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Research paper

Difference in absorption of the two structurally similar flavonoid glycosides, hyperoside and isoquercitrin, in rats

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Abstract

The present study was to investigate oral absorption of the two similar flavonoid glycosides, isoquercitrin (IQ, quercetin-3-*O*-glucoside) and hyperoside (HP, quercetin-3-*O*-galactoside) in rats. Two groups of male SD rats received an oral dose of either IQ (4.5 mg/kg) or HP (6.0 mg/kg). Blood samples were collected via jugular vein at time intervals after drug administration and the plasma concentrations of the studied compounds were analyzed by HPLC. The stability of IQ and HP in the GI tract was also measured by incubation with various GI contents from rats. The results showed that unchanged IQ was barely detectable whereas the glucuronidated quercetin (the aglycone of IQ) was found to be the major form in plasma after oral administration of IQ. In contrast, HP could not be detected in plasma neither as unchanged form nor its aglycone or conjugated aglycone form. Additional in vitro stability studies demonstrated that HP is more stable than IQ in the GI tract. This suggests that IQ could be hydrolyzed easier than HP to its aglycone in GI tract before being absorbed. In conclusion, IQ, as a flavonoid glucoside, could be rapidly absorbed and transformed into glucuronidated quercetin and such absorption might be related to the hydrolysis of the type of sugar moieties attached to its aglycone molecule.

Keywords: Isoquercitrin; Hyperoside; Flavonoids; Absorption

1. Introduction

Flavonoids are a large and complex group of polyphenolic compounds widely distributed throughout the plant kingdom. They are common dietary components of fruits, vegetables and beverages, and are usually present in plant tissue as the form of glycosides [1,2]. Flavonoids exhibit a multitude of biological activities, such as anti-bacteria, anti-inflammation, anti-allergy and anti-oxidation [3,4]. Epidemiological studies have shown that the dietary intake of flavonoids is inversely associated with the incidence of coronary heart disease and cancer [5,6]. Due to their abundance in dietary products and their potential beneficial pharmacological and nutritional effects, the flavonoids are of considerable interest for drug as well as health food supplement.

Even though a lot of investigations have been conducted for the absorption and bioavailability of flavonoid glycosides, the results are quite contradictory. Early studies hypothesized that flavonoid glycosides would not enter the systemic circulation, neither as the natural glycosides nor as the aglycone hydrolysis products. It was believed that cleavage at the central heterocyclic ring by intestinal bacteria would occur effectively generating phenolic acid fission products [7,8]. At the later stage of flavonoid study, it was generally believed that the flavonoid glycosides have to be hydrolyzed to its aglycone before being absorbed [9–11]. However, recent studies reported that some flavonoids glycosides can be detected in human or rat plasma as its intact form [12,13], indicating that flavonoid glycosides may be able to be absorbed before being hydrolyzed.

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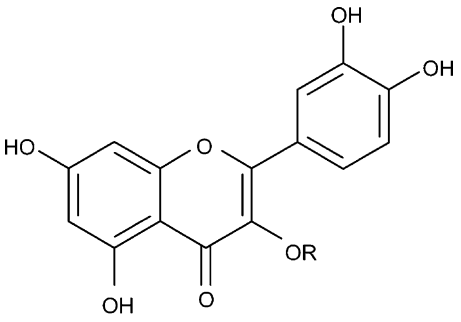

Hyperoside R = Galactose
Isoquercitrin R = Glucose

Fig. 1. Chemical structures of hyperoside and isoquercitrin.

In most of the above studies, the plasma samples were all subjected to the hydrolysis and the concentrations of the aglycone in the plasma were examined. However, both flavonoid glycoside and the glucuronidated conjugate of the aglycone could be hydrolyzed by the β-glucuronidase [8,14], which was generally used for the hydrolysis treatment. Thus, the study could not confirm whether the detected aglycone is from flavonoid glycoside absorbed as its intact form or from the flavonoid glucuronide formed during the absorption of its aglycone after the hydrolysis of flavonoid glycoside in GI tract.

Therefore, in the present study, we plan to investigate the oral absorption of the two flavonoid glycosides, isoquercitrin (IQ, quercetin-3-*O*-glucoside) and hyperoside (HP, quercetin-3-*O*-galactoside) (Fig. 1), by measuring their intact forms as well as their glucuronidated metabolite in plasma. The doses of IQ and HP were chosen as 4.5 and 6.0 mg/kg, respectively, which ratio was based on their contents in hawthorn phenolic extract [15] and our previous pharmacokinetic study on hawthorn extract [16]. The purposes of the current studies are to: (1) find out the detailed absorption processes of the studied natural flavonoids; (2) investigate the influence of the sugar moieties on the absorption of flavonoids.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade isoquercitrin (IQ, quercetin-3-*O*-glucoside) and hyperoside (HP, quercetin-3-*O*-galatoside) were purchased from Carl Roth GmbH (Karlsruhe, Germany). Naringin used as internal standard for HPLC assay was obtained from Signa (St. Louis, MO, USA). HPLC-grade Acetonitrile was obtained from Labscan (Labscan Asia Co. Ltd, Thailand). All other chemicals and solvents used were of analytical grade and were obtained from Sigma or BDH Laboratory Supplies (Poole, Dorset, England). Distilled and deionized water was used throughout the study.

2.2. Animals

Male Sprague–Dawley rats aged 7 weeks (200–220 g) were supplied by and bred at the Laboratory Animal Service Center at the Chinese University of Hong Kong. The rats were housed in an air-conditioned room (temperature, 23 ± 2 °C; relative humidity, 55 ± 5%) and kept on a light/dark cycle of 12/12 h. They had free access to standard rodent diet (Prolab RHM 3000; PMI Nutrition International, Inc., Brentwood, MO, USA) and water before the experiment.

On the day before the experiment, a light surgery for the rat was performed. A polyethylene catheter (0.40-mm ID, 0.80-mm OD, Portex Limited, Hythe, Kent, England) was cannulated into the right jugular vein under light anaesthesia. After surgery, the rat was placed individually in metabolic cages. The animal was then allowed to recover for 24 h and fasted overnight prior to each experiment.

2.3. Drug administration and blood sampling

The dosing solutions of IQ and HP were freshly prepared by dissolving in saline containing 2% DMSO before experiment. Two groups of male Sprague–Dawley rats (200–220 g) received an oral dose of IQ (4.5 mg/kg, $n = 9$) and HP (6.0 mg/kg, $n = 4$), respectively, by gastric gavages. In addition, a higher dose of HP (30 mg/kg, $n = 2$) was also given to rats to further confirm the findings. Blood samples (0.2 ml) were withdrawn via the catheter before and at 2, 5, 10, 15, 20, 30, 40 and 50 min post-dosing and collected into heparinized centrifuge tubes. At the last time point, the collection volume of blood was doubled in order to improve the detection sensitivity of compounds for HPLC analysis. After each blood sampling, 0.2 ml of heparinized normal saline solution (20 IU/ml) was immediately injected back into the catheter to prevent coagulation. The collected blood samples were immediately centrifuged at 4000 rpm for 5 min. The plasma was then separated and stored at −80 °C for analysis. The urine and faeces samples were collected over 12 and 24 h, respectively, post-dose. All the faeces collected were homogenized in 100 ml of water. The mixture was sonicated for 30 min and centrifuged at 6000 rpm for 10 min. The supernatant was collected and stored at −80 °C until analysis.

2.4. In vitro stability of IQ and HP

The stability of IQ and HP in the rat plasma and the gastrointestinal (GI) tract was evaluated by incubation in plasma, buffer solutions with pH 1.2 (similar to the pH in stomach) and 6.8 (similar to the pH in small intestine and colon), and following various GI content solutions (stomach, duodenum, jejunum, ileum), respectively.

2.4.1. In plasma

Fresh rat plasma obtained from rats was spiked with a standard solution of either IQ or HP at a final concentration