Transepithelial Permeability of Myricitrin and Its Degradation by Simulated Digestion in Human Intestinal Caco-2 Cell Monolayer

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Received February 18, 2005; Accepted May 30, 2005

Myricitrin permeated the human intestinal Caco-2 cell monolayer via the paracellular pathway in a time- and concentration-dependent manner. Myricitrin was not conjugated by Caco-2 cells. Myricitrin was degraded by simulated intestinal digestion, but permeability did not change significantly.

Key words: myricitrin; flavonoid; Caco-2 cells; transepithelial permeability

Flavonoids have various bioactivities associated with cancer, coronary heart disease, atherosclerosis, inflammation, and other oxidative stresses.1) Myricitrin is a rhamnose glycoside of myricetin contained in various plants, and it has strong antioxidative activity.2–4) Myricitrin and its alkaline degradation products (DM) prepared by mild alkaline treatment that simulated the human intestinal environment has a strong inhibitory effect on low-density lipoprotein (LDL) oxidation due to radical-scavenging and metal-ion chelating activities.5) Several flavonoids have recently been detected in human blood after oral intake, and the absorption mechanism of flavonoids has also been studied in vitro using cell lines.6–8) It is important to understand the mechanism of intestinal absorption of myricitrin in order to develop a method of increasing the bioavailability of myricitrin. The Caco-2 cell monolayer is a model of intestinal epithelial cells and has the same enzymes, transporters, and morphology as intact human intestinal epithelial cells.9) In this study, we investigated the intestinal absorption of myricitrin using a human colon adenocarcinoma cell line Caco-2 monolayer.

Myricitrin was purchased from Funakoshi (Tokyo). Figure 1 shows the structure of myricitrin. DM was prepared by mild alkaline treatment (pH 8.5, 60 min) that simulated intestinal digestion, and was analyzed by high performance liquid chromatography (HPLC)/mass spectrometry (MS) (Alliance ZQ2000 LC/MS System, Waters, Milford, MA) using a reversed-phase column XTerra MS C18 (2.1 × 150 mm, Waters), as reported previously.5) The human colon adenocarcinoma cell line Caco-2 was obtained from American Type Culture Collection (Rockville, MD). A culture medium consisting of Dulbecco’s modified Eagle’s medium, 10% fetal bovine serum, 1% nonessential amino acids, 4 mm l-glutamine, 100 units/ml of penicillin, and 100 μg/ml of streptomycin, and Hanks’ balanced salt solution (HBSS), were purchased from Invitrogen (Carlsbad, CA). The Caco-2 monolayer culture was grown in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C. For permeation studies, Caco-2 cells at passage 56–67 were seeded in a 24-well polycarbonate membrane (HTS Transwell-24 systems, 0.4 μm pore, 6.5 mm in diameter and 0.33 cm²/insert, Corning, One riverfront plaza, NY) at a density of 5 × 10⁵ cells/cm². Before sample addition, the medium was removed and the cell monolayer was rinsed with HBSS. The sample in DMSO solution was diluted with HBSS and added to the apical side, and the solution in the basal side was HBSS. After each incubation, the basal solution was analyzed by HPLC. The transepithelial electrical resistance (TEER) of the Caco-2 monolayer was measured with a Millicell-ERS (Millipore, Bedford, MA). A Caco-2 cell monolayer with various degrees of TEER value was obtained by Cytochalasin D treatment.

Certain food-derived components affect the tight junction of Caco-2 cells and lower the TEER value due to a cytotoxic effect.10) Myricitrin did not cause a significant change in the TEER value of the Caco-2 cell monolayer under this experimental condition. Several absorption routes such as specific transporter-mediated transport, transcytosis, transcellular pathway, and passive diffusion through the tight junction (paracellular pathway) have been proposed for the human intestinal epithelium. Small water-soluble molecules, such as water and lucifer yellow permeate the Caco-2 cell monolayer via the paracellular pathway.11) To investigate the absorption route of myricitrin, we incubated the Caco-2 cell monolayer with various TEER values in a solution containing 100 μM of myricitrin and lucifer yellow in the apical side (Fig. 1). The TEER value represents the intensity of intercellular junction and is

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Abbreviations: DM, degraded myricitrin; LDL, low-density lipoprotein; HPLC, high performance liquid chromatography; MS, mass spectrometry; HBSS, hanks’ balanced salt solution; TEER, transepithelial electrical resistance
related to permeability through the paracellular pathway. The amounts of myricitrin and lucifer yellow in the basal solution were determined after a 2-h incubation. Myricitrin showed permeability similar to lucifer yellow, and myricitrin permeability increased with decreases in TEER value. The effects of metabolic inhibitor, sodium azide, and 2-deoxy-D-glucose on permeability were also examined. The results showed that the presence of sodium azide plus 2-deoxy-D-glucose in the apical has no significant effect on myricitrin permeability. Moreover, the Caco-2 cell monolayer after treatment with myricitrin was washed with HBSS and incubated with methanol for 30 min to extract the incorporated myricitrin into the Caco-2 cells, but the amount of myricitrin in the cellular extracts was below the detection limit. These results suggest that myricitrin permeates the Caco-2 cell monolayer via the paracellular pathway by passive diffusion. Kim et al. have reported that hesperidin glycoside permeates the Caco-2 cell monolayer via the paracellular pathway.\textsuperscript{12)

Ikeno et al. reported that quercetin was easily conjugated in Caco-2 cells.\textsuperscript{13) Hence we analyzed by HPLC a myricitrin solution treated with \textit{C12-glucuronidase and sulfatase after incubation for 2 h with the Caco-2 cell monolayer, but no conjugated myricitrin was detected.\textsuperscript{12,13}

We also investigated the effect of incubation time and concentration on myricitrin permeability using a Caco-2 cell monolayer with a TEER of more than 250 \( \Omega \cdot \text{cm}^2 \) (Fig. 2). Myricitrin at various concentrations was added to the apical side, and after each incubation time the basal solution was recovered and analyzed by HPLC. Most of the myricitrin was retained in the apical side, but myricitrin permeated to the basal side in a time- and concentration-dependent manner. About 0.68% of the 100 \( \mu \text{M} \) myricitrin in the apical side permeated to the

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Fig. 1. Correlation between Transepithelial Permeability and TEER of Myricitrin in the Caco-2 Cell Monolayer. A solution containing 100 \( \mu \text{M} \) myricitrin and lucifer yellow was added to the apical side of the Caco-2 cell monolayer with various TEER values. After a 2-h incubation, the basal solution was analyzed by HPLC.

Fig. 2. Effect of Myricitrin Incubation Time and Concentration on Transepithelial Permeability of Caco-2 Cell Monolayer. A myricitrin solution 100 \( \mu \text{M} \) (A) or 10 to 100 \( \mu \text{M} \) (B) was added to the apical side of the Caco-2 cell monolayer. After each incubation time, 15 to 120 min (A) or 120 min (B), the basal solution was analyzed by HPLC. Each data point shows the mean \( \pm \) SD (n = 4–6). Significance of difference was investigated by ANOVA. Posthoc analysis was done using Tukey-Kramer’s HSD test. Values with different letters are significantly different from each other (\( p < 0.05 \)).
basal side within 2 h of the start of incubation. In humans, Miyazawa et al. reported that 30 min after intake of 2.7 mg/kg cyaniding-3-glucoside, an average of 11 μg/l was detected in the plasma. As for (−)-epigallocatechin-3-gallate and (−)-epigallocatechin, the plasma contained 0.2 to 2.0% of the total intake. These results suggest that although intestinal absorption of flavonoids is poor, intake of myricitrin-rich foods can promote the incorporation of myricitrin into the blood.

Previously, we reported that myricitrin is unstable and degrades under an alkaline condition. To investigate the effect of degradation on Caco-2 cell monolayer permeability, we prepared DM by simulated digestion. Mildly alkaline-treated myricitrin produced certain components, as shown in Fig. 3. The main peak had a molecular weight higher than that of myricitrin, but the permeability of DM was not significantly different from that of intact myricitrin.

In conclusion, the absorption mechanism of flavonoids is complex and differs with the kind of flavonoid. But the present results suggest that myricitrin is absorbed via the paracellular pathway in a time- and concentration-dependent manner, even after degradation by the mildly alkaline intestinal environment. Myricitrin shows strong antioxidant activity as a radical scavenger and metal-ion chelator and is widely distributed in plants. Previously, we found that myricitrin and its alkaline degradation products (DM) strongly inhibited LDL oxidation. These findings suggest that the intake of myricitrin-rich foods might help inhibit harmful oxidation in vivo.

References