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Transport of Proanthocyanidin Dimer, Trimer, and Polymer Across Monolayers of Human Intestinal Epithelial Caco-2 Cells

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ABSTRACT

The gut absorption of proanthocyanidins (PAs) and of the related (+)-catechin monomer was investigated with colonic carcinoma (Caco-2) cells of a human origin, grown in monolayers on permeable filters. Permeability of various radiolabeled PAs differing in their molecular weight was compared with that of the radiolabeled (+)-catechin. No toxicity was observed at PA concentrations up to the physiological concentration of 1 m*M*. (+)-Catechin and PA dimer and trimer had similar permeability coefficients (P_{app} = 0.9–2.0 × 10^{-6} cm s^{-1}) close to that of mannitol, a marker of paracellular transport. Paracellular transport was also indicated by the increase of absorption after reduction of the transepithelial electric resistance through calcium ion removal. In contrast, permeability of a PA polymer with an average polymerization degree of 6 (molecular weight 1,740) was ~10 times lower (P_{app} = 0.10 ± 0.04 × 10^{-6} cm s^{-1}). PAs, particularly the most astringent PA polymer, were also adsorbed on the epithelial cells. These results suggest that PA dimers and trimers could be absorbed *in vivo* and that polymer bioavailability is limited to the gut lumen. Antioxid. Redox Signal. 3, 957–967.

INTRODUCTION

ABOUT 1 G OF POLYPHENOLS is ingested every day with our diet (28). Together with other dietary antioxidants (vitamins E and C, carotenoids, etc.), they contribute to limit oxidative stress in human tissues (45). Their consumption should therefore contribute to prevent cancers, cardiovascular diseases, and other chronic diseases associated with oxidative stress (20, 49). Two main classes of polyphenols, hydroxycinnamic acids and flavonoids, are found in food and beverages (48). Among flavonoids, compounds such as quercetin, catechin, or genistein have been largely studied, whereas others such as proanthocyanidins (PAs; *syn* condensed tannins), despite their abundance in our diet, have received much less attention. This is largely explained by their polymeric nature and high structural complexity.

PAs are common constituents of many foodstuffs (fruits, legume seeds, chocolate) and bev-

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erages (fruit juices, wine, beer, cider, tea) (44, 48, 54). They are flavonoid polymers structurally related to the catechin monomers. They constitute the flavanol group among flavonoids. The average polymerization degree of PAs generally varies between 3 and 11 (Fig. 1) (44) and may reach values as high as 17, as has been shown in a cider apple extract by liquid chromatography–mass spectrometry analysis (21). Two main types of PAs can be distinguished according to their hydroxylation pattern: procyanidins (PCs) with a 3′,4′-dihydroxy substitution on the B ring, and the less common prodelphinidins with a 3′,4′,5′-trihydroxy substitution. The phenolic and polymeric nature of PAs makes them good complexants of proteins and explains the astringent character of foods and beverages rich in PAs (skin and seeds of grape, unripe fruits, wine, cider, etc.) (23). The daily intake of PAs has been estimated at 0.1–0.5 g (28, 44, 46). However, the complexity of their chemical structure and the lack of a suitable method for their estimation make these figures uncertain.

To assess their role in human nutrition and disease prevention (see 44 for a review), it is essential to improve our knowledge on their bioavailability. Once ingested, polyphenols may either be directly absorbed in the small intestine or reach the colon where they can be metabolized by the colonic microflora. PA polymers (14) and a PA dimer (18) were shown to be degraded into aromatic acids, which are subsequently absorbed through the colon barrier. Very little is known on the direct absorption of PAs in the small intestine. Previous work in mice and rats suggested that PA dimers were absorbed (22, 30). One tenth of a mixture of ^{14}C-labeled monomers and dimers administered orally was recovered in the urine in an intact flavonoid form. On the other hand, ^{14}C-labeled PA polymers did not cross the intestinal barrier of chickens (26) and sheep (55) and were largely recovered in the feces. Unfortunately, poorly characterized substrates were used in these studies. Dimers were contaminated with some catechin monomers, polymers were poorly characterized, and no details on the radiochemical purity of the labeled PAs were reported.

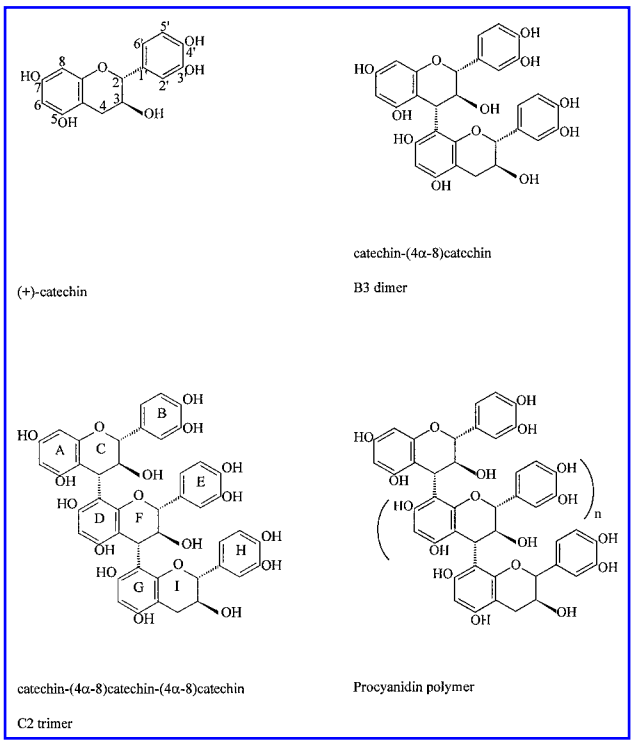

FIG. 1. Chemical structures of procyanidin dimer B3, trimer C2, and polymer and of the related monomer (+)-catechin.

The purpose of the present work is to examine the influence of PA polymerization degree on gut absorption. The Caco-2 cell model is used. Caco-2 cells originate from a human colon adenocarcinoma. When grown on plates or filters, they spontaneously differentiate into a continuous monolayer of cells presenting a morphology and functionality typical of normal ileal enterocytes (4, 24, 41). Such a model has been used to study the intestinal absorption of polyphenols (2, 29, 57, 58) or to predict the absorption of drugs (5, 52). Permeability of passively transported compounds can be predicted with a particularly high degree of accuracy (33). The permeability of three ^{14}C-labeled PCs (dimer B3, trimer C2, and a mixture of PCs of higher polymerization degree) is compared to that of the structurally related (+)-catechin monomer.

MATERIALS AND METHODS

General

D-(−)-mannitol and (+)-catechin were purchased from Prolabo and Fluka, respectively.

[2-^{3}H]-D-Mannitol [20 Ci/mmol; 1 mCi/ml in ethanol/water (9:1)] was purchased from Isotopchim (Peyruis, France). Cell culture reagents were obtained from GIBCO (Cergy Pontoise, France) and plastic dishes from Falcon (Strasbourg, France). All other reagents were purchased from Sigma unless specified. PA dimer, trimer, and polymer concentrations were expressed in catechin unit equivalents.

^{14}C-Labeled flavanols

^{14}C-Labeled flavanols (Table 1) were prepared by administration of [1-^{14}C]acetate to willow shoots and purified by chromatography on Sephadex LH 20; their purity was checked by paper chromatography and reverse-phase HPLC (13). PA polymers were analyzed by thiolysis (36). Yield of thioadducts formed from purified ^{14}C-PA polymers was 42.2%. The two major thioadducts (yield 95%) were 3,4-*cis*- and 3,4-*trans*-benzylthiocatechins typical of PCs based on (+)-catechin units. Only trace amounts (yield 3%) of 2,3-*cis* thioether (derived from epicatechin units) were formed. The ratio of terminal units [(+)-catechin] to thioethers allowed the calculation of an average degree of polymerization of 6. The PA polymer fraction was free of any PA dimer or trimer. All radiolabeled flavanols used in this study were radiochemically pure as no radioactive contaminant could be detected on any chromatogram (13).

Cell culture

The Caco-2 polyclonal cell line was supplied by Dr. G. Trugnan (INSERM, Paris, France). Cells were cultured routinely in HEPES-buffered Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose and supplemented with 12.5% heat-decomplemented fetal calf serum, 1% nonessential amino acids, 5 m*M* L-glutamine, 40 U/ml penicillin, 100 μg/ml gentamycin, and (40 μg/ml) streptomycin (DMEMc). Cells were maintained at 37°C in a humidified atmosphere of CO_2/air (5:95) and passaged every 7 days by trypsinization. They were seeded at 4×10^4 cells/cm^2 and grown in 75-cm^2 flasks. The medium (15 ml) was changed every 2 days until cells reached confluence (7–8 days). Cells were used between passages 43 and 63.

Metabolism of (+)-catechin by Caco-2 cells

The metabolism of (+)-catechin was studied in differentiated Caco-2 cultured in six-well plates (35-mm diameter, Falcon). In brief, cells were seeded at 4×10^4 cells/cm^2 and cultured as described above until day 15 after seeding. On day 15, the culture medium was aspirated and replaced by 3 ml of 1 m*M* (+)-catechin in either fresh culture medium or transport medium. The composition of the transport medium was 10 m*M* HEPES, 10 m*M* glucose, 137 m*M* NaCl, 5.4 m*M* KCl, 0.3 m*M* KH_2PO_4, 1.0 m*M* $MgSO_4$, 0.3 m*M* NaH_2PO_4, 2.8 m*M* $CaCl_2$, buffered to pH 7.4 with tris(hydroxymethyl)aminomethane (Tris). Culture plates were incubated at 37°C, and aliquots of the medium (500 μl) were removed after 4 h (transport medium) and 20 h (culture medium). The samples were immediately mixed with 1 ml of methanol and filtered on 0.45-μm Millex-HV filter units, and (+)-catechin and its metabolites were analyzed by reverse-phase HPLC. Negative controls were run in parallel by incubating (+)-catechin in both transport and culture media in the absence of Caco-2 cells.

TABLE 1. SOME CHARACTERISTICS OF ^{14}C-FLAVAN-3-OLS USED IN TRANSPORT STUDIES

^{14}C-Flavanols	*Polymerization degree*	*Molecular weight*	*Specific activity (μCi/g)*
(+)-Catechin	1	290	302
B3 dimer	2	578	150
C2 trimer	3	866	144
PA polymer	6*	1,740*	188

Concentrations are expressed as (+)-catechin unit equivalents.
*Average value estimated by thiolysis.

Transepithelial electrical resistance

To assess the effect of PA polymers on the transepithelial electrical resistance (TEER) across Caco-2 monolayers, Caco-2 cells were seeded at 2.4×10^4 cells/cm^2 on polyethylene terephthalate filters (0.4 μm; 4.9 cm^2) mounted on cell culture polycarbonate inserts (Falcon) and cultured six-well plates. Cells were grown in DMEMc with 2 ml of fresh medium added in the upper and lower compartments delimited by the filter every 2 days. On day 15 post seeding, TEER was measured using an epithelial volt-ohm-meter with dual electrodes (Endohm, World Precision Instruments Inc., New Haven, CT, U.S.A.). The potential difference was expressed as transmembrane resistance (Ω.cm^2) against a blank insert (intrinsic resistance obtained over a cell-free insert). The effect of PA polymers on TEER was measured as follow: The culture medium was aspirated, cell monolayers were rinsed with transport medium, and 1.8 ml of this medium was added in both the apical and basolateral compartment. After 15 min, a first determination of TEER was done and PA polymers were added to the apical compartment (0.2 ml, final concentration 1 m*M*). Subsequent measurement of TEER were then performed every 15 min for 2 h.

Measuring TEER was essential to interpret the results as previously stressed (12). TEER values varied largely from one well to the other, possibly due to variations in passage number at the time of the experiments [TEER values usually increase with the number of passages (8)] and to the polyclonal nature of the Caco-2 cell line (4).

Transepithelial permeability experiments

Caco-2 cells were seeded at 2.4×10^4 cells/cm^2 on polyethylene terephthalate filters (0.4 μm; 4.9 cm^2) mounted on cell culture polycarbonate inserts (Falcon) and cultured in six-well plates. Cells were grown in DMEMc with 2 ml of fresh medium added in the upper and lower compartments delimited by the filter every 2 days. After 15–17 days, TEER was measured and only monolayers with TEER in the 350–750 Ω.cm^2 range were used for permeability measurements. Culture medium was then removed, and 2 ml of transport medium was added to both the apical and the basolateral compartments of the bicameral chamber. In selected experiments, a modified transport medium with no calcium was used. ^{14}C-Flavanols [0.04–0.18 μCi, 0.5 or 1 m*M* of equivalent (+)-catechin] or [2-^{3}H]-D-mannitol (0.2 μCi, 1 m*M*) was added to the apical compartment of bicameral chambers. Aliquots (400–700 μl) were removed from the basolateral compartment at different time points over 3–4 h and replaced by an equivalent volume of transport medium. The amount of radiolabeled material appearing in the basolateral compartment as a function of time was measured by liquid scintillation counting. At the end of the experiment, the media were aspirated from the apical and basolateral compartments, the filters were rinsed with fresh transport medium, and the amount of radioactivity associated with the cell monolayers was determined by liquid scintillation counting.

Apparent permeability coefficients (P_{app}, cm s^{-1}) were calculated according to the following equation:

$$P_{app} = \Delta Q/\Delta t \times 1/AC_o$$

where $\Delta Q/\Delta t$ is the permeability rate (μg/s), C_0 the initial concentration in the donor (apical) chamber (μg/ml), and A the surface area of the filter (cm^2). All permeability and adsorption values are the means of at least triplicate measurements with separate filters.

Radioactivity counting

Carbon-14 and tritium radioactive countings were carried out with a Beckman 2200 CA Tri-Carb liquid scintillation counter (Villepinte, France) with Ultima Gold LSC-cocktail (Packard, Meriden, CT, U.S.A.). All values were corrected for background.

Statistics

To determine differences between groups, a Kruskall–Wallis test followed by a Mann–Whitney–Wilcoxon *U* test was performed.

RESULTS

TEER values and Caco-2 cell permeability

TEER values are commonly used to evaluate the integrity of Caco-2 cell monolayers and are generally considered acceptable when they exceed 200 (40) or 350 $\Omega.cm^2$ (57). TEER of the monolayers was systematically measured at 15–17 days post seeding before permeability was estimated. It varied widely between 200 and 1,100 $\Omega.cm^2$, and permeability of the monolayer to a PA polymer decreased as TEER increased over the whole interval (Fig. 2). Only monolayers with TEER values higher than 350 and lower than 750 $\Omega.cm^2$ were selected for permeability measurements.

Permeability of mannitol, a hydrophilic and small molecule crossing the cell monolayer by the paracellular route (7, 12) commonly used as a marker of medium permeability, was assessed. A permeability coefficient (P_{app}) of 1.1×10^{-6} $cm.s^{-1}$ was measured at TEER values higher than 350 $\Omega.cm^2$ and lower than 750 $\Omega.cm^2$ and is close to that reported by Walgren *et al.* (57): 0.5×10^{-6} $cm.s^{-1}$ at a TEER higher than 350 $\Omega.cm^2$.

Influence of PAs on Caco-2 cell functionality

No cytotoxicity of flavanols could be observed after incubation of Caco-2 cells in the presence of 1 m*M* (+)-catechin or PA polymers for 3 h, as indicated by the absence of lactate dehydrogenase activity released in the medium (Mila, Scalbert, Huneau, and Tomé, unpublished observation).

Variations of TEER in the presence of 1 m*M* PA polymers were also followed as a function of time. PA polymers significantly increased TEER as compared with the control with no polymer, and TEER showed little variation in the following 2 h (Fig. 3). PA flux through the monolayer also remained constant over the 2–3 h of transport experiments at any initial TEER value (Fig. 2). This observation confirms the lack of toxicity of PAs at a 1 m*M* concentration.

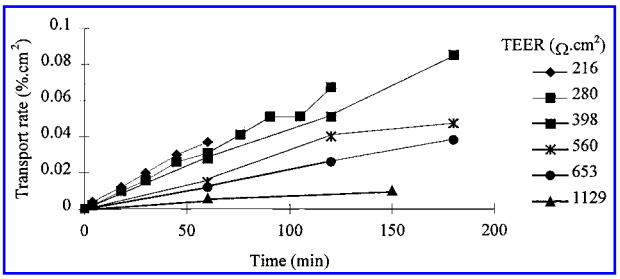

FIG. 2. Kinetics of ^{14}C-PA polymer transport across Caco-2 cell monolayers according to the TEER.

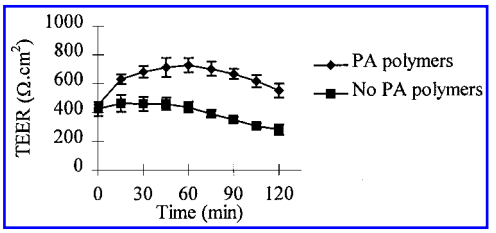

FIG. 3. Effect of the addition of PA polymers (1 m*M*) on TEER of Caco-2 cell monolayers. Values reported are the means ± SD of triplicate wells.

It was checked that radioactivity measurement truly reflected the flux of radiolabeled compounds through the cell monolayer. The average recovery of radioactive substances in the apical and basolateral compartments was on average 90 ± 5% upon termination of incubations. This value is similar to those reported for several drugs with Caco-2 cells (39). Furthermore, flavanols were not metabolized by the cells during the few hours of the transport experiment: no metabolite could be observed by HPLC after 4-h-incubations of (+)-catechin in the transport buffer or 20 h in the culture medium, and all catechin was recovered intact.

Permeability of (+)-catechin and PAs through the Caco-2 cell monolayer

Permeability of [^{14}C]-(+)-catechin was first compared with that of [^{3}H]mannitol in single wells. They showed very similar apparent permeabilities of 0.8×10^{-6} and 0.9×10^{-6} $cm.s^{-1}$ respectively (average TEER: 509 ± 54 $\Omega.cm^2$). Their permeabilities were also measured in separate wells and compared with those of PAs (Fig. 4). No significative difference was observed between permeabilities of the (+)-catechin monomer, mannitol, and the PA dimer and trimer (P_{app} = 0.9–2.0 $\times 10^{-6}$ $cm.s^{-1}$). In con-

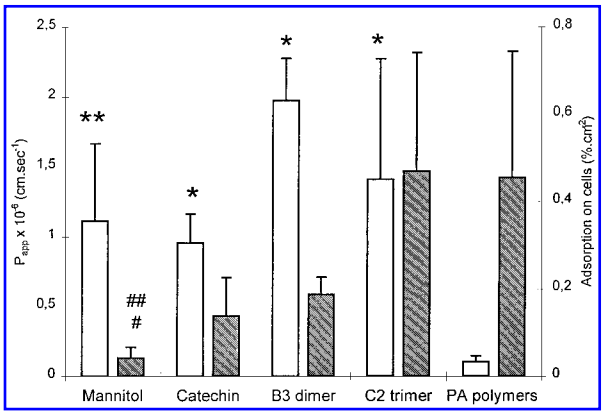

FIG. 4. Apical to basolateral permeabilities through Caco-2 cell monolayers (empty columns) and adsorption on cells (gray columns) of PAs, (+)-catechin, and mannitol (means ± SD). *$p < 0.05$ versus permeability of PA polymers; **$p < 0.005$ versus permeability of PA polymers; #$p < 0.05$ versus adsorption of PA polymers; ##$p <$ 0.005 versus adsorption of C2 trimer.

trast, permeability of the PA polymers was ~10 times lower ($P_{app} = 0.10 \pm 0.04 \times 10^{-6}$ cm.s^{-1}).

The effect of calcium removal from the transport buffer on the transepithelial transport of B3 dimer and C2 trimer was also assessed. Calcium ions contribute to the integrity and functions of tight junctions (3), and their removal leads to an enlargement of intercellular spaces (17). Calcium removal resulted in an increase in permeability particularly for the PA dimer B3 and trimer C2 (Fig. 5). The low permeability measured in this experiment in the absence of calcium as compared with the data shown in Fig. 4 is explained by a particularly high initial TEER (1,160 ± 70 Ω.cm^2) in this experiment. Opening of the intercellular spaces induced a decrease of TEER values to 594 ± 119 Ω.cm^2 and an increase of permeability to values similar to those of the previous experiment shown in Fig. 4. TEER thus influences widely the permeability of (+)-catechin and PAs. All permeability values from Figs. 4 and 5 were replotted according to TEER values (Fig. 6). At high TEERs, PA dimer, trimer, and polymer were hardly permeable. The permability of the dimer, trimer, and (+)-catechin monomer, as well as that of mannitol, increased as TEER decreased. Permeability of the PA polymer was in comparison virtually nil at any TEER value.

Adsorption of (+)-catechin and PA on Caco-2 cells

Measurement of the radioactivity retained on the cells after they were rinsed with the transport buffer allowed their affinity for the cells to be estimated. The fraction of (+)-catechin retained on the cells was three times higher than that of mannitol (Fig. 4). Retention increased with the polymerization degree. It was about three times higher for the trimer C2 and the PA polymer as compared with (+)-catechin.

DISCUSSION

Absence of toxicity of (+)-catechin and PAs on Caco-2 cells

No toxicity of (+)-catechin or PA polymers could be observed at concentrations as high as 1 m*M*. (+)-Catechin and PAs thus appear less toxic than quercetin, which exhibits cytotoxic effects on Caco-2 cells at 15–120 μM concentrations (1). The higher toxicity of quercetin is possibly explained by its higher lipophilicity, which facilitates its penetration in the cell (see below). A 0.5–1 m*M* concentration was selected in all experiments as close to the one expected to occur in the gut. One glass of red wine contains a minimum of 45 mg of PA (44). Once ingested, PAs will be diluted in an intestinal volume of 600 ml (34), which would correspond to a minimum concentration of 0.3 m*M*, close

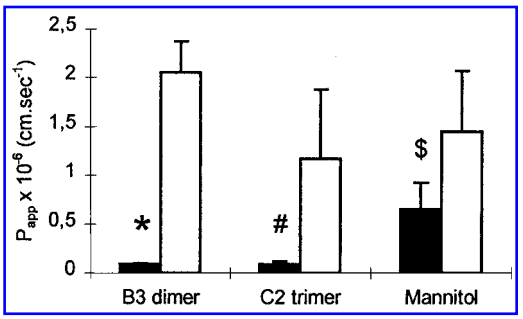

FIG. 5. Apical to basolateral permeability of PA dimer B3 and trimer C2 and of mannitol in the presence (solid columns) and in the absence (empty columns) of calcium ions. TEER values were 1,161 ± 69 and 594 ± 119 Ω.cm^2 in the presence and absence of calcium, respectively (means ± SD). *$p < 0.05$ versus B3 dimer without calcium; #$p < 0.05$ versus C2 trimer without calcium; \$$p <$ 0.05 versus mannitol without calcium.

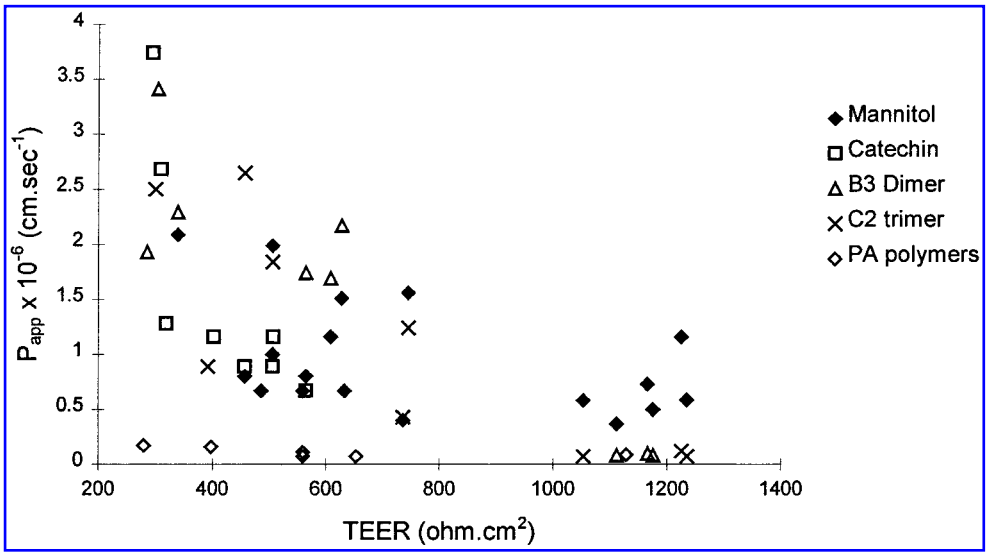

FIG. 6. Apical to basolateral permeabilities through Caco-2 cell monolayers of PAs, (+)-catechin, and mannitol according to the TEER.

to the concentrations selected here. The concentration in the gut lumen could be significantly higher when considering the proposed daily consumption of 0.1–0.5 g/day (44).

Preferential absorption of (+)-catechin and PAs through the intestinal epithelium by the paracellular route

The low permeability coefficients of (+)-catechin and PAs indicate a preferential transepithelial transport through the paracellular route. Permeability was similar or lower than that of mannitol, a marker of the paracellular transport (Fig. 4), and the paracellular pathway is known to be of quantitative importance for such solutes of low permeability (4, 56) generally too polar (PAs are water-soluble) to cross the cell membrane. In contrast, quercetin, a more lipophilic flavonoid, was 10 times more easily absorbed by Caco-2 cells than mannitol (57). The partition coefficient in a water/octanol system of quercetin is twofold higher than that of epicatechin [a diastereoisomer of (+)-catechin] (43). Chrysin, a poorly hydroxylated flavone, is also 10 times more easily absorbed than mannitol (58). Quercetin and chrysin are largely absorbed by transcellular diffusion and differ in this respect from (+)-catechin and PAs.

An inverse association between permeability coefficients of (+)-catechin and PAs and TEER values also advocates for the paracellular route of absorption. Permeability was strongly reduced for all flavanols at high TEER values (>1,000 Ω.cm^2) observed in certain cell cultures (Fig. 6) or when calcium was removed from the culture medium (Fig. 5). A decrease of permeability when TEER increases is even more clearly seen for the poorly absorbed PA polymer (Fig. 2).

Influence of the polymerization degree on the intestinal absorption of PAs

Absorption through the paracellular route is controlled by the size of the intercellular spaces and tight junctions, which behave as a molecular sieve. Polymers such as polyethylene glycol or inulin of molecular weight of 5,000 are not absorbed by confluent Caco-2 cells (7, 24). Similarly, PA polymers with an average polymerization of 6 (average molecular weight, 1,740) are poorly absorbed in comparison with the monomer, dimer, or trimer (Fig. 4). This discrimination according to molecular weight is evident at low TEER, whereas it is not observed at high TEER, where the pores are too tight to allow absorption of any PAs, dimer and trimer included (Fig. 6). This low permeability of PA polymer may also be the consequence of their effect on TEER values, which increase by 300 Ω.cm^2 at a 1 m*M* concentration (Fig. 3). PAs, particularly those of high polymerization de-

gree, are astringent (44). They may complex the membrane proteins and strengthen the tight junctions. Whatever the explanation for the poor absorption of PA polymers, it is in good agreement with previous results obtained on animals (26, 55).

Various attempts have been made to predict *in vivo* absorption in humans by using *in vitro* absorption through Caco-2 cell monolayers (51). However, very little is known about gut absorption of polyphenols in humans, and it is difficult to evaluate the predictive value of *in vitro* results. The only data available on gut absorption of polyphenols in humans concern quercetin: 52% of the 90 mg of quercetin glucosides contained in onions and fed to ileostomized volunteers was absorbed in the small intestine (25). Walgren *et al.* (57) found no transport of these glucosides in Caco-2 cells, possibly because of the lack of expression of transporters probably involved in their gut absorption (11).

Nonglucosylated polyphenols are likely absorbed through diffusion. Their *in vitro* permeability can be compared with their recovery in urine after ingestion by humans. Recovery in urine of (+)-catechin ingested with tea (32) and (−)-epicatechin ingested with wine (Donovan and Waterhouse, personnal communication) was measured: a value of 5–6% was obtained with the two catechin sources. This value is similar to the 5% value reported for mannitol (51). An intestinal absorption of 24% quercetin aglycone was determined after ingestion of 100 mg of quercetin by ileostomized volunteers. These figures rank in the same order as the permeability coefficients determined in the present work and by Walgren *et al.* (57): quercetin > catechin = mannitol. However, this should be interpreted with care due to the dependence of recovery in urine on metabolism or enterohepatic cycling (47).

Similar permeability coefficients for (+)-catechin and PA oligomers suggest a possible absorption of PA dimers and trimers, whereas the low permeability coefficients for PA polymers of higher polymerization degree suggest that they are not absorbed *in vivo*. Other polymers like inulin or polyethylene glycol with molecular weights of 4,000–5,000 are not absorbed *in vivo* (7, 24). Intercellular spaces in the epithelium behave like a molecular sieve. The pores are too small to accommodate such polymers. This would explain the lack of absorption of PA polymers of high molecular weight. A tight epithelium like in the colon may even prevent the absorption of oligomers as was suggested by the low absorption of PA dimer B3 and trimer C2 at high TEER values (Fig. 6).

Complexation of PA with luminal and mucosa proteins may also influence absorption. Addition of proteins to the apical compartment of Caco-2 cells inhibited the transport rate of flavone, a nonhydroxylated synthetic flavonoid (29). Affinity of PAs for proteins increases with their polymerization degree (44). Complexation with proteins in gut lumen may thus prevent absorption of PA of high molecular weight in agreement with the present results (Fig. 4). However, little is known about the stability of PA/protein complexes in the gut. It is known that a high consumption of tannins does not inhibit the digestion of dietary proteins (9), and PA would be freed from the complexes when proteins are hydrolyzed into amino acids. Astringency may diminish absorption, but it does not prevent it, as can be deduced from the absorption in humans of epigallocatechin gallate (32), a highly astringent polyphenol abundant in tea (38).

Adsorption of PAs on the gut epithelium

PAs of high polymerization degree are preferentially retained on the Caco-2 cells (Fig. 4), in agreement with their known affinity for proteins. This high affinity for mucosa proteins explains the long-lasting feeling of astringency in the mouth when consuming PA-rich foods or beverages (31, 37). PAs retained by the mucosa may also protect the mucosa against agression by toxic compounds in food. Ethanol is a well established risk factor of oropharyngeal and esophageal cancers. However, the risk differs according to alcoholic beverages. An epidemiological study has revealed that it decreases with the proportion of wine in the total alcohol intake (19). The relative risk even decreased to 0.5 for moderate drinkers (7–21 drinks per week) who include >30% wine in their alcohol intake, as compared with nondrinkers. Some compounds present in wine seem to counter-

act the toxic effects of alcohol. PA and other wine phenolic polymers bound to the mucosa might be these protective compounds (44).

The mucus on the gut epithelium may limit PA binding to the gut epithelium. Small hydrophilic molecules can usually diffuse easily through the mucus, whereas diffusion of larger molecules may be affected (4). Nothing is known on the affinity of PA for the mucus glycoproteins, and glycosylation of proteins has been shown to either increase (6) or decrease (27, 53) affinity for tannins.

Metabolism of (+)-catechin by the gut epithelium

No metabolites could be detected after exposure of the Caco-2 cells to (+)-catechin. Opposite results were obtained on chrysin by Walle *et al.* (58), who observed glucuronidation and the formation of sulfate esters in similar conditions (16). Hydroxytyrosol, another plant phenolic compound, was methylated by Caco-2 cells (35). A few studies on rats also showed metabolism of flavonoids at the gut level: quercetin (10), genistein (47), and most likely (−)-epicatechin (42) are conjugated as glucuronides or sulfate esters, and quercetin is weakly methylated (10). Recent results in one of our laboratories showed that (+)-catechin, when perfused through the small intestine, is recovered in the mesenteric vein as methylated and glucuronidated metabolites (15). This indicates that in rats, (+)-catechin likely enters the cells of the gut epithelium and is reexcreted as conjugates on the serosal side. The absence of metabolism of (+)-catechin and PAs by Caco-2 cells could be explained either by the lack of penetration of such hydrophilic flavonoids within the cells or by the lack of expression of the conjugating enzymes in the cells under the conditions of the experiment.

Conclusions

The present results on the Caco-2 cell model show that (+)-catechin and PA oligomers are absorbed through the epithelium by the paracellular route. PA dimers and trimers are possibly absorbed *in vivo*. Confirmation still awaits rigorous evaluation in humans. PA polymers are likely not absorbed due to their high molecular weight. Only their degradation products, either monomers and oligomers supposedly formed in the stomach (50) or the aromatic acids formed in the colon (14), would be absorbed through the gut barrier. More data on PA bioavailability are needed to evaluate their possible effects on human health.

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ABBREVIATIONS

DMEM, Dulbecco's modified Eagle's medium; P_{app}, apparent permeability coefficient; PA, proanthocyanidin; PC, procyanidin; TEER, transepithelial electrical resistance.

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