Increased Absorption of the Antiviral Ester Prodrug Tenofovir Disoproxil in Rat Ileum by Inhibiting Its Intestinal Metabolism

(Received May 12, 2000; accepted August 17, 2000)

This paper is available online at http://www.dmd.org

ABSTRACT:

Previous studies have shown that strawberry extract increases the transepithelial transport of tenofovir disoproxil, an esterase-sensitive prodrug of the antiviral compound tenofovir (formerly PMPA), across Caco-2 monolayers. This increase in transport was at least partially due to inhibition of its intestinal metabolism. To further study the feasibility of this absorption enhancing strategy, the influence of various concentrations of strawberry extract (0–2%) on the intestinal absorption of tenofovir disoproxil (100 $\mu$M) was assessed using an in situ perfusion model with immediate blood sampling from the mesenteric vein, a model closer to the in vivo situation than the in vitro Caco-2 system. Inclusion of strawberry extract (1%) resulted in a 7-fold increase in the appearance of tenofovir equivalents. The metabolism of tenofovir disoproxil in the intestinal perfusate was significantly lower in the presence of strawberry extract (1%), showing that the metabolism of tenofovir disoproxil is reduced by the flavoring extract.

Materials and Methods

Chemicals. Tenofovir [(R)-PMPA] and tenofovir disoproxil (fumarate salt) were obtained from Gilead Sciences (Foster City, CA). Transport medium
consisted of Hanks’ balanced salt solution supplemented with Hepes, 10 mM, adjusted to a pH of 7.4. Strawberry extract was provided by Givaudan-Roure (Dortmund, Germany). Its quantitative and qualitative composition is well defined and is nature-identical, which means that it consists of a mixture of synthetic compounds of which the composition resembles that of the natural fruit extract. For the composition of natural strawberry extract, the reader is referred to the literature on food chemistry (e.g., Belitz and Grosch, 1999). Disodium heparin (185 IU/mg) was purchased from Sigma (Bornem, Belgium). Chloroacetaldehyde was obtained from Fluka Chemie (Buchs, Switzerland). All other chemicals were of reagent grade and of the highest purity commercially available (as described in Augustijns et al., 1998).

In Situ Rat Intestinal Perfusion Studies with Tenofovir Disoproxil and Tenofovir. In situ perfusion experiments were performed based on a previously described method (Annaert et al., 2000). Male Wistar rats (≤300 g, Animalium, K. U. Leuven, Leuven, Belgium) were used. Rats were housed under standard laboratory conditions with free access to water and food. Before the start of the experiment, the intestinal segment was perfused for 15 min with transport medium containing various concentrations of strawberry extract (0–2% v/v). The flow rate of the perfusate amounted to 3 ml/min. This relatively high flow rate was used to obtain quick steady-state perfusate concentrations and to obtain a homogeneous distribution of the drug in the perfused segment throughout the whole experiment. At the beginning of the perfusion with the antiviral compound, the mesenteric-jugular shunt was opened and donor blood supply initiated via the jugular vein at a rate of 0.5 ml/min. The intestinal segment was perfused with tenofovir disoproxil or free tenofovir (0.1 mM) in the presence of various concentrations of strawberry extract (0–2%). Blood from the mesenteric vein was collected in heparinized tubes over 5-min time intervals for 30 min. In addition, samples were taken from the perfusion medium in the middle of each time interval. Blood samples were centrifuged at 5000 g for 10 min (4°C), and the obtained plasma fractions were weighed. Because no significant accumulation of tenofovir disoproxil nor its metabolites occurs in red blood cells (<1.5%), and because higher sensitivity could be obtained when using plasma samples, concentrations of tenofovir and tenofovir mono(ester) were determined in plasma instead of total blood. Perfusate samples were tested for lactate dehydrogenase (LDH) release to measure cellular membrane damage using the LD-L 20 kit (Sigma Diagnostics).

HPLC Analysis of Tenofovir Disoproxil and Metabolites. Perfusate samples of tenofovir disoproxil and metabolites were analyzed following a previously described method (Van Gelder et al., 1999a). Tenofovir and tenofovir mono(ester)-prodrug concentrations in plasma were determined according to a previously described method (Naens et al., 1992) with some modifications. After deproteinization of the sample (100 μl) with acetonitrile (100 μl) and centrifugation, supernatant was used for derivatization. Briefly, the adenine moiety of the nucleotide analogs was converted with chloroacetalddehyde at 60°C for 6 h into a fluorescent 1,4-thioacetaldehyde derivative. Analysis of the derivatized samples was performed using a HPLC gradient method with fluorescence detection (Jasco FP-920 detector). The excitation wavelength was 260 nm and emission was followed at 425 nm. The column used was a Waters SymmetryShield RP8 (10 × 0.46 cm, i. d., 3.5-μm particle size) and a Waters Symmetry RP precolumn. The analytical range was between 2 and 0.01 μM for tenofovir and tenofovir mono(ester). In both cases, inter- and intraday variability was lower than 10%.

Calculations. Results of the perfusion experiments with tenofovir and tenofovir disoproxil were expressed as tenofovir equivalents appearing in the mesenteric plasma, corrected for time and length of the segment. Due to slight variations in perfusate concentrations, the calculated steady-state perfusate concentration of each experiment was normalized to a target concentration of 0.1 mM.

Statistical Analysis. Data are expressed as average value ± S.E.M. (n ≥ 3). A Student’s t test was performed to compare two data sets. A one-way ANOVA combined with a Tukey’s post-test was used to compare more than two data sets. A P value of <.05 was considered significant.

Results and Discussion

Because of the similar degradation rate of tenofovir disoproxil in homogenates from rat ileum compared with homogenates from human ileum (Van Gelder et al., 2000), rat ileum perfusion was used to study the effect of strawberry extract on the intestinal absorption of this ester prodrug. A significant increase in intestinal absorption of tenofovir equivalents was obtained when it was used as its ester prodrug: a 12-fold increase in tenofovir equivalents appearing in the plasma was observed when tenofovir was perfused through rat ileum as its disoproxil prodrug (6.39 ± 1.47 pmol/cm² min compared with 0.51 ± 0.15 pmol/cm² min when free tenofovir was used). The very low absorption of tenofovir when perfused as the free compound confirms previous results with the in vitro Caco-2 system (Naens et al., 1998; Van Gelder et al., 1999b). As tenofovir has two negative charges at physiological pH, one can assume that its transport is limited to the paracellular pathway, which may explain its low intestinal absorption. The increase in absorption when using tenofovir disoproxil indicates that the prodrug has the ability to cross the epithelial cell monolayer through the transcellular pathway.

A limiting factor to the efficiency of tenofovir disoproxil as an absorption-promoting approach for tenofovir is, however, its esterase-mediated, intra-epithelial degradation. In dogs, the oral administration of tenofovir as its disoproxil prodrug has been reported to increase the bioavailability of the antiviral drug candidate from 17.1% for free tenofovir to only 30.1% for tenofovir disoproxil (Shaw et al., 1997; Cundy et al., 1998). An interesting strategy to further increase the intestinal absorption of tenofovir disoproxil may be to inhibit its intestinal metabolism by using nature-identical strawberry extract, because it contains a multitude of small esters that may competitively inhibit the esterase-mediated degradation of tenofovir disoproxil. Figure 2 shows the percentage of tenofovir mono(ester) formed in the lumen during perfusion of the rat intestinal segment with tenofovir disoproxil in the absence or presence of fruit extract (1%). A significant (P < .05) decrease in the appearance of tenofovir mono(ester) was observed when the extract was present in the perfusate, indicating that tenofovir disoproxil was protected against the esterase-mediated degradation by compounds present in the fruit extract. Simultaneously, a 7.5-fold increase in tenofovir equivalents appearing in the mesenteric plasma was observed when tenofovir disoproxil was coperfused with 1% strawberry extract (Fig. 3). No further improvement was observed upon increasing the concentration of the extract to 2%, suggesting that the increase in intestinal absorption is not due to a concentration-dependent toxic effect of the extract. The inclusion of strawberry extract 1% did not result in a significant increase in
absorption when free tenofovir was used (0.66 ± 0.24 pmol/cm · min). As stated previously, tenofovir is believed to cross the cell monolayers through the paracellular pathway; therefore, the absence of any effect of strawberry extract on the ideal absorption of tenofovir indicates that the increase in intestinal absorption of tenofovir disoproxil by the fruit extract is not due to an effect on the tight junctions. The enhancing effect of the fruit extract is considered to be mainly due to inhibition of the metabolism of the prodrug. This conclusion is based on our previous studies, showing an 80% inhibition of the esterase-mediated degradation of tenofovir disoproxil (0.1 mM) in intestinal homogenates after inclusion of 1% strawberry extract. Furthermore, transport studies with the in vitro Caco-2 system also showed an increase in transepithelial transport of tenofovir disoproxil when coincubated with strawberry extract. This increase in transport was not accompanied by a reduction in transepithelial electrical resistance values or an increase in sodium fluorescein transport, which was used as a paracellular marker (Van Gelder et al., 1999a).

The increase in the prodrug’s intestinal absorption by strawberry extract was not accompanied by any considerable cell membrane damage. This was shown by measuring the effect of strawberry extract on the release of LDH in the perfusate as an indication of cell membrane disruption (Wu and Robinson, 1999). No significant increase in LDH activity was observed when tenofovir disoproxil was copерфused with strawberry extract (0.77 ± 0.07 U/l · cm in the absence of strawberry extract, compared with 0.75 ± 0.09 U/l · cm in the presence of 1% strawberry extract). A slight increase (P < .05) was observed in the presence of 2% fruit extract (2.34 ± 0.47 U/l · cm). This increase was still small compared with a control perfusion experiment with 2% Tween 80 (4.73 ± 0.26 U/l · cm), which has been described to be a surfactant with only mild cytotoxicity (Oberle et al., 1995). Therefore, it can be concluded that strawberry extract, up to 1%, did not affect the intestinal cell membranes.

In conclusion, strawberry extract strongly increases the intestinal absorption of tenofovir disoproxil in rat ileum. This increase in absorption is mainly due to inhibition of the esterase-mediated degradation of the prodrug. The increase in intestinal absorption of the prodrug by the extract was not accompanied by an effect on the integrity of the epithelium (opening of the tight junctions or influence on membrane integrity). Therefore, metabolic inhibition by components in fruit extract appears to be a promising strategy to enhance the intestinal absorption of tenofovir disoproxil and other ester prodrugs.

References


