Inhibition of Intestinal Metabolism of the Antiviral Ester Prodrug bis(POC)-PMPA by Nature-Identical Fruit Extracts as a Strategy to Enhance Its Oral Absorption: An *In Vitro* Study

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Purpose. To explore the usefulness of fruit extracts as enhancers of the oral absorption of esterase-sensitive prodrugs.

Methods. Inhibition of esterase-mediated degradation by nature-identical fruit extracts was evaluated using 1) *p*-nitrophenylacetate (model substrate for esterase-activity) in rat intestinal homogenates and 2) bis(isopropyloxycarbonyloxymethyl)-(*R*)-9-[(2-phosphonomethoxy)propyl]adenine [bis(POC)-PMPA] (esterase-sensitive prodrug of the antiviral agent PMPA) in Caco-2 cell homogenates and in intestinal homogenates from rat, pig and man. Subsequently, transport of the ester prodrug was studied across Caco-2 monolayers in the presence or absence of fruit extracts.

Results. In homogenates from rat ileum, the esterase activity could be reduced significantly by the inclusion of fruit extracts (1%): the initial enzymatic degradation of *p*-nitrophenylacetate was inhibited by 77% (strawberry), 16% (passion fruit) and 57% (banana). A similar inhibition of bis(POC)-PMPA metabolism by fruit extracts was observed in intestinal homogenates from several species and in homogenates from Caco-2 cells. Transport of total PMPA across Caco-2 monolayers was enhanced 3-fold by co-incubation with strawberry extract (1%). The fraction of intact prodrug appearing in the acceptor compartment increased from virtually zero to 67%.

Conclusions. The results suggest that co-incubation with nature-identical fruit extracts might be useful as a strategy to enhance the transepithelial transport of esterase-sensitive prodrugs through inhibition of intracellular metabolism of the prodrug.

KEY WORDS: fruit extracts; esterase inhibition; bis(POC)-PMPA; ester prodrug; Caco-2 cells; absorption enhancer.

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ABBREVIATIONS: (*R*)-PMPA, (*R*)-9-[(2-phosphonomethoxy) propyl]adenine; PMEA, 9-[(2-phosphonomethoxy)ethyl]adenine; Bis(POC)-PMPA, bis(isopropyloxycarbonyloxymethyl)-PMPA; Bis(POM)-PMEA, bis(pivaloyloxymethyl)-PMEA; Mono(POC)-PMPA, mono(isopropyloxycarbonyloxymethyl)-PMPA; Mono(POM)-PMEA, mono(pivaloyloxymethyl)-PMEA; TEER, transepithelial electrical resistance; TM, transport medium; HIV, human immunodeficiency virus.

INTRODUCTION

(*R*)-9-[(2-phosphonomethoxy)propyl]adenine (PMPA) is an acyclic nucleoside phosphonate analogue with potent and selective inhibitory activity against retroviruses (1). A major drawback of PMPA is its low permeation across biological membranes due to its hydrophilic nature, resulting in low oral bioavailability (2). It is obvious that the chronic character of several viral diseases necessitates an easy treatment schedule in order to obtain optimal patient compliance, thus requiring an oral drug formulation. To overcome low absorption, a lipophilic ester derivative, bis(isopropyloxycarbonyloxymethyl)-PMPA [bis(POC)-PMPA] (Fig. 1) was designed and is currently being evaluated in phase II clinical trials in HIV-infected patients (2). Recent studies in which the Caco-2 system was used as an *in vitro* simulation of the intestinal mucosa have also shown enhanced transepithelial transport of total PMPA after addition of the bis-ester prodrug (3). However, bis(POC)-PMPA was significantly metabolized by intracellular esterases, offsetting the absorption enhancing effect of the ester prodrug. A similar degradation profile and subsequent intracellular entrapment of the negatively charged metabolites have been observed previously for bis(POM)-PMEA (4). In order to further improve the absorption enhancement of the ester prodrug, the feasibility of interfering with ester-hydrolysis during absorption was explored in this study using nature-identical fruit extracts. These extracts contain a variety of flavouring esters, which may be substrates for the esterases present in the intestinal mucosa. In the Literature, several specific esterase inhibitors have been described, including diethyl *p*-nitrophenyl phosphate (paraoxon) (5), and bis-p-nitrophenylphosphate (6) (both carboxylesterase inhibitors), neostigmine (a cholinesterase inhibitor) (7) and *p*-hydroxymercuribenzoate (an arylesterase inhibitor) (8). However, the extreme toxicity of these compounds precludes their clinical use; besides, it would be very difficult to obtain a specific inhibition of degradation of bis(POC)-PMPA by using just one inhibitor. A more realistic strategy would be to use a cocktail of several competitive esters to obtain a reasonable enzymatic inhibition without having a toxic effect of the inhibitor itself. Therefore, the use of nature-identical fruit extracts is promising as a conceptually simple and attractive approach to enhance the oral absorption of esterase-sensitive prodrugs. Inhibition of intestinal metabolism by fruit juices has already been reported in the Literature: it has recently been shown that grapefruit juice increases the oral bioavailability of several compounds by interfering with their CYP450 3A4-mediated oxidative metabolism (9).

In this study, we first explored the influence of nature-identical flavouring extracts on esterase-mediated metabolism using a model substrate (*p*-nitrophenylacetate) for esterase activity (10); next, the stabilizing effect of fruit extracts was investigated by co-incubating bis(POC)-PMPA with homogenates of Caco-2 cells and of the intestinal mucosa from man, pig and rat. Finally, the effect of these flavouring esters on transepithelial transport of bis(POC)-PMPA was evaluated in an *in vitro* model of the intestinal mucosa (Caco-2) (11).

MATERIALS AND METHODS

Materials

(*R*)-PMPA and bis(POC)-PMPA were obtained from Gilead Sciences (Foster City, CA). All chemicals used for culturing

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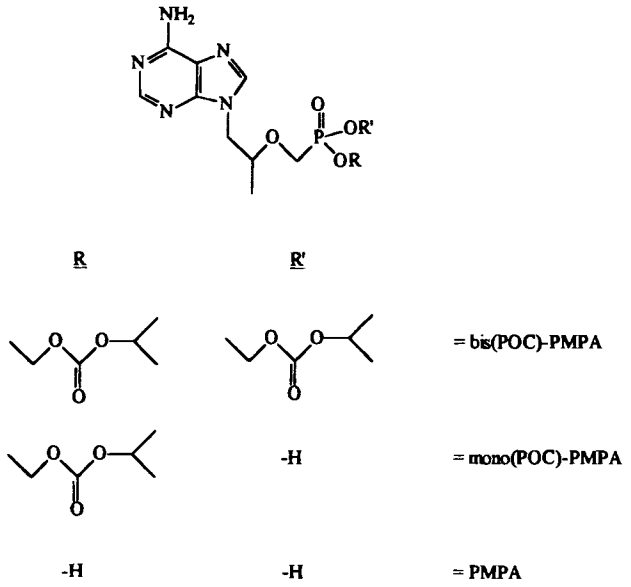

Fig. 1. Structural formula of 9-[(2-phosphonomethoxy)propyl]adenine [PMPA], the bis-ester prodrug bis(POC)-PMPA and its metabolite mono(POC)-PMPA.

the cells were purchased from Gibco Inc. (Life Technologies Inc., Merelbeke, Belgium). Transport medium (TM) consisted of 500 ml Hanks' Balanced Salt Solution containing 25 mM glucose and 10mM Hepes. All fruit extracts (strawberry, passion fruit, banana, apricot) were provided by Givaudan-Roure (Dortmund, Germany). Their quantitative and qualitative composition is well defined and is nature-identical. This means that they consist of a mixture of synthetic compounds of which the qualitative and quantitative composition is comparable to the composition of natural fruit extracts. All other chemicals were of reagent grade or the highest purity commercially available [as described in (4)].

Caco-2 Cell Culture

Caco-2 cells were kindly provided by Dr.Y. Schneider (UCL, Louvain-La-Neuve, Belgium). Cells were grown in 75 cm^2 culture flasks at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. Cells were passaged every 3–4 days (at 70–80% confluence) at a split ratio of 1 to 7. The Caco-2 cells were maintained in high glucose (4.5 g/l) Dulbecco's Minimum Essential Medium containing glutaMAX™, 100 U/ml of penicillin, 100 mg/ml of streptomycin, 1% non-essential amino acids and 10% fetal bovine serum and grown in tissue culture flasks (75 cm^2, Nunc, Roskilde, Denmark). The 'in-house' validation of the Caco-2 system has been described previously (12).

Growth of Cells on Membrane Inserts

For transport experiments, Caco-2 cells were plated at a density of 60,000 cells/cm^2 on Transwell membrane inserts (3 μm pore diameter, 12 mm diameter; Corning Costar Corporation, Cambridge, MA). The monolayers were used for experiments between days 23–25 post-seeding. Cells between passage number 96 and 129 were used in the experiments. Apical and basolateral chamber volumes were maintained at 0.5 and 1.5 ml, respectively. Transepithelial electrical resistance (TEER) was measured using an EVOM Voltohmmeter (WPI, Aston, UK). Only monolayers having TEER values above 200 ohm.cm^2 were used. Sodium fluorescein was used as a hydrophilic marker for cell monolayer integrity. Typical sodium fluorescein flux values across Caco-2 monolayers after the transport experiments with test compound were below 0.5%.hr^{-1}.

Transepithelial Transport Studies

For the determination of the transepithelial flux of PMPA and its prodrug ester bis(POC)-PMPA across Caco-2 monolayers, the polarized monolayers were preincubated with TM for 30 min with or without fruit extracts, after which TEER values were measured to check cell monolayer integrity. The medium was then replaced by TM with test compound (30–1000 μM) at the donor side in the presence or absence of inhibitor. Samples were taken from the apical and basolateral side (100 μl), and the volume withdrawn was replaced with fresh TM, which was corrected for in further calculations. Samples were adjusted with HCl 0.05 M to pH 3.3 and kept at 4°C to reduce chemical degradation of bis(POC)-PMPA during storage. Unless stated otherwise, all flux experiments were conducted in triplicate. Transport is expressed as percentage of the initial amount appearing at the receptor side (average ± sd). "Transport of total PMPA" is the sum of the concentrations of bis(POC)-PMPA, mono(POC)-PMPA and PMPA appearing at the receptor side.

Preparation of Intestinal Homogenates

Freshly scraped Caco-2 cells grown in a 75 cm^2 flask were homogenized in 5 ml TM using a Potter Elvejhem tube and pestle at 0°C. Duodenum and ileum of rat and pig were excised (within 30 min after sacrifying the animals), a segment (±5 cm) was cut along the longitudinal axis and washed with ice-cold TM to remove the intestinal contents. Human duodenum was obtained after Whipple operations; the tissue was evaluated on anatomo-pathological basis to assure that the tissue was non-pathological; the procedure was approved by the Committee of Medical Ethics and Clinical Research, University Hospital Leuven, Belgium. The intestinal mucosa was removed by scraping the intestine from different species with a glass microscope slide. The scrapings were homogenized at 0°C in 5 ml of cold TM using a Potter Elvejhem tube and pestle.

After centrifugation of the crude Caco-2 homogenate and intestinal tissue homogenates at 10,000g for 10 min, the supernatants were harvested and kept at 0°C. Protein content of all preparations was determined according to the method of Lowry (13) using bovine serum albumin as a standard.

Degradation Studies of *p*-Nitrophenylacetate in Purified Esterase Solutions and in Homogenates From Rat Intestine

Prior to use in enzyme assays, protein content of all homogenates was adjusted to 1 mg protein/ml. Linearity of the reaction speed of ester hydrolysis with protein concentration was obtained up to 2 mg protein/ml. Initial velocities for degradation of 1 mM *p*-nitrophenylacetate by purified esterases or by esterases in homogenates from rat intestinal mucosa were determined by adding 50 μl enzyme preparation to 950 μl of prewarmed (37°C) substrate solution with or without esterase inhibitors

[fruit extracts (1%) or specific inhibitors (paraoxon, *p*-hydroxymercuribenzoate, neostigmine or bis-p-nitrophenylphosphate at a concentration of 200 μM)]. The final concentration of DMSO was always adjusted to 1.33% and did not appear to affect the esterase activity (data not shown). Solutions of fruit extracts were always adjusted to pH 7.4 with NaOH 1 M. In order to monitor the release of *p*-nitrophenol, the absorbance of the mixture was followed at 400 nm using a diode array spectrophotometer (HP 8452A). Initial velocities (nmol/sec) of *p*-nitrophenol formation were calculated from the slope of the linear part of the absorbance *versus* time curve. Preliminary studies had shown linearity for the degradation of 1 mM *p*-nitrophenylacetate between 15 and 60 sec for all enzyme preparations. The concentrations of *p*-nitrophenylacetate for determining the kinetic parameters ranged between 3 and 1000 μM. All results are expressed as nmol/s.mg protein.

Degradation of Bis(POC)-PMPA in Homogenates of Caco-2 Monolayers and of Duodenum From Rat, Pig and Man

Metabolism studies were performed at 37°C at a concentration of bis(POC)-PMPA of 100 μM in homogenates containing various concentrations of fruit extracts (0–1%). When inhibition studies were performed, reaction time was always 5 min. This reaction time appeared to fall in the linear part of the concentration versus time curve. Preliminary experiments were performed to obtain linear conditions for each species in further experiments. The esterase-activity appeared to be highly species-dependent; therefore, the protein content for each species was adapted to balance between sensitivity of the analytical method and linearity of the esterase activity of the preparations of each species. Protein content was adjusted to 0.05 mg protein/ml (rat intestine), 0.5 mg protein/ml (pig intestine and Caco-2 homogenates) and 0.1 mg protein/ml (human intestine). At predetermined time points, the reaction was stopped by spiking 100 μl of the incubation solution to 900 μl of ice-cold methanol. After centrifugation at 10000g (10 min, 4°C), 800 μl of the supernatant was evaporated by a gentle stream of air and redissolved in 800 μl of TM adjusted to pH 3.3 with HCl 0.05 M; 50 μl was injected into the HPLC system. Degradation rates were expressed as nmol/s.mg protein (average ± sd; n = 3). The values were corrected for chemical hydrolysis.

HPLC-Analysis of bis(POC)-PMPA and Its Metabolites

Bis(POC)-PMPA and its metabolites mono(POC)-PMPA and PMPA were analyzed using a high-performance liquid chromatographic system equipped with a model 600E Controller and Pump, a model 717plus autosampler and a model Lambda-Max UV detector at 260 nm (Waters, Milford, MA). UV signals were monitored and the obtained peaks integrated using a Digital personal computer running Waters Millenium 32 chromatography software. The column used was a homepacked polystyrene/divinylbenzene column (5 × 0.46 cm i.d., 8 μm particle size, 100 nm pore size; PLRPS, Polymer Labs, The Netherlands). The flow rate amounted to 1.2 ml/min. Mobile phase A consisted of a mixture of 980 ml buffer (10 mM potassium dihydrogenphosphate and 1 mM tetrabutylammoniumhydrogen sulfate adjusted to pH 8 with ammonia 25%) and 20 ml of acetonitrile. Mobile phase B consisted of 970 ml of the same aqueous buffer (adjusted to pH 5.5 with ammonia 2.5%) and 30 ml of acetonitrile. Mobile phase C consisted of 80% acetonitrile and 20% water. Separation was carried out with a gradient starting with 100% mobile phase A, changing to 91% of mobile phase B and 9% of mobile phase C after 4 min, followed by a linear gradient from 9% to 11% of mobile phase C over 5 min. After 9 min, the percentage of mobile phase C was increased to 23%, followed by a linear gradient to 30% mobile phase C after 16 min, and a return to the initial conditions over 1 min. Concentrations of bis(POC)-PMPA and its metabolites were determined using a calibration graph of each compound. Mono(POC)-PMPA concentrations were determined using bis(POC)-PMPA calibration curves, which was allowed because both esters show the same UV response at 260 nm.

RESULTS

Inhibition of Esterase-Mediated Degradation of *p*-Nitrophenylacetate

The stabilizing effect of fruit extracts and selective inhibitors of esterase activity (paraoxon, *p*-hydroxymercuribenzoate, neostigmine or bis-*p*-nitrophenylphosphate) on the enzymatic degradation of the model ester *p*-nitrophenylacetate after incubation with purified esterase or homogenates of rat ileum is summarized in Table I. Experiments conducted with purified esterase revealed a significant stabilization of the model substrate after inclusion of fruit extracts, varying from 79.9% (strawberry) to 52.5% (passion fruit) inhibition of degradation. Fruit extracts also appeared to be very efficient in inhibiting esterase-mediated degradation of *p*-nitrophenylacetate in homogenates from rat ileum: a pronounced inhibition of degradation was observed, varying from 76.6% (strawberry) to 15.7% (passion fruit).

Inhibition of Metabolism of bis(POC)-PMPA in Different Species

The stabilizing effect of fruit extracts on the esterase-mediated degradation of the prodrug ester bis(POC)-PMPA was

Table I. Inhibitory Effect of Esterase Inhibitors on Degradation of *p*-Nitrophenylacetate (1 mM) in Purified Esterase Solution (0.1 IU/ml) or in Homogenates From Rat Ileum (0.05 mg protein/ml)

	concentration	Purified esterase	Rat ileum
p-Hydroxymercuribenzoate	200 μM	N.D.**	23.0 ± 1.8
Paraoxon	200 μM	N.D.	81.9 ± 3.4
bis-*p*-Nitrophenylphosphate	200 μM	N.D.	80.3 ± 3.7
Neostigmine	200 μM	N.D.	5.6 ± 0.1
Strawberry	1%	79.9 ± 4.7	76.6 ± 5.6
Banana	1%	73.7 ± 8.1	57.0 ± 4.6
Passion fruit	1%	52.5 ± 4.9	15.7 ± 1.2

Note: Results are expressed as the percentage inhibition as compared to the degradation rate in the absence of inhibitor * (n = 3; average ± sd).

* Degradation rates for p-nitrophenylacetate 1 mM without inhibitor were 34.7 ± 1.2 and 14.0 ± 0.7 nmol/s.mg protein for purified esterase and rat ileum, respectively; in rat ileum, K_m = 238 ± 20 μM and V_{max} = 19.0 ± 2.3 nmol/s.mg protein.

** N.D. = Not Determined.

assessed in homogenates of duodenum from rat, man and pig. The enzymatic degradation rates of bis(POC)-PMPA (100 μM) in duodenum of different species amounted to 1.37, 0.51 and 0.14 nmol/s.mg protein for rat, man and pig, respectively. The degradation rate of bis(POC)-PMPA (100 μM) in Caco-2 homogenate was 0.02 nmol/s.mg protein. The effect of the addition of various fruit extracts (1%) on enzymatic degradation of bis(POC)-PMPA (100 μM) upon exposure to homogenates of rat duodenum is shown in Table II. The inhibition of enzymatic degradation of bis(POC)-PMPA when fruit extracts were included in the incubation medium, ranged from 0.6% (passion fruit) to 94.4% (apricot). A similar inhibition was obtained when bis(POC)-PMPA was incubated in homogenates prepared from pig and man duodenum and from Caco-2 monolayers in the presence of strawberry extract 1% (data not shown). As illustrated in Table II for strawberry extract, the inhibitory effect was found to be concentration-dependent.

Transport of bis(POC)-PMPA Across Caco-2 Monolayers: Influence of Fruit Extracts

The effect of the selected fruit extracts on the intestinal absorption of bis(POC)-PMPA was assessed using an *in vitro* system of the intestinal mucosa, Caco-2. Preliminary experiments had shown that the four flavouring extracts tested had a similar effect on transport and metabolism of bis(POC)-PMPA (data not shown). Based on these results and on analytical considerations, strawberry extract was selected for further experiments. Figure 2 shows the time-dependent transport of bis(POC)-PMPA (50 μM initial concentration) and its metabolites across Caco-2 monolayers in the presence or absence of strawberry extract (1%). The inclusion of strawberry extract resulted in a 3-fold higher transport as compared to the control condition. The fraction of intact prodrug appearing in the acceptor compartment increased from virtually zero to 67.1%. No toxic effect of fruit extracts on the Caco-2 monolayers was observed at a concentration up to 1%, as could be concluded from the TEER values measured before and after the transport experiments, and from the sodium fluorescein (a paracellular leakage marker) transport. TEER values appeared to slightly increase when fruit extracts were added into the medium; the reason for this increase remains unclear. In the absence of

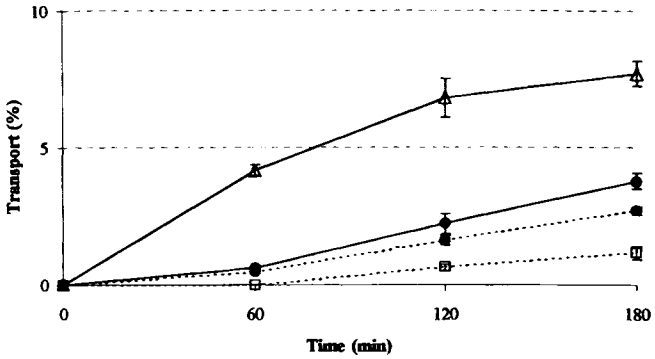

Fig. 2. Time course of apical to basolateral transport across Caco-2 monolayers for bis(POC)-PMPA and its metabolites [mono(POC)-PMPA and PMPA] after adding 50 μM of bis(POC)-PMPA to the apical side (Δ, prodrug; ●, mono-ester; □, PMPA). Dotted lines represent control transport of bis(POC)-PMPA and its metabolites, while solid lines represent transport of the prodrug and metabolites in the presence of 1% strawberry extract. No detectable amounts of the bis-ester prodrug (in the absence of flavouring extract) and of PMPA (in the presence of flavouring extract) were observed. Values are averages ± sd; n = 3.

strawberry extract, transport of bis(POC)-PMPA increased with increasing concentrations initially added to the apical side (Fig. 3); nevertheless, at all concentrations of bis(POC)-PMPA tested, an absorption enhancing effect was observed upon inclusion of strawberry extracts (1%). The effect of strawberry extract (0–1%) on transport of total bis(POC)-PMPA also appeared to be concentration-dependent (Fig. 4).

DISCUSSION

The ester prodrug concept is commonly used to increase the oral absorption of charged compounds. In the case of bis(POC)-PMPA (ester prodrug of PMPA), the two lipophilic side chains mask the anionic charges of the phosphonate moiety. It is crucial that the active drug is released once the prodrug reaches the bloodstream. The ubiquitous presence of esterases in the body substantiates the choice of using ester derivatives: the ester prodrug will be readily cleaved after entering the

Table II. Inhibition of Metabolism of bis(POC)-PMPA 100 μM by Fruit Extracts in Homogenates of Rat Duodenum (0.05 mg protein/ml)

Extract	Concentration (%)	% inhibition
Passion fruit	1	0.6 ± 0.1*
Apricot	1	94.4 ± 18.9
Banana	1	72.1 ± 8.2
Strawberry	1	90.6 ± 4.5
	0.5	75.1 ± 11.7
	0.25	49.3 ± 1.7
	0.125	28.2 ± 1.6

Note: Values are expressed as percentage inhibition of the degradation rate of bis(POC)-PMPA in the absence of fruit extracts (n = 3; average ± sd).

* Degradation rate of bis(POC)-PMPA 100 μM in the absence of fruit extract was 1.25 ± 0.02 nmol/s.mg protein.

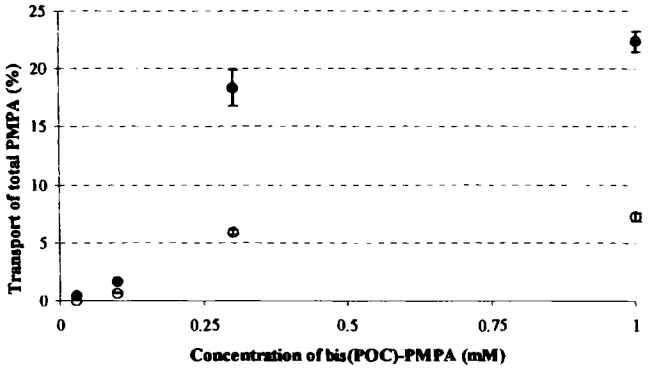

Fig. 3. Transport of total PMPA (= bis + mono + PMPA) across Caco-2 monolayers at 60 min after addition of various initial concentrations of the bis-ester prodrug in the presence (●) or absence (○) of strawberry extract 1%. Transport is expressed as the average percentage (± sd) of the amount of bis(POC)-PMPA initially added to the donor compartment (n = 3).

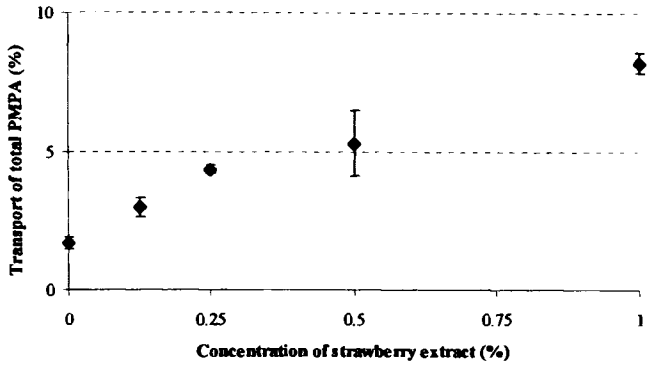

Fig. 4. Transport of total PMPA across Caco-2 monolayers at 120 min after addition of 100 μM bis(POC)-PMPA in the presence of increasing concentrations of strawberry extract (0–1%). Transport is expressed as average percentage (± sd) of the amount of bis(POC)-PMPA initially added to the donor compartment (n = 2).

systemic circulation. However, esterases in the intestinal epithelia may convert the lipophilic prodrug to the parent drug even before entering the blood stream, thus reducing the efficiency of the ester prodrug to enhance the oral absorption. Several intestinal esterases have been described [e.g., carboxylesterases (14), cholinesterases (10), paraoxonases (15)], suggesting that different esterases may be responsible for the rapid intracellular metabolism of ester prodrugs. In this study, we evaluated inhibition of esterase-activity by fruit extracts as a conceptually simple strategy to enhance transport of bis(POC)-PMPA through epithelial cells. The fruit extracts used contain a large series of small esters, which could be competitive inhibitors for the esterase-mediated degradation of bis(POC)-PMPA. Because of the variety of esterases present in the intestinal mucosa, the use of such ester cocktails might be useful to obtain broad-spectrum inhibition without any toxicity of the inhibitor itself.

In a first set of experiments, *p*-nitrophenylacetate was used as a model substrate for studies on esterase activity (12). The results show that degradation of this substrate in rat ileum was significantly reduced when specific esterase inhibitors were included in the incubation medium. The results also suggest that *p*-nitrophenylacetate is mainly degraded by carboxylesterases, since bis-*p*-nitrophenylphosphate and paraoxon, both inhibitors of carboxylesterase, elicited a much higher inhibition than neostigmine (cholinesterase inhibitor) or *p*-hydroxymercuribenzoate (arylesterase inhibitor). A substantial protection against esterase-mediated degradation in homogenates from rat ileum was also obtained when the fruit extracts (1%) were included in the incubation medium.

In a second set of experiments, the concept of esterase inhibition by inclusion of fruit extracts was studied for bis-(POC)-PMPA. First, the degradation of bis(POC)-PMPA was evaluated in duodenal homogenates from several species. Its metabolism was found to be species-dependent, in the order rat > man > pig > Caco-2. Because of its strong esterase activity, rat duodenum was selected for further inhibition studies with fruit extracts. The esterase-mediated degradation of bis(POC)-PMPA was markedly inhibited by the inclusion of certain fruit extracts (mainly by strawberry and by apricot); passion fruit extract caused a rather minor inhibition of the degradation of bis(POC)-PMPA. The differences between these fruit extracts should be attributed to their specific composition. It is not yet known which esters are responsible for the inhibitory effect on the metabolism of bis(POC)-PMPA and p-nitrophenylacetate. It should be noted that, despite the different quantitative and qualitative inhibitory effects of the fruit extracts on degradation of *p*-nitrophenylacetate and bis(POC)-PMPA metabolism, the inhibition by fruit extracts showed a similar trend for both compounds.

The effect of stabilization of bis(POC)-PMPA by fruit extracts on intestinal absorption was evaluated in the Caco-2 model. At an initial concentration of 50 μM bis(POC)-PMPA, a 3-fold increase in transport of total PMPA was obtained in the presence of strawberry extract (1%). The inclusion of the extract also resulted in the appearance of intact prodrug at the acceptor compartment, indicating that inhibition of metabolism is at least partially responsible for this absorption enhancement. The fact that the transport of total PMPA increases at higher initial concentrations of bis(POC)-PMPA (Fig. 3) can probably be attributed to partial saturation of the intestinal metabolism. However, even at the highest concentrations of bis(POC)-PMPA tested (1 mM), the absorption-enhancing effect of the fruit extracts was still observed. The finding that the effect of strawberry extract on transport of bis(POC)-PMPA is dependent on the concentration of the extract is in agreement with its concentration-dependent effect on the metabolism of bis(POC)-PMPA upon incubation with intestinal homogenates (Fig. 4).

In conclusion, the results of this study clearly demonstrate that fruit extracts inhibit the degradation of the ester prodrug bis(POC)-PMPA and the model compound, p-nitrophenylacetate. Notwithstanding large interspecies differences in metabolism, a stabilizing effect by fruit extracts was observed in all the experimental conditions. Transport of total PMPA across Caco-2 monolayers was significantly enhanced when bis(POC)-PMPA was combined with fruit extracts. This conceptually simple approach appears to be a promising strategy for the design of an efficient oral drug delivery system for the antiviral ester prodrug bis(POC)-PMPA. The same approach may be equally valid for the delivery of bis(POM)-PMEA. *In vivo* studies will have to demonstrate the applicability of this absorption enhancing approach for these and other ester prodrugs.

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