of hawthorn flavonoids loading solutions

Solutions for individual pure compounds of the three studied hawthorn flavonoids were prepared by dissolving appropriate amount of each compound in the PBS^+ transport buffer to reach a final concentration of 50 μM. HPE solution was prepared by dissolving HPE powder in the PBS^+ transport buffer (pH 6.8) to reach a concentration of 1.1 mg/ml. The final HPE solution contained 23.3 μg/ml (50 μM) of IQ, 32.5 μg/ml (70 μM) of HP, 175 μg/ml (603 μM) of EC. Mixture form of pure compounds solution was prepared by dissolving the three pure hawthorn flavonoids in the PBS^+ transport buffer to make each flavonoid reach the same molar concentration as those in the HPE solution. DMSO at a concentration of 1% in the final loading