Eur J Nutr (2006) 45:376–382
DOI 10.1007/s00394-006-0609-8

ORIGINAL CONTRIBUTION

Caroline Henry-Vitrac
Alexis Desmoulière
Delphine Girard
Jean-Michel Mérillon
Stéphanie Krisa

Transport, deglycosylation, and metabolism of *trans*-piceid by small intestinal epithelial cells

Abstract *Background:* Numerous epidemiological and animal studies have shown that consumption of red wine is related to reduced incidence of cardiovascular diseases and cancer. *Trans*-resveratrol (3, 5, 4′-trihydroxystilbene), a phenolic compound present in wine, has been reported to have a potential cancer chemopreventive activity. Moreover, it may exert a protective effect against atherogenesis through its antioxidant properties. *Trans*-piceid (3-ß glucoside of *trans*-resveratrol) is present to a greater extent than its aglycone in red wine, but hydrolysis of this glycosylated derivative can occur in small intestine and liver, which would enhance the amount of the biological active *trans*-resveratrol. *Aims:* The present study aimed to investigate the rate of transepithelial transport of *trans*-piceid using human intestinal Caco-2 cell monolayers and metabolism of this compound during its absorption across the small intestine. *Methods:* The transport of *trans*-piceid was evaluated in the human epithelial cell line Caco-2, which possesses enterocyte-like properties in vitro. For transepithelial experiments, confluent monolayers of Caco-2 cells were grown on Transwell® inserts. For metabolic studies, we used both Caco-2 cells seeded on 6-well plates and rat small intestine cell-free extracts. *Results:* The time course of apical (AP) to basolateral (BL) transport of *trans*-piceid showed that the favorable apparent permeability coefficient (Papp) declined rapidly during the 6 h of the experiment. This observation could be correlated with the appearance of metabolites. After incubation of Caco-2 cells with *trans*-piceid, *trans*-resveratrol was detected on both AP and BL sides. By using protein extracts obtained from rat, we conclude that the Lactase Phlorizin Hydrolase (LPH) and Cytosolic-ß-Glucosidase (CBG) are involved in the hydrolysis of *trans*-piceid. Furthermore, we show that after deglycosylation, the resulting aglycone is metabolized in *trans*-resveratrol-3-*O*-ß-glucuronide and to a lesser extent in *trans*-resveratrol-4′-*O*-ß-glucuronide, and that UGT1A1 is mainly involved in this metabolism. *Conclusions:* This study demonstrates that the transepithelial transport of *trans*-piceid occurs at a high rate and that the compound is deglycosylated in *trans*-resveratrol. There are two possible pathways by which *trans*-piceid is hydrolyzed in the intestine. The first is a cleavage by the CBG, after passing the brush-border membrane by SGLT1. The second is deglycosylation on the luminal side of the epithelium by the membrane-bound enzyme LPH, followed by passive diffusion of the released aglycone, which is further metabolized inside the cells into two glucuronoconjugates.

Key words *trans*-piceid – *trans*-resveratrol – Caco-2 – transepithelial transport – metabolites

Received: 21 July 2005
Accepted: 1 June 2006
Published online: 28 September 2006

C. Henry-Vitrac · D. Girard
J.-M. Mérillon · S. Krisa (✉)
Groupe d'Etude des Substances Végétales à Activités Biologiques, EA 3675
Université Victor-Segalen Bordeaux II,
UFR Sciences Pharmaceutiques
146 rue Léo Saignat
33076 Bordeaux Cedex, France
Tel./Fax: +33-55/757-4688
E-Mail: stephanie.krisa@phyto.u-bordeaux2.fr

A. Desmoulière
GREF, INSERM E 0362
Université Victor-Segalen Bordeaux II
Bordeaux, France

EJN 609

Introduction

Trans-hydroxystilbenes are naturally occurring polyphenolic compounds, which have been reported to have potential preventive activities in human diseases. Among these stilbenes, *trans*-resveratrol, which is mainly found in grapes and red wine, is one of the most important in terms of biological activities, since it has been reported to exert anticarcinogenic, antioxidant, and cardioprotective activities [1]. Although there is considerable evidence that *trans*-resveratrol possesses strong biological activities, it is present in small quantities in wine compared to one of its glucosides, *trans*-piceid [2]. However, hydrolysis of this glycosylated derivative by ß-glucosidases may occur in the major sites of biotransformation (liver and intestine), thus allowing the quantity of *trans*-resveratrol available from the diet to be greater. Although the biological activities in vitro of these compounds are understood, little is known about their absorption and bioavailability in human. Indeed, their potential biological activities in vivo are dependent on their intestinal absorption and subsequent access to the target tissues. Numerous studies have shown that dietary polyphenols are subjected to metabolic conversion not only in the liver, but also during their absorption in the intestinal epithelial cells before reaching the systemic circulation [3].

Recently, we studied the distribution of *trans*-resveratrol in organs and tissues after oral administration of this ^{14}C-labeled compound in the mouse. Intact resveratrol together with metabolites (glucuronides and/or sulfated derivatives) were detected in liver 3 h after administration [4]. This result corroborates our previous studies showing that *trans*-resveratrol can be glucuronidated in human and rat liver microsomes [5]. Moreover, Kuhnle et al. [6] reported a similar metabolism during the uptake of *trans*-resveratrol in the small intestine of rat.

The present study aimed to investigate the rate of transepithelial absorption of *trans*-piceid using human intestinal Caco-2 cell monolayers, a model that has been fully used to study the transepithelial transport of polyphenols [7, 8]. In our previous study, we evaluated the apical (AP) uptake of *trans*-piceid by using Caco-2 cells [9], and we will now study the metabolism of *trans*-piceid during its absorption across the small intestine.

Materials and methods

Materials

Trans-resveratrol, chrysin (5,7-dihydroxyflavone), [^{14}C]-mannitol, ß-glucuronidase (bovine liver, 1.10^6 units/g), D-Gluconolactone, Triton X100, ß-mercaptoethanol, EDTA, and protease inhibitor cocktail were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). *Trans*-piceid was extracted and purified from *Vitis vinifera* cell suspension cultures as previously described [10]. All the solvents were of HPLC grade quality (Scharlaud, Barcelona, Spain). *Trans*-resveratrol glucuronides were obtained from human liver microsomes as reported [5].

Caco-2 cell culture

Human colon adenocarcinoma Caco-2 cells were purchased from the American Type Culture Collection (ATCC, Molsheim, France) and were cultured in high Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, 100 Units/ml penicillin, and 100 µg/ml of streptomycin (Sigma-Aldrich, St. Quentin Fallavier, France). Caco-2 cells were grown to confluence at 37°C in a humidified atmosphere of 5% CO_2–95% air and subcultured using 0.02% EDTA and 0.05% trypsin.

Transport experiments

For transport experiments, Caco-2 cells (at passages 40–90) were seeded on polycarbonate membrane filter Transwell® inserts (0.4-µm pore size, 1.13-cm^2 growth area, Corning Costar Corp.) in 12-well plastic plates at a density of 9×10^4 cells per cm^2. Culture medium was replaced by fresh medium every 2 days and 1 day before transport studies. Experiments were conducted 18–24 days postconfluence after measuring the transepithelial electrical resistance (TEER) using a Millicell-ERS Voltohmmeter (Millipore Corp). Only inserts with TEER values >300 Ω cm^2 in culture medium were selected for transport experiments. The integrity of the monolayers was also measured with 50-µM [^{14}C]-mannitol added to the AP chamber as a marker of low paracellular permeability. The cell monolayers were considered tight when the Papp for mannitol was less than 0.5×10^{-6} cm/s. Before experiments, culture medium was removed from both the AP and basolateral (BL) chambers. Caco-2 cell monolayers were incubated twice with prewarmed transport buffer consisting of (mM) NaCl (140), KCl (5.33), $CaCl_2$ (1.26), $MgSO_4$ (0.84), KH_2PO_4 (0.44), and mannitol (5.48) buffered at pH 7.4, for 30 min at 37°C. Then, the preincubation buffer was removed and the cells were incubated with stilbenes dissolved in transport buffer/DMSO (dimethyl sulfoxide) (99.5/0.5, v/v). The solutions containing *trans*-piceid were added either to the AP chambers for AP → BL measurements (0.5 ml in the AP chambers) or to the BL chambers for BL → AP measurements (1.5 ml in the

BL chambers). Transport buffer alone was added to the other side. At the end of the incubation period, the media (AP and BL) were collected for HPLC analysis.

Deglycosylation studies

For deglycosylation studies, two models were used. In the first one, Caco-2 cells grown on 6-well plates were used on days 12–14 postconfluence and were incubated at 37°C with 100 μM of *trans*-piceid dissolved in complete medium/DMSO (99.5/0.5, v/v) for different times (0.5, 1, 3, 6, 9 and 24 h). At each time point, the medium together with the cells (medium + cells) were recovered by scraping the cells with a plastic spatula, sonicated for 15 s and centrifuged at 20,000 × *g* for 10 min to obtain the supernatant used for HPLC analysis.

In the second model, male Wistar rats (Charles Rivers, St Aubin les Elbeuf, France) weighting approximately 300 g were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg; Imalgen, Rhône Mérieux, Lyon, France) and xylazine (10 mg/kg; Rompun, Bayer Pharma, Puteaux, France). Small intestine was removed via an abdominal incision. All experiments were carried out using accepted ethical guidelines. The small intestine was washed with ice-cold NaCl solution (0.9%) and the mucosa was removed by scraping. The separated cells were homogenized in 3 ml of 50-mM phosphate buffer, pH 7, containing EDTA (1 mM), $CaCl_2$ (1 mM), $MgCl_2$ (10 mM), ß-mercaptoethanol (10 mM), and a protease inhibitor cocktail (15 μl). To separate soluble (CBG) and membrane-bound Lactase Phlorizin Hydrolase (LPH) ß-glucosidases, we used the method described by Nemeth et al. [11]. The homogenate was centrifuged at 48,000 × *g* for 60 min at 4°C. The supernatant (S containing CBG) was retained. The pellet was resuspended in buffer containing 4% (w/v) Triton X100 and incubated for 60 min at 4°C with agitation in order to solubilize the membrane-bound proteins. The solubilized pellet was centrifuged (48,000 × *g*, 60 min, 4°C) and the supernatant (ST containing LPH) retained. A reaction mixture (final volume of 500 μl in 50-mM phosphate buffer, pH 6.5) containing *trans*-piceid (50 μM) was incubated with 300-μg protein for 60 min at 37°C. The reaction was stopped by addition of 0.5 ml of methanol containing ascorbic acid (1 mM), followed by centrifugation at 13,600 × *g* for 5 min at 4°C. The supernatant was collected for HPLC analysis.

Metabolism experiments

After seeding of Caco-2 cells in 6-well plates for 4–6 days (when cells were confluent), we started pretreatment with 50-μM chrysin in complete cell culture medium (chrysin dissolved in ethanol/DMSO (80/20, v/v) at a final concentration not exceeding 0.5%). Complete culture medium with 50 μM chrysin was replaced in each well every 24 h, and the cells were used for metabolism assays 24 h after the last medium change. Control cells were treated with the same volume of complete medium alone. The cell layer was then rinsed with culture medium (to remove chrysin used in pretreatment of cells) and the treatment consisted of incubation of the cells for 24 h with 3 ml of *trans*-piceid or *trans*-resveratrol (50 μM dissolved in culture medium, max 1% DMSO) in both chrysin-treated and control cells. D-saccharic acid lactone (5 mM), an inhibitor of the ß-glucuronidases, was added to pretreated Caco-2 cells. After 24 h of treatment, the medium + cells were recovered and treated as described in "Deglycosylation studies".

HPLC analysis

Before HPLC analysis, samples were filtered through Millipore filters (0.45 μm). HPLC was performed on a system equipped with a 250 × 4-mm Prontosil C_{18} (5 μm) reverse-phase column (Bischoff Chromatography, Leonberg, Germany) protected by a guard column of the same material. Separation was performed at a flow rate of 1 ml/min with a mobile phase composed of (A) H_2O:TFA (97.5:2.5, v/v) and (B) ACN:A (80:20, v/v). The run was set as follows; 0–10 min, 15% B; 10–13 min, from 15% B to 18% B; 13–15 min, 18% B; 15–17 min, from 18% to 22% B; 17–30 min, from 22% B to 25% B; 30–34 min, from 25% B to 32% B; 34–36 min, 32% B; 36–39 min, from 32% B to 40% B; 39–40 min, 40% B; 40–47 min, from 40% B to 70% B; 47–50 min, from 70% B to 100% B. Chromatographic peaks were monitored using a fluorescence detector (ProStar 363, Varian, USA) set at optimal wavelengths for detection of both *trans*-resveratrol and *trans*-piceid (λ_{exc} 300 nm; λ_{em} 390 nm) [2]. Quantification of compounds was estimated from calibration curves that were prepared with standards.

Statistical analysis

Data were expressed as the means ± standard deviation of three to six determinations. Statistical analysis was performed using Student's *t*-test and $P < 0.05$ was considered to be significant.

Results

Transepithelial transport of *trans*-piceid

The transepithelial transport of *trans*-piceid (100 μM) in Transwell® inserts was measured for 1 h at 37°C in

Table 1 Transport rates, for different times, of *trans*-piceid in Caco-2 cells grown on Transwell® inserts

Time (min)	A–B transport	B–A transport
30	15.7 ± 0.4	19.5 ± 0.9*
60	10.5 ± 0.1[a]	15.3 ± 0.6*,[a]
180	7.7 ± 0.6[a,b]	9.8 ± 0.4[a,b]
360	5.6 ± 0.2[a,b,c]	6.5 ± 0.1[a,b,c]

* Indicates values of Papp (B–A) transport significantly different than Papp (A–B) transport
a, b, c indicate values of Papp significantly different than value of Papp at times 30, 60 and 180 respectively
Papp is expressed in cm/s ($\times 10^{-6}$); values are means ± SEM, $n = 3$

AP → BL and BL → AP directions (Table 1). During this time, transcellular absorption clearly occurred in both directions. The apparent permeability coefficients (Papp) expressed in cm/s were calculated according to the following equation: Papp $= (dC/dt) \times (V/A\text{Co})$, where dC/dt is the change in concentration on the receiving chamber over time (μM/s), V is the volume of the solution in the receiving compartment (cm^3), Co is the initial concentration in the donor chamber (μM), and A is the growth area of the monolayer (1.13 cm^2). The AP → BL transport of *trans*-piceid ($10.5 \times 10^{-6} \pm 0.1$ cm/s) was significantly weaker than in the opposite direction (BL → AP) ($15.3 \times 10^{-6} \pm 0.6$ cm/s). The Papp value of $\approx 10 \times 10^{-6}$ cm/s at 1 h would predict a high absorption in vivo [12].

We also determined the AP → BL and BL → AP transport of *trans*-piceid over 6 h (Table 1). When *trans*-piceid was loaded on the AP or BL sides of the cells, the compound could be detected on the opposite side as early as 30 min and throughout the 6 h of incubation. Moreover, we show that during this time, there was a reduction in transport, with a rapid decline in Papp. This could be associated with an important efflux out of the cells, as already shown in our previous studies [9] and/or a metabolism of *trans*-piceid as reported by Walle et al. [13] for the flavonoid chrysin. Indeed, the aglycone of *trans*-piceid (*trans*-resveratrol) appeared in detectable but not quantifiable quantities on the AP and BL side of the cells after 6 h of incubation. For further investigations, larger amounts of compounds were generated by seeding Caco-2 cells in 6-well plates in order to facilitate the detection and identification of new compounds.

Deglycosylation studies

The time course of hydrolysis of *trans*-piceid was examined by HPLC (Fig. 1). After 30 min at 37°C *trans*-resveratrol was already detected in medium + cells. In addition, the greatest amount of *trans*-resveratrol appeared after 24 h of incubation (4.5 ± 0.1 pmol/cm^2). Although Caco-2 cells have been reported to express membrane-bound LPH and cytosolic CBG, the expression of these two proteins is weak in this model [11, 14]. Therefore, to investigate the mechanisms of the hydrolysis of *trans*-piceid, we used enterocytes from the small intestine of the rat to perform assays for glycosidase activity.

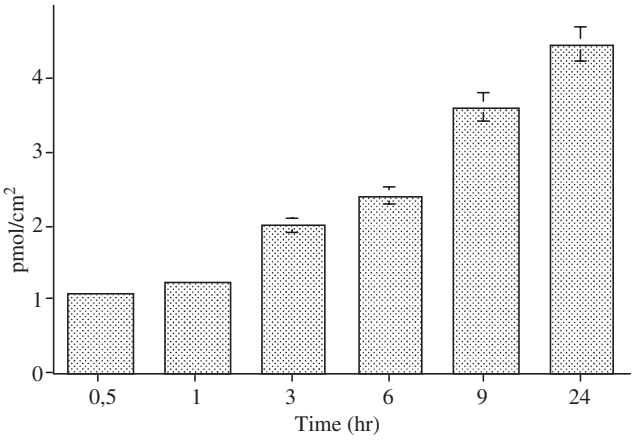

Fig. 1 Time course of deglycosylation of *trans*-piceid in *trans*-resveratrol by Caco-2 cells cultured in 6-well plates. The amount of *trans*-resveratrol was determined in pmol/cm^2 for 0.5, 1, 3, 6, 9 and 24 h. *Trans*-piceid was dissolved in transport buffer/DMSO (99.5/0.5, v/v), and this percentage of DMSO didn't affect the transport of the stilbene (data not shown). Values are expressed as means ± standard deviations ($n \geq 3$)

The small intestine of the rat was separated into a soluble fraction (S containing CBG) and an insoluble fraction solubilized by treatment with Triton X100 (ST containing LPH). After incubation of *trans*-piceid for 1 h at 37°C with the two fractions, *trans*-resveratrol was detected in both assays. The level of ß-glucosidase activity in the ST fraction (311.6 ± 10.2 pmol/mg protein/min) was about 5-fold higher than in the S fraction (63.0 ± 9.6 pmol/mg protein/min). Moreover, incubation with D-gluconolactone (2 mM), an inhibitor of both cytosolic and membrane-bound glucosidases, reduced trans-piceid hydrolysis by 93% and 69% in the ST and S fractions, respectively. This suggests that hydrolysis of *trans*-piceid is carried out by both CBG and LPH proteins.

Metabolism experiments

The metabolism of *trans*-piceid in Caco-2 cells was analyzed by HPLC. Figure 2A shows the appearance of *trans*-resveratrol in medium + cells when *trans*-piceid was added to the culture medium for 24 h. Two other compounds named G1 and G2 were also detected, with retention times close to that of *trans*-piceid. The same experiments were conducted with *trans*-resveratrol and showed that G1 and G2 were

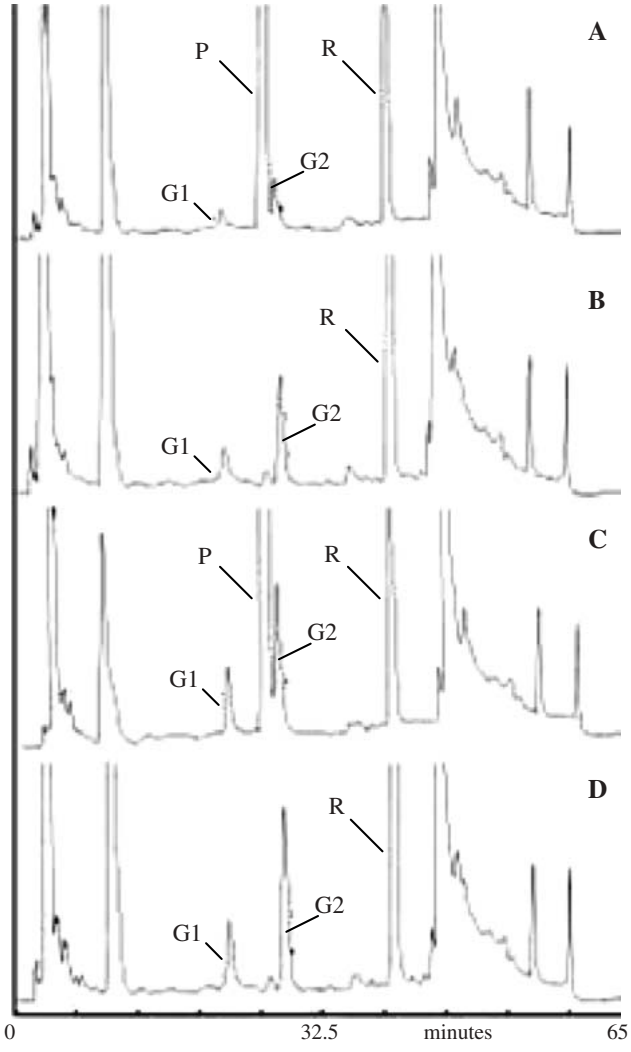

Fig. 2 HPLC of *trans*-piceid (P), *trans*-resveratrol (R) and metabolites G1 and G2 in medium + cells. (**A**) and (**B**) represent 24-h incubation of the cells with (**A**) *trans*-piceid (100 μM) and (**B**) *trans*-resveratrol (100 μM), without pretreatment with chrysin. (**C**) and (**D**) represent the metabolism by Caco-2 cells after pretreatment with 50 μM chrysin for 3 days, followed by treatment for 24 h with (**C**) *trans*-piceid (100 μM) or (**D**) *trans*-resveratrol (100 μM)

also detected (Fig. 2B). Since phase-II metabolism such as glucuronidation seems to be the major metabolic pathway in the liver for *trans*-resveratrol [5], we anticipated that UDP-glucuronosyltransferases (UGTs) would actively participate in the metabolism of stilbenes in the intestinal Caco-2 model. Several studies have shown that pretreatment of Caco-2 cells with chrysin can induce the UGT1A1 family [15]. Pretreatment of Caco-2 cells with 50 μM of chrysin for 3 days, followed by treatment with 100 μM of *trans*-piceid or *trans*-resveratrol for 24 h, resulted in a large increase in G1 and G2 compounds (Fig. 2C, D). Furthermore, incubation with ß-glucuronidase led to the disappearance of the G1 and G2 peaks and co-injection with standard glucuronides of *trans*-resveratrol suggested that G1 was *trans*-resveratrol-4′-*O*-ß-glucuronide and the major metabolite G2 was *trans*-resveratrol-3-*O*-ß-glucuronide (data not shown).

Discussion

It is essential to know the bioavailability of stilbenes in order to understand their potential actions *in vivo*. In this study, we used Caco-2 cells, a well-accepted model of human intestinal absorption [7]. Transepithelial transport of *trans*-piceid clearly occurred with a favorable Papp of about 10×10^{-6} cm/s for the AP to BL flux, which suggests virtually complete absorption of *trans*-piceid in humans [12]. Furthermore, considerable evidence is now available supporting the hypothesis that deglycosylation by the small intestine is the first step in the absorption and metabolism of dietary polyphenol glycosides [11, 14, 16]. In this study, we show that *trans*-piceid is deglycosylated by both small intestinal LPH and CBG. Two potential pathways of passage through the enterocytes appear to be available to *trans*-piceid. The first is the involvement of the LPH, which is present on the AP membrane. It might release the aglycone *trans*-resveratrol into the lumen, where the latter might then passively diffuse across the AP side of Caco-2 cells. The second is cleavage of the glucoside by the CBG after passing the brush-border membrane via the sodium-dependent glucose transporter 1 (SGLT1), as reported by several authors for numerous polyphenol glucosides [9, 17]. In conclusion, deglycosylation seems to be the most likely step during the transport and metabolism of *trans*-piceid in this intestinal cellular model. Once absorbed, released aglycone compounds might be metabolized by phase-I or phase-II enzymes.

The liver plays a major role in the metabolism of xenobiotics. However, the contribution of the small intestine should not be underestimated. Conjugation with glucuronide or sulfate is the most likely metabolic pathways for several polyphenols [18]. In this study, we show that after deglycosylation of *trans*-piceid, the resulting *trans*-resveratrol is further metabolized in the enterocytes, and that the new compounds are glucuronic conjugates, the major glucuronide being *trans*-resveratrol-3-*O*-ß-glucuronide and the minor one *trans*-resveratrol-4′-*O*-ß-glucuronide, as already shown by Aumont et al. [5] using human liver microsomes.

The UGTs have long been known to be inducible by various chemicals in Caco-2 cells [15, 19]. Walle and Walle [20] observed a 4-fold increase in glucuronidation after pretreating the cells with chrysin for 3 days, whereas sulfate conjugation was not affected.

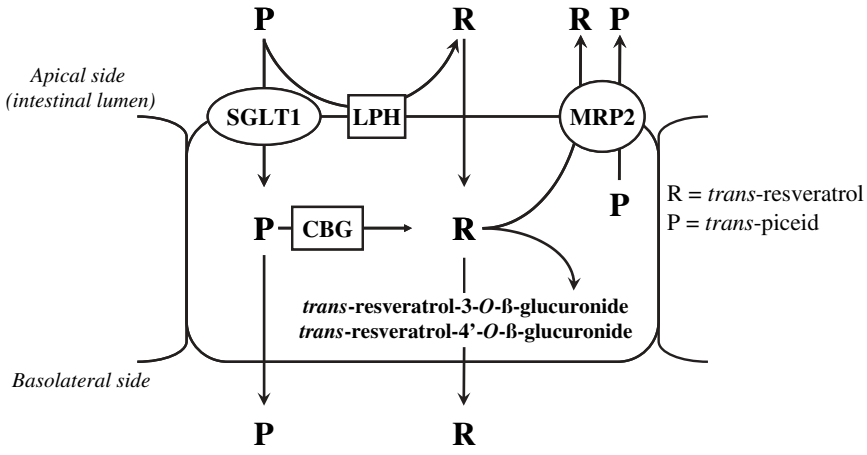

Fig. 3 Proposed pathways for intestinal absorption and metabolism of *trans*-resveratrol and *trans*-piceid

Our results corroborate these observations since the amount of glucuronides of *trans*-resveratrol formed were significantly higher in chrysin-treated assays than in the untreated assays. These results confirm that *trans*-resveratrol is glucuronidated mainly by UGT1A1, not only in the liver, as already shown [5], but also in the enterocytes. Moreover, no sulfate conjugates were detected in these studies with our HPLC methods, contrary to the other authors [21, 22].

Generally, the glucuronoconjugates are excreted from the enterocytes back to the lumen. The metabolites are unlikely to diffuse passively across the AP membrane, owing to a negative charge at physiological pH [23]. However, these compounds are excreted mostly by ATP-binding cassette transporters of the multidrug resistance associated protein family (MRP), in particular the MRP2 localized exclusively in the AP membrane of the cells [8, 19, 24]. Moreover, in this study, in order to localize the metabolites, medium from the AP side and the cells were treated separately. The medium was removed from the plates and the cells were lysed with methanol. No glucuronides were detected on the AP side of the cells (medium) but only within (methanolic extract). Previous work demonstrated that the efflux pump MRP2 is involved in the efflux of both *trans*-piceid and *trans*-resveratrol by Caco-2 cells [9, 25]. The amount of the native form in the cells (piceid or resveratrol) was higher than the glucuronides, so their efflux via MRP2 could be competitively inhibited by their parent forms.

Since the metabolites are not excreted on the luminal side, there might be a shift in glucuronide export towards the BL side via MRP3 [21]. However, ß-glucuronidases, which are widely expressed in human organs, tissues and body fluids, might modulate the rate of glucuronides and release active parent aglycone [26]. This pathway would enhance the amount of resveratrol and therefore increase its effectiveness in humans.

In conclusion, we have established a model of absorption of *trans*-piceid in the enterocyte (Fig. 3). This compound is actively absorbed across the AP membrane via SGLT1 and is also actively effluxed via MRP2 [9]. The results observed in this study suggest that *trans*-piceid has favorable capacities for biological membrane penetration, and that efficient metabolism observed in Caco-2 cells could suggest a low bioavailability of this compound in human. We have also demonstrated, that the first step of metabolism of *trans*-piceid is hydrolysis of the sugar moiety, which is common to most phenolics. This may occur in the small intestine by action of membrane-bound LPH, or by CBG after transport into the enterocyte via SGLT1. The resulting *trans*-resveratrol may also undergo further metabolism, such as conjugation with glucuronic acid inside the enterocytes. However, further studies are required to investigate a possible pharmacological action of these metabolites in human.

Acknowledgments This study was supported by a grant from the Région Aquitaine, France. We are grateful to Dr. Ray Cooke for reading the manuscript.

References

1. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275:218–220
2. Vitrac X, Monti JP, Vercauteren J, Deffieux G, Merillon JM (2002) Direct liquid chromatographic analysis of resveratrol derivatives and flavanonols in wines with absorbance and fluorescence detection. Anal Chim Acta 458:103–110
3. Silberberg M, Morand C, Mathevon T, Besson C, Manach C, Scalbert A, Remesy C (2006) The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. Eur J Nutr 45:88–96
4. Vitrac X, Desmoulière A, Brouillaud B, Krisa S, Deffieux G, Barthe N, Rosenbaum J, Merillon JM (2003) Distribution of [14C]-*trans*-resveratrol, a cancer chemopreventive polyphenol, in mouse tissues after oral administration. Life Sci 72:2219–2233
5. Aumont V, Krisa S, Battaglia E, Netter P, Richard T, Merillon JM, Magdalou J, Sabolovic N (2001) Regioselective and stereospecific glucuronidation of *trans*- and *cis*-resveratrol in human. Arch Biochem Biophys 393:281–289
6. Kuhnle G, Spencer J, Chowrimootoo G, Schroeter H, Debnam E, Srai KS, Rice-Evans C, Hahn U (2000) Resveratrol is absorbed in the small intestine as resveratrol glucuronide. Biochem Biophys Res Commun 272:212–217
7. Artursson P, Karlsson J (1991) Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochem Biophys Res Commun 175:880–885
8. Walgren RA, Karnaky KJ, Lindenmayer GE, Walle T (2000) Efflux of dietary flavonoid quercetin 4′-beta-glucoside across human intestinal Caco-2 cell monolayers by apical multidrug resistance-associated protein-2. J Pharmacol Exp Ther 294:830–836
9. Henry C, Vitrac X, Decendit A, Ennamany R, Krisa S, Merillon JM (2005) Cellular uptake and efflux of *trans*-piceid and its aglycone *trans*-resveratrol on the apical membrane of human intestinal Caco-2 cells. J Agric Food Chem 53:798–803
10. Waffo Téguo P, Decendit A, Vercauteren J, Deffieux G, Merillon JM (1996) *Trans*-Resveratrol-3-*O*-beta-Glucoside (Piceid) in cell suspension cultures of *Vitis vinifera*. Psychochemistry 42: 1591–1593
11. Nemeth K, Plumb GW, Berrin JG, Juge N, Jacob R, Naim HY, Williamson G, Swallow DM, Kroon PA (2003) Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. Eur J Nutr 42:29–42
12. Yee S (1997) *In vitro* permeability across Caco-2 cells (colonic) can predict *in vivo* (small intestinal) absorption in man – fact or myth. Pharm Res 14:763–766
13. Walle UK, Galijatovic A, Walle T (1999) Transport of the flavonoid chrysin and its conjugated metabolites by the human intestinal cell line Caco-2. Biochem Pharmacol 58:431–438
14. Day AJ, Gee JM, DuPont MS, Johnson IT, Williamson G (2003) Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: the role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. Biochem Pharmacol 65:1199–1206
15. Galijatovic A, Otake Y, Walle UK, Walle T (2001) Induction of UDP-glucuronosyltransferase UGT1A1 by the flavonoid chrysin in Caco-2 cells – potential role in carcinogen bioinactivation. Pharm Res 18:374–379
16. Wilkinson AP, Gee JM, Dupont MS, Needs PW, Mellon FA, Williamson G, Johnson IT (2003) Hydrolysis by lactase phlorizin hydrolase is the first step in the uptake of daidzein glucosides by rat small intestine in vitro. Xenobiotica 33:255–264
17. Walgren RA, Lin JT, Kinne RK, Walle T (2000) Cellular uptake of dietary flavonoid quercetin 4′-beta-glucoside by sodium-dependent glucose transporter SGLT1. J Pharmacol Exp Ther 294:837–843
18. Chen J, Lin H, Hu M (2005) Absorption and metabolism of genistein and its five isoflavone analogs in the human intestinal Caco-2 model. Cancer Chemother Pharmacol. 55:159–169
19. Ng SP, Wong KY, Zhang L, Zuo Z, Lin G (2004) Evaluation of the first-pass glucuronidation of selected flavones in gut by Caco-2 monolayer model. J Pharm Pharm Sci 8:1–9
20. Walle UK, Walle T (2002) Induction of human UDP-glucuronosyltransferase UGT1A1 by flavonoids-structural requirements. Drug Metab Disp 30:564–569
21. Kaldas MI, Walle UK, Walle T (2003) Resveratrol transport and metabolism by human intestinal Caco-2 cells. J Pharm Pharmacol 55:307–312
22. Li Y, Shin YG, Yu C, Kosmeder JW, Hirschelman WH, Pezzuto JM, van Breemen RB (2003) Increasing the throughput and productivity of Caco-2 cell permeability assays using liquid chromatography-mass spectrometry: application to resveratrol absorption and metabolism. Comb Chem High Throughput Screen 6:757–767
23. Williamson G, Day AJ, Plumb GW, Couteau D (2000) Human metabolic pathways of dietary flavonoids and cinnamates. Biochem Soc Trans 28:16–22
24. Walle UK, French KL, Walgren RA, Walle T (1999) Transport of genistein-7-glucoside by human intestinal Caco-2 cells: potential role for MRP2. Res Commun Mol Pathol Pharmacol 103:45–46
25. Sergent T, Garsou S, Schaut A, Saeger SD, Pussemier L, Peteghem CV, Larondelle Y, Schneider YJ (2005) Differential modulation of ochratoxin A absorption across Caco-2 cells by dietary polyphenols, used at realistic intestinal concentrations. Toxicol Lett 159:60–70
26. Sperker B, Backman JT, Kroemer HK (1997) The role of beta-glucuronidase in drug disposition and drug targeting in humans. Clin Pharmacokinet 33:18–31