Acerola (Malpighia emarginata DC.) Promotes Ascorbic Acid Uptake into Human Intestinal Caco-2 Cells via Enhancing the Gene Expression of Sodium-Dependent Vitamin C Transporter 1

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Summary Acerola (Malpighia emarginata DC.) is a fruit containing abundant ascorbic acid (AsA) and numerous functional phytochemicals. We previously reported that the intake of acerola juice increased the absorption of AsA in plasma of healthy Japanese subjects. The functional phytochemicals in acerola may influence the intestinal epithelial cells to increase the cellular uptake of AsA. Therefore, in this study, we compared the AsA uptake into Caco-2 cells between AsA alone and that in acerola juice at the same concentration using a human intestinal model. Caco-2 cells were incubated with 3 mM AsA and 3 mM AsA in acerola juice. Intracellular AsA contents gradually increased until 24 h upon incubation with both AsA alone and AsA in acerola juice; however, these contents when incubated with AsA in acerola juice, were significantly higher than those incubated with AsA alone at 2, 3, 4, 8, and 24 h. Furthermore, the mRNA expression level of the sodium-dependent vitamin C transporter (SVCT) 1 was significantly higher in the cells incubated with AsA in acerola juice than those incubated with AsA alone. Moreover, polyphenols such as cyanidin-3-glucoside chloride and quercetin enhanced the SVCT1 gene expression in Caco-2 cells. Collectively, these results suggest that acerola polyphenols enhances the SVCT1 gene expression in Caco-2 cells and promotes AsA uptake.

Key Words acerola, ascorbic acid, Caco-2 cells, functional phytochemical, polyphenol, SVCT1, vitamin C

Acerola (Malpighia emarginata DC.) is a fruit containing abundant vitamin C (L-ascorbic acid, AsA) and numerous functional phytochemicals such as carotenoids and polyphenols (1–4). We previously reported that acerola comprises various polyphenols, such as cyanidin-3-O-rhamnoside, pelargonidin-3-O-rhamnoside, quercetin-3-O-rhamnoside, kaempferol glycosides, astilbin, and proanthocyanidin (1, 2, 4). Furthermore, we have demonstrated the beneficial effects of acerola in humans, that is, the intake of acerola juice increased the AsA absorption in plasma and reduced its excretion via urine in healthy Japanese subjects, which indicated an enhanced AsA bioavailability in humans (5). High AsA concentration in plasma may be due to the functional phytochemicals in acerola juice that contribute to the increased cellular AsA uptake in the intestinal epithelial cells. Therefore, in the present study, we compared the AsA uptake into Caco-2 cells between AsA alone and that in acerola juice at the same concentration using a human intestinal model, and found that AsA in acerola juice had higher cellular uptake accompanied by an enhanced sodium-dependent vitamin C transporter (SVCT) 1 gene expression.

MATERIALS AND METHODS

Chemicals. AsA, cyanidin chloride, cyanidin-3-glucoside chloride, quercetin, and quercetin-3-glucoside were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). AsA was dissolved in water at a concentration of 300 mM and diluted with the culture medium at a concentration of 3 mM. Cyanidin chloride, cyanidin-3-glucoside chloride, quercetin, and quercetin-3-glucoside were dissolved in dimethyl sulfoxide at a concentration of 10 mM and diluted with the culture medium at concentrations of 1 μM and 5 μM.

Acerola juice. Acerola juice was prepared from frozen mature fruit procured from Nichirei Suco Vietnam Co. Ltd. (Tien Giang Province, Vietnam). The frozen acerola fruit was defrosted and squeezed by using a juice extractor (GP-E1503, Green Power Co. Ltd., South Korea), followed by filtration (No. 5C, Toyo Advantec Co., Tokyo, Japan). The prepared acerola juice was immediately frozen and stored at −30°C until further use.

AsA uptake into Caco-2 cells. AsA uptake was performed with differentiated Caco-2 (human intestinal epithelial cell line) monolayers to replicate the intestinal barrier. Caco-2 was purchased from KAC Co. Ltd. (Kyoto, Japan). Cells were plated in 24-well plates (Corning...
Acerola Promotes Ascorbic Acid Uptake into Cells

Incorporated, Corning, NY, USA) at a density of 1.8×10^4 cells/well and were cultured for 21 d. The apical (upper) and basolateral (lower) compartments received 0.5 mL and 1.5 mL of the culture medium, respectively. For the time-course study of AsA uptake, 3 mM AsA alone and 3 mM of AsA in acerola juice in the medium were added to the apical side of inserts and were incubated for 1, 2, 3, 4, 8, or 24 h. At a specific duration, the cells were washed with the Hank’s balanced salt solution and were collected using 0.1% sodium dodecyl sulfate (FUJIFILM Wako Pure Chemical Corporation) to determine the AsA content in the cells. Protein concentration was determined with a BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA) using bovine serum albumin as the standard.

**AsA determination.** AsA was evaluated by using high-performance liquid chromatography coupled with electrochemical detection, as per a method described previously (6). For determining the AsA in acerola juice, acerola juice was diluted 10,000-fold and mixed with 5% metaphosphoric acid containing 1 mM ethylenediaminetetraacetic acid; whereas for assessing the AsA in acerola juice in the medium were added to the apical side of inserts and were incubated for 1, 2, 3, 4, 8, or 24 h. At a specific duration, the cells were washed with the Hank’s balanced salt solution and were collected using 0.1% sodium dodecyl sulfate (FUJIFILM Wako Pure Chemical Corporation) to determine the AsA content in the cells. Protein concentration was determined with a BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) using bovine serum albumin as the standard.

**Quantitative real-time RT-PCR analysis.** Using THUNDERBIRD® Probe qPCR Mix (Toyobo, Osaka, Japan), qPCR was performed according to the manufacturer’s instructions. The primers and 5′-carboxyfluorescein (6-FAM)/N, Ndiethyl-4-(4-nitrophenyl)-1-ylazo)-phenylamine (ZEN)/Iowa Black® FQ-3′ double-quenched probes for SVCT1, SVCT2, and glycerolaldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Integrated DNA Technologies, Inc. (Coralville, IA, USA). The primer sequences have been provided in Table 1. The reactions were performed using a real-time PCR instrument (StepOne Plus, Applied Biosystems, Foster City, CA, USA). The amplification protocol comprised denaturation at 95°C for 1 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. For the quantitative analysis of each mRNA expression level, a standard curve was designed; an aliquot of each experimental sample was used to generate the standard curves. The mRNA expression levels of SVCT1 and SVCT2 were evaluated relative to the levels of GAPDH.

**Statistical analysis.** The experimental results have been shown as the means±standard error of the mean. The probability of significant differences between the experimental groups was determined with the Welch’s t-test using the GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA, USA). Differences were considered as statistically significant at p<0.05.

**RESULTS AND DISCUSSION**

**AsA uptake into Caco-2 cells**

Acerola is known to contain high amounts of AsA. To adjust the AsA concentration, we initially determined its concentration in 100% acerola juice used, which was 49 mM. In the present study, we used 3 mM AsA, assuming that the consumption of acerola juice bever-
was significantly higher than AsA; moreover, the intracellular AsA, although these two AsA in acerola juice (magenta bars) for 24 h.

AsA (orange bars) and AsA (orange bars) and AsA in acerola juice (magenta bars) for 1, 2, 3, and 8, and 24 h, and the intracellular AsA contents were determined at the indicated times. Values have been expressed as the mean±standard error of the mean of three to four plates. * \( p<0.05 \), ** \( p<0.01 \), *** \( p<0.001 \). N.D., not detected.

Fig. 1. AsA uptake into Caco-2 cells during incubation. Cells were incubated with 3 mM AsA (orange bars) and 3 mM AsA in acerola juice (magenta bars) for 1, 2, 3, 4, 8, and 24 h, and the intracellular AsA contents were determined. Intracellular AsA contents gradually increased until 24 h after incubation with both AsA alone and AsA in acerola juice (Fig. 1); however, these contents incubated with AsA in acerola juice were significantly higher (1.7-, 1.7-, 1.4-, 1.3-, and 1.3-fold) compared to those incubated with AsA alone at 2, 3, 4, 8, and 24 h, respectively. Moreover, no significant difference was observed after 1 h.

In our previous report using human skin fibroblasts, the cellular uptake of AsA increased until 12 h and then it gradually decreased until 24 h when incubated with 0.5 mM AsA; moreover, the intracellular AsA contents measured 29.8 nmol/mg protein at 12-h peak time (8). In the present study, intracellular AsA contents of Caco-2 cells measured 107.2 nmol/mg at 24 h, when incubated with 3 mM AsA alone. Although the AsA concentration added to the cells differed, the difference in intracellular AsA concentration between human skin fibroblasts and Caco-2 cells may arise due to the variation in the maximum intracellular AsA concentration that can be maintained. In particular, the AsA concentration differs in various tissues (6).

Effects of acerola on SVCT1 and SVCT2 mRNA expression

AsA is known to migrate through cell membranes using two special transporters, SVCT1 and SVCT2, both being 12-transmembrane proteins (9). SVCT1 and SVCT2 play pivotal roles in the sodium-dependent and secondary active transport of AsA from the outer to the interior of cells (10). Therefore, we further examined whether acerola affected the SVCT1 and SVCT2 gene expression. Caco-2 cells were incubated with 3 mM AsA and AsA in acerola juice for 24 h, and the SVCT1 and SVCT2 mRNA expression levels were analyzed using quantitative real-time RT-PCR analysis. SVCT1 mRNA expression levels incubated with AsA in acerola juice were 1.8-fold higher than those incubated with AsA alone (Fig. 2A); however, no differences were observed in the SVCT2 mRNA expression levels between AsA and AsA in acerola juice (Fig. 2B).

SVCT1 is involved in the whole-body homeostasis of AsA and exhibits a higher maximum velocity than SVCT2 (11). Human SVCT1 is distributed in numerous tissues, including the intestine, the lung, the liver, the kidneys, and the skin. These results strongly suggested the possibility that acerola enhanced the SVCT1 gene expression in the Caco-2 cells and contributed to a high AsA uptake.

Effects of polyphenols on SVCT1 mRNA expression

Acerola is known to contain numerous functional phytochemicals, such as carotenoids and polyphenols (1–4). Previously, we found that acerola comprised various polyphenols, such as cyanidin-3-\( \alpha \)-O-rhamnoside, pelargonidin-3-\( \alpha \)-O-rhamnoside, quercetin-3-\( \alpha \)-O-rhamnoside, kaempferol glycosides, astilbin, and proanthocyanidin (1, 2, 4). Therefore, presumably, these polyphenols may affect SVCT1 gene expression in Caco-2 cells. To confirm this possibility, we examined SVCT1 mRNA expression levels in Caco-2 cells by incubating with commercially available polyphenols including cyanidin chloride, cyanidin-3-glucoside chloride, quercetin, and quercetin-3-glucoside. In this study, we used 1 \( \mu \)M and 5 \( \mu \)M polyphenols, assuming that the consumption of acerola juice beverage corresponded to 6% acerola juice (2). The SVCT1 mRNA expression level after incubation with cyanidin-3-glucoside chloride and quercetin at a concentration of 5 \( \mu \)M was significantly higher than that with AsA (Fig. 3). No differences were observed after incubation with cyanidin chloride and quercetin-3-glucoside at a concentration of 5 \( \mu \)M, although these two

Fig. 2. Effects of AsA and AsA in acerola juice on the SVCT1 and SVCT2 mRNA expression in Caco-2 cells. Cells were incubated with 3 mM AsA (orange bars) and 3 mM AsA in acerola juice (magenta bars) for 24 h. SVCT1 (A) and SVCT2 (B) mRNA expression levels were measured by quantitative real-time RT-PCR analysis. The mRNA expression of SVCT1 and SVCT2 was evaluated relative to the GAPDH level. Values have been expressed as the means±standard errors of the mean of three to four plates. * \( p<0.05 \).
polyphenols exhibited higher SVCT1 mRNA expression levels than that by AsA alone. Thus, certain polyphenols in acerola may affect the SVCT1 mRNA expression.

In a previous study, we reported that crude acerola polyphenol fraction had a preventive effect on hyperglycemia in the postprandial state, wherein the suppression of the intestinal glucose transport and inhibition of α-glucosidase by using Caco-2 cells and KK.Ay mice served as an animal model for obesity and diabetes study (J2). Moreover, we have demonstrated that the intake of acerola juice increased the AsA absorption in plasma and minimized its excretion via urine in healthy Japanese subjects (J5). These preventive effects on hyperglycemia and an enhanced AsA bioavailability may occur due to an enhanced AsA absorption from the small intestine by polyphenols.

In conclusion, acerola promoted an AsA uptake into the Caco-2 cells by enhancing the gene expression of SVCT1.

**Authorship**

YT, HA, YK, and AI designed the research. YT, HA, and AI conducted the experiments, and YT, HA, and AI analyzed the data. YT, HA, YK, and AI wrote the manuscript and had primary responsibility for the final content of the manuscript.

**Disclosure of state of COI**

HA is an employee of Nichirei Foods Inc. and is dedicated to the functional evaluation of food. The remaining authors declare no competing interests.

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