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Transepithelial transport of artepillin C in intestinal Caco-2 cell monolayers

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Abstract

The absorption characteristics of artepillin C (AC), an active ingredient of Brazilian propolis, were examined by measuring permeation across Caco-2 cell monolayers. The permeation rate in the basolateral-to-apical direction, $J_{bl \rightarrow ap}$, in the presence of proton gradient was 0.14 nmol/min/mg protein, whereas $J_{bl \rightarrow ap}$ in the absence of proton gradient was 1.14 nmol/min/mg protein. The latter value is nearly the same as the permeation rate in the apical-to-basolateral direction, $J_{ap \rightarrow bl}$, both in the presence and absence of proton gradient. In the presence of proton gradient, $J_{ap \rightarrow bl}$ was almost constant, irrespective of NaN_3 or benzoic acid. However, $J_{bl \rightarrow ap}$ dramatically increased upon the addition of NaN_3 or benzoic acid specifically to the apical side. In both the presence and absence of proton gradient, $J_{ap \rightarrow bl}$ also appeared to be constant irrespective of the paracellular permeability of Caco-2 cells. After AC was loaded apically in the presence of proton gradient, the intracellular AC increased with time. This accumulation was inhibited by apically loaded NaN_3. These indicate that AC transport occurs mainly via transcellular passive diffusion, although a considerable amount of AC was taken up intracellularly by monocarboxylic acid transporter (MCT) on the apical side and not transported out across the basolateral membrane, suggesting that different subtypes of MCT are involved.

Keywords: Artepillin C; Monocarboxylic acid transporter; Transcellular passive diffusion; Caco-2

1. Introduction

Propolis is the generic name for the resinous hive product collected by honeybees from various plants sources. It is generally composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen and 5% various other substances, including organic debris [1]. Processed propolis, such as propolis tincture or extract made by removing the wax and organic debris, is extensively used in foods, beverages and traditional medicines, especially in Brazil and Eastern Europe. Specifically, these products are known to contain antibacterial, antiviral, anticarcinogenic, anti-inflammatory and immuno-stimulatory agents [2–6]. Since propolis contains a mixture of plant-derived compounds such as flavonoids, phenolic acids and esters, aromatic aldehydes and alcohols, terpenoids and β-steroids, the precise composition varies depending on the source of the plant species. To date, more than 200 compounds have been isolated from propolis [7]. However, the most important constituents appear to be phenolic compounds, which account for more than ca. 50% of the mixture [8].

Artepillin C (AC; 3,5-diprenyl-4-hydroxycinnamic acid), one of the principal phenolic acids found in propolis extract, exhibits various biological activities, such as antibacterial, antiviral and anticarcinogenic properties [2–6]. The physiological significance and health benefit associated with AC is dependent on the intestinal absorption and subsequent interactions with target tissues. However, there are very few reports in the literature

Abbreviations: AC, artepillin C; HBSS, Hanks' balanced salt solution; TER, transepithelial electrical resistance; MCT, monocarboxylic acid transporter; ECD, electrochemical detector

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concerning the absorption, distribution and excretion of AC. Recently, Shimizu and coworkers reported that intact AC is readily absorbed by the intestine and gives protection against oxidative stress. The study used human intestinal Caco-2 cells as an in vitro model of intestinal absorption and metabolism [9–11]. They concluded that AC is the principal bioactive compound in propolis [12]. Although the preliminary absorption characteristics of AC were examined [12], further work is required to fully understand the effect of AC on human health.

Recently, it has been shown that certain phenolic acids, such as ferulic or *p*-coumaric acids, are actively absorbed by the monocarboxylic acid transporter (MCT) in Caco-2 cells [13,14]. The key components of a substrate for MCTs are thought to be monoanionic carboxylic acid group and a nonpolar side chain or aromatic hydrophobic moiety [15]. However, dihydroxy and trihydroxy derivatives of benzoic or cinnamic acids, such as caffeic and gallic acids, have either no affinity or a very low affinity for MCT. These compounds are mainly absorbed by paracellular diffusion [14,16]. The hydroxyl groups of these derivatives are thought to interfere with the molecular recognition between MCT and the phenolic acid [17]. Similarly, esterified phenolic acids, such as chlorogenic acid and rosmarinic acid, have no affinity for MCT and are absorbed by paracellular diffusion [16,18]. Indeed, the absorption efficiency of phenolic acids in vivo increases in the order: gallic acid = rosmarinic acid < caffeic acid < *p*-coumaric acid, according to the affinity for MCT. Indeed, this correlates with the results of in vitro studies using Caco-2 cells. This illustrates the high absorption efficiency and physiological significance of MCT-mediated transport in vivo [19,20].

AC, having a monocarboxylic acid group and an aromatic group, seems to fulfill the structural criteria for an MCT substrate (Fig. 1), but there is a paucity of experimental data to verify whether AC is recognized and transported by MCT. This study was designed to identify the absorption mechanism of AC by directly measuring transepithelial transport across Caco-2 cells. We have used an HPLC-electrochemical detector (ECD) fitted with a coulometric detection system, which enables the measurement of AC with high sensitivity and accuracy. Our results suggest, for the first time, that different subtypes of MCTs participate in the absorption of phenolic acids in Caco-2 cells. MCT on the apical side appears to be different from MCT on the basolateral side in terms of the affinity for AC.

2. Materials and methods

2.1. Materials

The human colon adenocarcinoma cell line, Caco-2, was obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceuticals (Tokyo, Japan). Fetal calf serum, glutamine, nonessential amino acids, penicillin (10 000 units/mL in 0.9% NaCl), streptomycin (10 mg/mL in 0.9% NaCl), phosphate-buffered saline and Hank's balanced salt solution (HBSS) were all purchased from Invitrogen Corp. (Carlsbad, CA). Type I collagen was purchased from Nitta Gelatin Inc. (Osaka, Japan). Plastic dishes, plates and Transwell inserts with 0.4-μm polycarbonate membranes (12 mm in

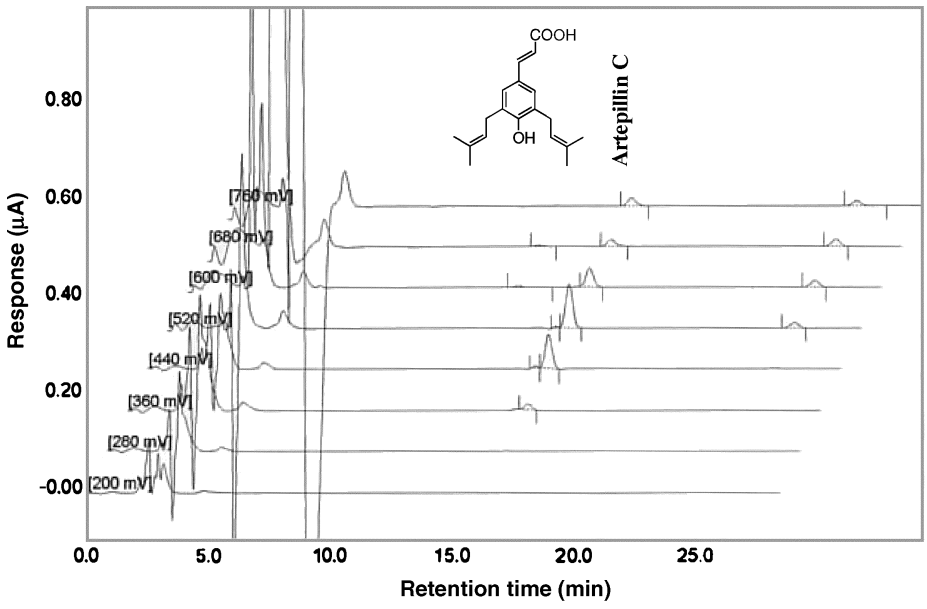

Fig. 1. Chromatograms of AC transported across Caco-2 cell monolayers.

diameter) were obtained from Corning (Corning, NY). AC was from Wako Pure Chemicals Inc., Ltd. (Osaka, Japan). All other chemicals used in this study were of analytical grade.

2.2. Cell culture

Caco-2 cells were cultured in DMEM containing 10% fetal calf serum, 1% nonessential amino acids, 4 mM L-glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin (pH 7.4). Cells were grown in a humidified atmosphere of 5% CO_2 at 37 °C. All cells used were between passages 57 and 68.

2.3. Transepithelial transport experiments

Cells were grown in Transwell inserts with the semi-permeable membrane first coated with type I collagen (12-mm diameter and 0.4-μm pore size, Corning Costar, NY). The cells were seeded at a density of $1 \times 10^5/cm^2$ and a monolayer was formed after culturing for 2 weeks. The integrity of the cell layer was evaluated by measurement of transepithelial electrical resistance (TER) with Millicell-ERS equipment (Millipore, MA). A monolayer with a TER of more than 250 Ω cm^2 was used for the transepithelial transport experiments.

To measure the apical-to-basolateral permeability, 1.5 ml of HBSS (pH 7.4, 37 °C) was added to the basal chamber of the Transwell insert and then 0.5 ml of the test solution (pH 6.0 or 7.4, 37 °C) containing AC (0.1 mmol/L) was added to the apical side. After the desired incubation time at 37 °C, the basal solution was collected and then replaced with an equal volume of HBSS. The amount of AC transported by the Caco-2 cells was determined using a HPLC-electrochemical detector (ECD) with an ESA coulometric detection system (ESA Inc., Boston, MA). The results were expressed in terms of specific permeability (μL/cm^2), which was calculated as the amount transported divided by the initial concentration in the donor compartment.

To examine the basolateral-to-apical transport, HBSS (pH 6.0 or 7.4, 37 °C) was added to the apical side and 1.5 ml of the test solution (pH 7.4, 37 °C) was added to the basolateral side.

2.4. Chromatographic conditions

HPLC-ECD fitted with a coulometric detection system was used for analysis as previously reported [13,14]. Chromatographic separation was performed on a C18 column (ODS150, MC Medical, Inc., Tokyo, Japan). The mobile phase A (Solvent A) was 50 mM sodium acetate containing 40% acetonitrile and 20% methanol (pH 3.0), while mobile phase B (Solvent B) was 50 mM sodium acetate containing 80% methanol (pH 3.5). The elution profile (0.6 mL/min) was as follows: 0–28.5 min, linear gradient from 85% Solvent A/15% Solvent B to 0% Solvent A/100% Solvent B; 28.5–32 min, isocratic elution 0% Solvent A/100% Solvent B; 32–35 min, isocratic elution 85% Solvent A/15% Solvent B. Eight electrode detector potentials (200 to 760 mV in increments of 80 mV) were used to measure the amount of AC.

2.5. Distribution of AC after transport experiments

AC was loaded on the apical or basolateral side of the cell monolayer for the desired incubation time at 37 °C. The level of AC in the apical and basolateral solutions was then measured. The monolayer cells were rinsed with HBSS (pH 6.0 or 7.4) and extracted with methanol/Solvent B (10:1, v/v) for 30 min. AC in this extract was measured and used as an index of the intracellular fractions taken up by the Caco-2 cells.

2.6. Data Analysis

The permeation rate [nmol/min/mg protein], J, was evaluated from the slope of the initial linear part of the plot of the amount transported [nmol/min/mg protein] against time (in minutes), calculated by linear regression analysis. Results are expressed as the mean ± S.D. Statistical analysis was done with Student's two-tailed t-test, and differences with $P < 0.01$ were considered significant.

3. Results

3.1. HPLC analysis of AC transported across Caco-2 cell monolayers

A representative chromatogram of AC transported into the basolateral solution is presented in Fig. 1. AC was determined at a detection limit <0.5 pmol on the column. The results were reproducible without requiring any sample pre-treatment. The purity of the peaks was assessed using peak area ratio accuracies for the adjacent oxidation channels (lower or upper) to the dominant oxidation channel. The voltammetric response of the analyte across these channels was unique for each compound. A greater than 70% ratio accuracy was considered to constitute peak purity [21]. The retention time (RT) and dominant oxidation potential for AC were 16.6 min and 520 mV, respectively.

3.2. Characteristics of transepithelial transport of AC

The bi-directional permeation of AC (0.1 mM) across a Caco-2 cell monolayer was examined in the presence and absence of an inwardly directed proton gradient (Fig. 2A). AC exhibited directional transport from the apical to the basolateral side in the presence of a proton gradient ($J_{ap \rightarrow bl}$ and $J_{bl \rightarrow ap}$ being 0.91 and 0.14 nmol/min/mg protein, respectively, with an apical pH, 6.0, and basolateral pH,

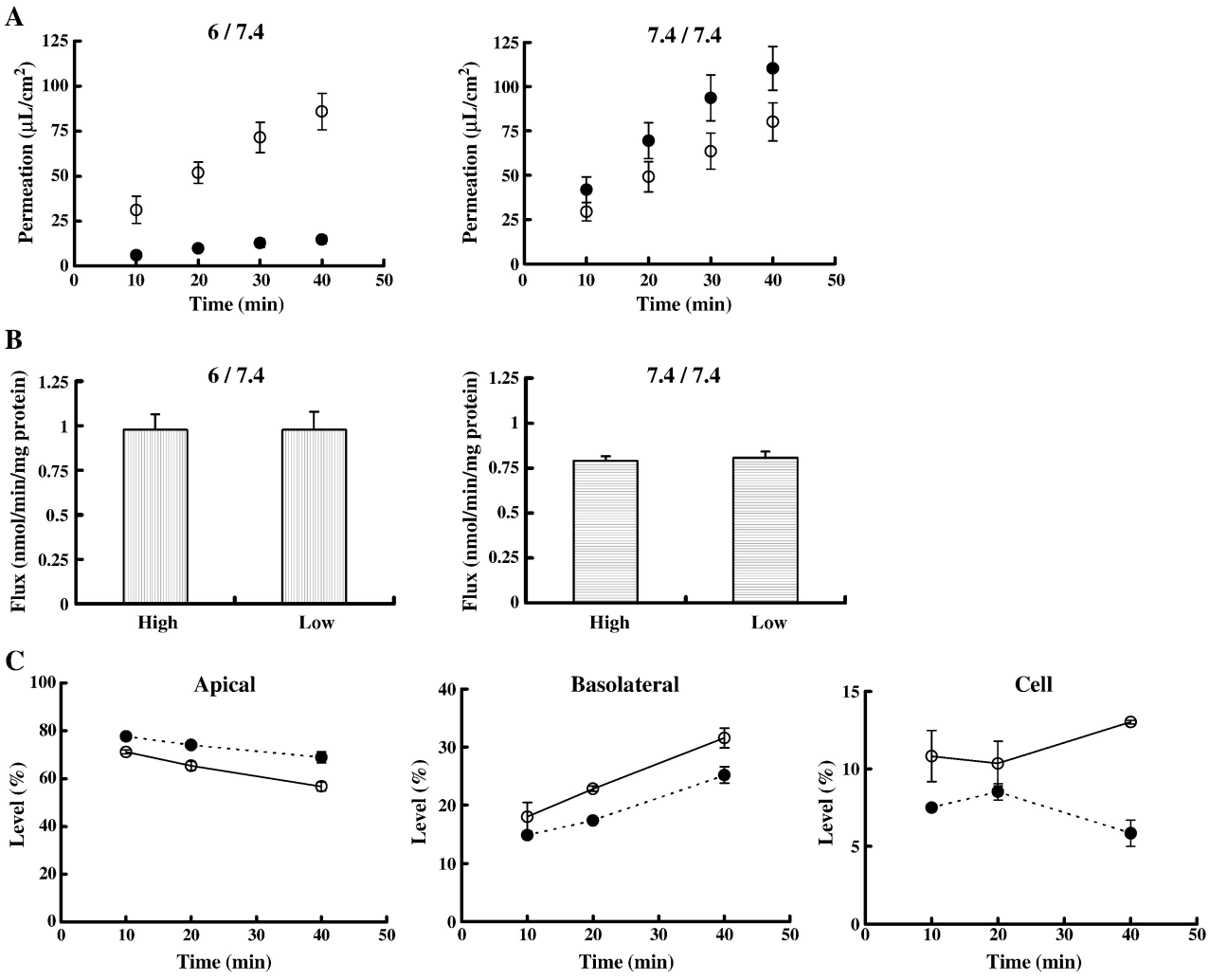

Fig. 2. Transport characteristics and changes of distribution of AC in Caco-2 cell monolayers. (A) The permeation of AC (0.1 mM) from the apical to the basolateral side (○) and from the basolateral to the apical side (●) was measured at 37 °C in the presence (left panel) and absence (right panel) of a proton gradient (apical pH, 6.0 or 7.4; basolateral pH, 7.4). Each point is the mean ± S.D. of three experiments. (B) AC (0.1 mM) was loaded in the apical side, and the flux from the apical side to the basolateral side was measured at 37 °C in the presence (left panel) and absence (right panel) of a proton gradient (apical pH, 6.0 or 7.4; basolateral pH, 7.4). Each point is the mean ± S.D. of three experiments. TER values are indicated as follows: (left panel) high: 1110 ± 67, low: 273 ± 8; (right panel) high: 965 ± 36, low: 259 ± 13. (C) AC (0.1 mM) was loaded in the apical side, and changes of distribution with incubation time were measured at 37 °C in the presence (●) and absence (○) of 5 mM NaN_3 (apical pH, 6.0; basolateral pH, 7.4). Each point is the mean ± S.D. of three or more experiments.

7.4). However, in the absence of a proton gradient (apical pH, 7.4; basolateral pH, 7.4), $J_{ap \rightarrow bl}$ (0.83 nmol/min/mg protein) was slightly less than $J_{bl \rightarrow ap}$ (1.14 nmol/min/mg protein). However, $J_{ap \rightarrow bl}$ (0.83 nmol/min/mg protein) was nearly the same as that of $J_{ap \rightarrow bl}$ (0.91 nmol/min/mg protein) in the presence of a proton gradient. AC appears to exhibit polarized transport in an apical-to-basolateral direction in the presence of a proton gradient. In contrast, polarized transport in a basolateral-to-apical direction appears to occur in the absence of proton gradient. These results indicate that the permeation of AC is complex.

Caco-2 cell monolayers exhibiting different TER values were prepared by treating the cells with cytochalasin D [22]. Using these monolayers, the apical-to-basolateral transport of AC was then examined in the presence or absence of a proton gradient. As illustrated in Fig. 2B, the transepithelial flux was almost constant irrespective of the TER, both in the presence and absence of a proton gradient. However, the transepithelial flux was slightly greater in the presence of a proton gradient.

3.3. Effects of NaN_3 or benzoic acid on the transepithelial transport of AC

The effect of the metabolic inhibitor, NaN_3 (5 mM), or benzoic acid (10 mM), a substrate of MCT, was studied by addition to either the apical or basolateral solution in the presence of a proton gradient. The permeation of AC in both the apical-to-basolateral and basolateral-to-apical directions was examined. $J_{ap \rightarrow bl}$ was unaffected by the apical or

basolateral addition of NaN_3 or benzoic acid (Table 1). Likewise, $J_{bl\rightarrow ap}$ was constant when NaN_3 or benzoic acid was loaded basolaterally. However, $J_{bl\rightarrow ap}$ increased up to the same level as $J_{ap\rightarrow bl}$ when NaN_3 or benzoic acid was loaded apically (Table 1).

3.4. Distribution of AC

After AC was loaded on the apical side of Caco-2 cells in the presence of a proton gradient, considerable levels of dosed AC were detected in both intracellular (6.6%) and basolateral (36.7%) fractions, while the level of AC on the apical side was 56.7% (Table 2A). In the absence of a proton gradient, however, the intracellular level (2.9%) and basolateral level (25.6%) of AC decreased in parallel with increasing levels of AC in the apical fraction (71.5%). The apical addition of NaN_3 or benzoic acid together with AC in the presence of a proton gradient caused a decrease in the level of AC in both the intracellular and basolateral fractions and an increase in the apical fraction (Table 2A). This result resembles the experiment conducted in the absence of a proton gradient (apical level, 71.5%; basolateral level, 25.6%; intracellular level, 2.9%). Basolaterally loaded NaN_3 or benzoic acid did not affect the distribution of AC in the presence of a proton gradient (Table 2A). Then, the distribution of AC was examined after AC was basolaterally loaded in the presence of a proton gradient. The levels of AC in the apical and intracellular fractions were low, while a large amount of AC remained on the basolateral side (Table 2B). However, when NaN_3 or benzoic acid was loaded apically, a dramatic increase in the level of AC in the apical fraction and a corresponding decrease in the basolateral fraction was observed (Table 2B).

Furthermore, changes in the distribution of apically loaded AC in the presence of a proton gradient were measured. The level of AC in the intracellular fraction increased with time, while the levels on the apical and basolateral sides decreased (Fig. 2C). Although the distribution of AC on the apical and basolateral sides showed a similar trend in the presence of apically loaded NaN_3, a decrease in the intracellular levels of AC was observed (Fig. 2C).

Table 1
Effects of NaN_3 and benzoic acid loaded apically or basolaterally on the permeation of AC loaded apically or basolaterally in Caco-2 cell monolayers in the presence of a proton gradient

	$Benzoate_{ap}$	$Benzoate_{bl}$	NaN_{3ap}	NaN_{3bl}
Relative permeation (% of control)				
$J_{ap\rightarrow bl}$	118.9±9.8	131.9±11.7	117.4±8.5	126.3±13.2
$J_{bl\rightarrow ap}$	508.5±38.6*	53.5±8.5*	413.3±42.1*	59.8±6.8*

Transepithelial transport experiments were done as described in Materials and methods in the apical-to-basolateral and basolateral-to-apical directions in the presence of a proton gradient (apical pH, 6.0; basolateral pH, 7.4) with or without (control) benzoate (10 mM) and NaN_3 (5 mM), loaded apically or basolaterally. Values are the mean±S.D. of three or more experiments. *$P<0.01$.
$Benzoate_{ap}$, benzoate apically loaded; $Benzoate_{bl}$, benzoate basolaterally loaded NaN_{3ap}, NaN_3 apically loaded; NaN_{3bl}, NaN_3 basolaterally loaded.

Table 2
Effects of NaN_3 and benzoic acid loaded apically or basolaterally on the distribution of AC loaded apically or basolaterally in Caco-2 cell monolayers in the presence of a proton gradient

	Control	$Benzoate_{ap}$	$Benzoate_{bl}$	NaN_{3ap}	NaN_{3bl}
(A) Distribution (%)					
Ap	56.7±2.5	68.7±1.4	58.4±3.1	68.5±3.1	57.5±3.5
Bl	36.7±3.7	26.5±2.7	35.8±3.8	27.7±3.7	37.5±5.1
Cell	6.6±1.9	4.8±1.3	5.9±1.0	3.8±1.0	5.0±1.9
(B) Distribution (%)					
Ap	1.7±0.1	7.7±0.9	1.1±0.5	6.5±0.4	1.7±0.0
Bl	97.3±0.1	90.9±0.9	98.0±0.5	92.6±0.4	97.8±0.1
Cell	1.0±0.1	1.4±0.1	0.9±0.1	0.9±0.1	0.6±0.0

(A) After AC was loaded apically, transepithelial transport experiments were done and distribution were examined in the presence of a proton gradient (apical pH, 6.0; basolateral pH, 7.4) with or without (control) benzoate (10 mM) and NaN_3 (5 mM), loaded apically or basolaterally. Levels of apical, basolateral, and intracellular pools (%) in the absence of proton gradient (apical pH, 7.4; basolateral pH, 7.4) are 71.5±3.2%, 25.6±3.8%, and 2.9±1.3%, respectively.
$Benzoate_{ap}$, benzoate apically loaded; $Benzoate_{bl}$, benzoate basolaterally loaded NaN_{3ap}, NaN_3 apically loaded; NaN_{3bl}, NaN_3 basolaterally loaded.
(B) After AC was loaded basolaterally, transepithelial transport experiments were done and distribution were examined in the presence of a proton gradient (apical pH, 6.0; basolateral pH, 7.4) with or without (control) benzoate (10 mM) and NaN_3 (5 mM), loaded apically or basolaterally. Levels of apical, basolateral, and intracellular pools (%) in the absence of proton gradient (apical pH, 7.4; basolateral pH, 7.4) are 9.3±0.9%, 89.4±1.0%, and 1.3±0.1%, respectively.

4. Discussion

Because AC exhibited low solubility in HBSS, the permeation of 0.1 mM AC across Caco-2 cells was examined in this study. Except for $J_{bl\rightarrow ap}$ in the presence of a proton gradient (0.14 nmol/min/mg protein), $J_{ap\rightarrow bl}$ in the presence and absence of a proton gradient and $J_{bl\rightarrow ap}$ in the absence of a proton gradient, all gave similar values (0.83–1.14 nmol/min/mg protein). This value is approximately one-tenth that of MCT substrates with high affinity at a concentration of 1 mM (e.g. *m*-coumaric acid, 7.23 nmol/min/mg protein; *m*-hydroxyphenylpropionic acid (mHPP), 7.47 nmol/min/mg protein; ferulic acid, 9.79 nmol/min/mg protein) [13,23]. However, the absorption characteristics of AC are different from those of the MCT substrates, because the pH-dependent polarized transport in the apical-to-basolateral direction, characteristic of MCT-mediated transport, was not observed in the permeation of AC (Fig. 2A). The increase of $J_{bl\rightarrow ap}$ in the presence of a proton gradient up to the same level as $J_{ap\rightarrow bl}$ was detected only when NaN_3 or benzoic acid was loaded on the apical side of the monolayer (Table 1). The basolateral loading of NaN_3 or benzoic acid did not have this effect. Intriguingly, $J_{ap\rightarrow bl}$ was unchanged with and without NaN_3 or benzoic acid when loaded apically or basolaterally (Table 1). These results indicate that energy-dependent transport is involved

during the permeation of AC in the basolateral-to-apical direction only in the presence of a proton gradient, although AC mainly permeates across Caco-2 cells in a non-energy-dependent manner. It was also demonstrated that the apical-to-basolateral flux ($J_{ap \rightarrow bl}$) was almost constant irrespective of the paracellular permeability of Caco-2 cells, both in the presence and absence of a proton gradient. However, $J_{ap \rightarrow bl}$ in the presence of a proton gradient was slightly greater than $J_{ap \rightarrow bl}$ in the absence of a proton gradient (Fig. 2B). The results presented here together with the high liphophilicity of AC, due to the diprenyl moiety, suggest that this molecule permeates across Caco-2 cells by transcellular passive diffusion. This conclusion is also supported by the study of Shimizu et al., which indicated that AC was readily incorporated into cells and released intact to the basolateral side [12].

To characterize the pH- and energy-dependent low level permeation $J_{bl \rightarrow ap}$, distribution studies were performed after preloading AC either apically or basolaterally with and without NaN_3 or benzoic acid (Table 2A, B). When AC was loaded basolaterally, the presence of NaN_3 or benzoic acid on the apical side caused a dramatic redistribution. There was a marked increase in the level of AC on the apical side with a corresponding decrease on the basolateral side. Basolaterally loaded NaN_3 or benzoic acid, however, exerted no effect on the distribution of AC. The same trend was also observed for apically loaded AC. Furthermore, changes in the levels of AC in each fraction were measured with time (Fig. 2C). Changes in the intracellular level of AC were detected, which were inhibited by apically loaded NaN_3 (Fig. 2C). The presence or absence of apically loaded NaN_3 did not influence the apical and basolateral redistribution of AC (Fig. 2C). These results, together with our previous work [13,14,16,18], give new insight into the absorption characteristics of AC (Fig. 3). Although the permeation of AC is mainly via passive transcellular diffusion, we believe that a fraction of AC is transported into the cells by MCT on the apical side, which is not then transported out across the basolateral membrane by MCT. This scheme of absorption characteristics of AC is also consistent with the results obtained by Shimizu and cow-orkers [12].

Currently, 14 isoforms of MCT have been identified [24], but only MCT1 to MCT4 are characterized in terms of their substrate and inhibitor kinetics. It is known that glucose is taken up into enterocytes across the brush border membrane by Na^+-driven active transport with SGLT-1, and then transported out across the basolateral membrane by a facilitated diffusion with GLUT2 [25]. In contrast, fructose is taken up by a facilitated diffusion with GLUT5 at the brush border membrane and transported into the blood by a facilitated diffusion with GLUT2 in the basolateral membrane [25]. It is possible that different types of MCTs are involved in the absorption of phenolic acids, as is the case for glucose and fructose. Since apically loaded NaN_3 and benzoic acid are considered to inhibit intracellular AC uptake by MCT on the apical side (Table 2A, B, Fig. 2C), it is likely that active transport would be involved in the permeation process across brush border membrane. At the basolateral side, however, it is unknown that active transport or facilitated diffusion would occur by MCT on the basolateral side, although it was speculated that MCT with no affinity for AC, different from MCT on the apical side, participated in the permeation process. Indeed, it was reported that various subtypes of MCT, such as MCT1, MCT3, MCT4, MCT5 and MCT6, are expressed in Caco-2 cells [26].

Directional transport in the basolateral-to-apical direction appears to take place in the absence of a proton gradient (Fig. 2A). The reason for this polarized transport is not clear.

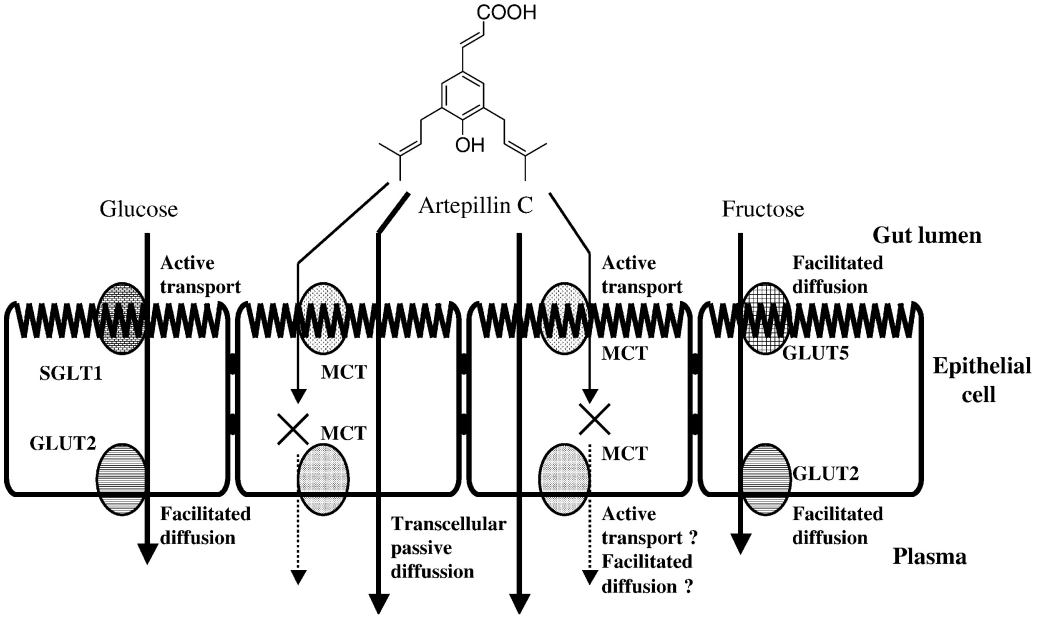

Fig. 3. Proposed pathways for intestinal absorption of AC together with glucose and fructose.

NaN_3 (5 mM) did not have any effect on the transport, suggesting that a specific transport system is not involved. Basolaterally loaded AC caused a change in the TER of Caco-2 cells, suggesting that the membrane integrity or fluidity had altered. This might account for the observed directional transport.

The biological activity and health effect of polyphenol must be reevaluated in terms of their bioavailability, and the physiological significance of microbial metabolites of the parent polyphenols has now been firmly established [27]. We have focused on the physiological impact of MCT-mediated absorption and distribution in human involving specific transport systems not only for phenolic acids but also for microbial metabolites of poorly absorbed polyphenols or dietary fibers [23,28]. To fully assess the health effects of phenolic acids and "metabo-nutrient", i.e. microbial metabolites with biological activities of polyphenols or dietary fibers [28], further studies to characterize various subtypes of MCT molecularly and clarify the tissue distribution and subcellular localization are required.

In conclusion, we have demonstrated that AC is mainly permeated across Caco-2 cells by transcellular passive diffusion. To a lesser extent, AC is also taken up intracellularly by MCT, but not transported out to the basolateral side. This also highlights the subtle absorption system, comprising different subtypes of MCT for dietary components.

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