Eppley Institute for Research in Cancer and Allied Diseases, and the
Departments of † Pharmacology and ‡ Biochemistry,
University of Nebraska Medical Center
Omaha, NE 68105, U.S.A.

ROBERT L. FOLDES*,†
EDWARD BRESNICK†,‡,§

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* Present address: Division of Cancer and Cell Biology, Mount Sinai Hospital Research Institute, 600 University Ave., Toronto, Ontario M5G-1X5, Canada.

§ Correspondence: Dr. Edward Bresnick, Department of Biochemistry, University of Nebraska Medical Center, 42nd & Dewey Ave., Omaha, NE 68105.

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Na^+ and pH dependent transport of foscarnet via the phosphate carrier system across intestinal brush-border membrane

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Foscarnet (trisodium phosphonoformic acid, PFA*, Fig. 1) is a potent inhibitor of herpesvirus replication [1, 2] and is also effective against the AIDS retrovirus (LAV/HTLV-III) [3]. PFA has been found to be absorbed completely in rabbits following oral administration [4], while approximately 30% absorption occurs in mice and rats (unpublished data from Astra Läkemedel AB.) and about 12–22% in humans†. These fairly high values of oral availability, despite the high hydrophilicity, and the considerable species difference in the availability suggest a participation of some specific mechanism for PFA absorption. The

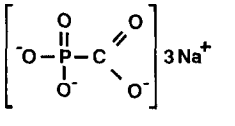

Fig. 1. Chemical structure of PFA.

* Abbreviations: PFA, foscarnet (trisodium phosphonoformic acid); Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid; Mes, 2-(*N*-morpholino)-ethanesulfonic adic; and FCCP, carbonylcyanide-4-trifluoromethoxyphenylhydrazone.

† Sjövall J, Karlsson A, Ogenstad S, Sandström E and Saarimäki M, 27th Interscience Conference of Antimicrobial Agents and Chemotherapy, New York, Abstr. No. 1083, p. 286, 1987.

chemical structure of PFA is very similar to phosphate; therefore, it is very likely that PFA is absorbed by the same mechanism as phosphate, which is known to be transported via a specific carrier-mediated system [5, 6]. However, the gastrointestinal absorption mechanism of this extremely hydrophilic drug has not been investigated.

The purpose of this study was to clarify the intestinal absorption mechanism of PFA, as well as its relationship to the phosphate transport system, using purified intestinal brush-border membrane vesicles.

Materials and methods

Male Sprague–Dawley rats weighing 220–250 g (Nihon-Clea, Tokyo, Japan) were used; they had access to a standard food and water prior to the experiments. ^{14}C-Labeled PFA (2.4 mCi/mmol) and unlabeled PFA were supplied by Astra Läkemedel AB., Södertälje, Sweden. Bovine serum albumin (BSA, Fraction V) and glycyl-L-proline were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.), and carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP) was from the Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Arsenate was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals were of reagent grade and were used without further purification.

Intestinal brush-border membrane vesicles were prepared by the method of Kessler *et al.* [7] with some modifications as described by Tsuji *et al.* [8]. The uptake of [^{14}C]PFA by membrane vesicles was measured using a rapid filtration technique described previously [8]. To minimize binding to membrane filters, 2 mM unlabeled PFA was added to the stop solution. The conditions employed for each experiment are given in the figure legends and the table footnotes.

Radioactivity of [^{14}C]PFA trapped on Millipore membrane filters (pore size, 0.45 μm) was counted with a liquid scintillation counter (LSC-671; Aloka, Tokyo, Japan). Protein was measured by the method of Bradford [9], using a Bio-Rad Protein Assay Kit with BSA as a standard.

Results and discussion

The equilibrium uptake of [^{14}C]PFA by brush-border membrane vesicles at 120 min decreased linearly with the increase of extravesicular osmolarity. The uptake obtained by extrapolating to infinite osmolarity was approximately 3% of the uptake under the isotonic conditions, indicating negligible binding to the brush-border membranes (Fig. 2). This result was also supported by an intravesicular space of 1.2 μl/mg of protein calculated from the equilibrium uptake under isotonic conditions, since the obtained intravesicular space was comparable to that derived in our laboratory from the uptake of D-glucose, 1.3 μl/mg protein.

As indicated in Table 1, the initial uptake of PFA at 30 sec was reduced in the presence of 5 mM unlabeled PFA, whereas D-glucose and glycyl-L-proline, which are absorbed by a specific carrier-mediated mechanism [10, 11], had no effect on PFA uptake. The specific inhibitory effect of PFA itself and the remarkable temperature dependency (Table 1) suggest the participation of a carrier-mediated transport mechanism in PFA uptake. Szczepanska-Konkel *et al.* [12] and Yusufi *et al.* [13] reported that PFA has a specific inhibitory effect on phosphate transport across renal brush-border membranes in both rats and humans. These results suggest the participation of a carrier-mediated reabsorption of PFA via the phosphate transport system in the renal proximal tubule. Phosphate is also absorbed via a carrier-mediated transport mechanism at the small intestinal brush-border membrane, though there are several differences in the transport characteristics between the two organs [5, 6, 14–16]. Therefore, it is possible that the antiviral agent PFA may be absorbed via the phosphate transport system existing at the intestinal brush-border membrane. Indeed, PFA transport was inhibited significantly by phosphate and arsenate (Table 1), which are specific inhibitors of phosphate transport [5]. These results suggest that PFA and phosphate share a common carrier-mediated transport system.

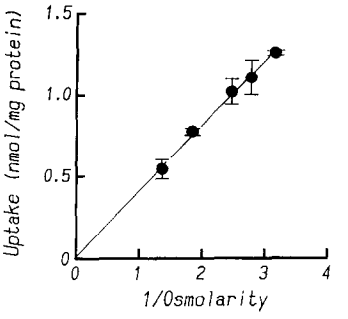

Fig. 2. Effect of osmolarity of the extravesicular medium on PFA uptake by brush-border membrane vesicles at the steady state. Membrane vesicles were preincubated with 10 mM Tris/Hepes buffer (pH 7.5) containing 270 mM mannitol. Uptake of PFA was measured by adding 80 μl of 10 mM Tris/Mes buffer (pH 6.0) containing 100 mM NaCl, various concentrations of mannitol and 1 mM [^{14}C]PFA to 20 μl of membrane vesicle suspenion containing approximately 80 μg protein at 37° for 180 min. Each point is the mean ± SE for three to five experiments.

The PFA transport was both pH and Na^+ dependent (Table 2). At an intravesicular pH (pH_{in}) of 7.5, the uptake at an acidic extravesicular pH (pH_{out}) of 6.0 was enhanced markedly (approximately 3-fold) compared to uptake at a neutral pH_{out} of 7.5. A similar pH effect was observed when pH_{in} was 6.0. These results indicate that an acidic extravesicular pH is favorable for PFA transport. Since the existence of a microclimate pH (approximately 5.5 to 6.2) is known in the small intestine [17, 18], the pH_{out} of 6.0 employed in this experiment is probably reasonable for evaluating the intestinal absorption mechanism of PFA. A similar pH dependence has been reported for the transport of phosphate [5, 6]. Possible mechanisms of this pH-dependent transport include: (1) the existence of a preferred ionic form of PFA or the carrier protein, (2) a requirement for a higher proton concentration in the binding or translocation of PFA, and (3) an H^+ or OH^- gradient dependent transport mechanism. Although the third mechanism has been suggested for phosphate transport [5], it is unlikely in the case of PFA. This is based on the fact that a protonophore, FCCP, had no significant effect on PFA uptake, and no significant difference in PFA uptake was observed in the presence (pH_{out}/pH_{in} = 6.0/7.5) and absence (pH_{out}/pH_{in} = 6.0/6.0) of a pH gradient (Table 2). PFA has three ionizable groups with pK_a values of 0.49 and 7.27 for the phosphate hydroxy groups and 3.41 for the carboxyl group [19]. At pH 7.5, more than 60% of total PFA exists in the trivalent form, whereas at pH 6.0 the divalent form accounts for more than 95%. If the divalent form of PFA is transported, then an approximately 2.5-fold increase in the rate of uptake would be expected upon lowering the pH_{out} from 7.5 to 6.0. This was actually the case (Table 2), supporting the first mechanism. However, the other mechanisms are not excluded as discussed for the case of phosphate transport [20].

Furthermore, the initial uptake of PFA at 30 sec was enhanced significantly in the presence of Na^+ (0.675 ± 0.032 nmol/30 sec/mg protein at a PFA concentration of 1 mM; mean ± SE, N = 3–4) in the extra-

Table 1. Effect of temperature and various compounds on the uptake of PFA by brush-border membranes vesicles

Test compound	Concn	PFA uptake (nmol/mg protein/30 sec)	N	Uptake (%)
Control (37°)		0.638 ± 0.017	4	100
Control (4°)		0.163 ± 0.016*	5	25.5
+ Glucose	10 mM	0.584 ± 0.017	4	91.5
+ Glycyl-L-proline	10 mM	0.589 ± 0.006	4	92.3
+ Unlabeled PFA	5 mM	0.413 ± 0.011*	5	64.7
+ Phosphte	5 mM	0.484 ± 0.019*	4	75.9
+ Arsente	5 mM	0.479 ± 0.021*	3	75.1

Membrane vesicles were preincubated as described in the legend to Fig. 2. Uptake of PFA was measured by adding 80 μl of 20 mM Tris/Mes buffer (pH 6.0) containing 100 mM mannitol, 100 mM NaCl and the corresponding compound to 20 μl of membrane vesicle suspension. The osmolarity of each solution was adjusted to that in the control study by reducing the mannitol concentration. Each value is the mean ± SE for (N) determinations.
* The level of significance, determined by Student's *t*-test, was $P < 0.05$.

Table 2. Effect of pH on the uptake of PFA by brush-border membrane vesicles

pH_{out}/pH_{in}	PFA uptake (nmol/mg protein/30 sec)	N
6.0/7.5	0.664 ± 0.005	4
7.5/7.5	0.222 ± 0.007	5
6.0/6.0	0.576 ± 0.019	3
7.5/6.0	0.210 ± 0.008	5
6.0/7.5 (+ FCCP)	0.597 ± 0.037	4
6.0/7.5 (− FCCP)	0.676 ± 0.023	4

Membrane vesicles were preincubated with 10 mM Tris/Hepes buffer (pH 7.5) or 10 mM Tris/Mes buffer (pH 6.0) each containing 270 mM mannitol. Uptake of PFA was measured by adding 80 μl of either 20 mM Tris/Mes buffer (pH 6.0) or 20 mM Tris/Hepes buffer (pH 7.5), each containing 100 mM mannitol, 100 mM NaCl and 1 mM [^{14}C]PFA to 20 μl of membrane vesicle suspension. In the study of FCCP effects, uptake of PFA was measured by adding 80 μl of 20 mM Tris/Mes buffer (pH 6.0) containing 100 mM mannitol, 100 mM NaCl, 1 mM [^{14}C]PFA and FCCP dissolved in ethanol to 20 μl of membrane vesicle suspension. The final concentrations of FCCP and ethanol were 40 μM and 0.4% respectively. Each value is the mean ± SE for (N) determinations.

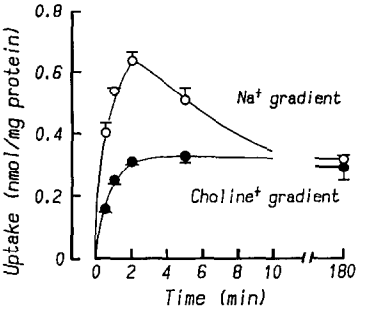

Fig. 3. Time course of PFA uptake by brush-border membrane vesicles. The membrane vesicles were preincubated as described in the legend to Fig. 2. Uptake of PFA was measured by adding 80 μl of 10 mM Tris/Mes buffer containing 100 mM mannitol, 0.5 mM [^{14}C]PFA mixed with either 100 mM NaCl (○) or 100 mM choline chloride (●) to 20 μl of membrane vesicle suspension. Each point is the mean ± SE of three to five experiments.

vesicular medium compared to those in the presence of K^+ (0.422 ± 0.043), Li^+ (0.418 ± 0.024) and choline$^+$ (0.394 ± 0.015). In the presence of an Na^+ gradient at pH_{out}/pH_{in} = 6.0/7.5, the uptake of PFA showed a clear overshoot phenomenon, indicating cotransport of PFA with Na^+ (Fig. 3). Although we only examined inward gradients of Na^+ and H^+, the Na^+ and pH dependencies of PFA transport resemble those reported for phosphate transport [5, 6].

The absorption of PFA from the *in situ* gastrointestinal loops of rats [21] exhibited significant regional specificity (data not shown). Absorption occurred primarily in the upper small intestine and was negligible from stomach and large intestine. Similarly, phosphate absorption in rats is highest in the jejunum, followed by the duodenum and ileum [22].

All the results obtained in the present experiments, utilizing the intestinal brush-border membrane vesicles and the *in situ* gastrointestinal loops, indicate the existence of a specialized transport system for PFA which is common to that for phosphate. This conclusion is supported by a recent investigation which identified a specific inhibitory effect of PFA on phosphate transport in the small intestine [23]. Therefore, it is concluded that the absorption of a highly hydrophilic drug, PFA, occurs mainly by a carrier-mediated process via the phosphate transport sytem existing in the intestinal brush-border membrane.

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* Correspondence: Prof. Akira Tsuji, Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan.

Faculty of Pharmaceutical Sciences
Kanazawa University
Kanazawa 920, Japan

AKIRA TSUJI*
IKUMI TAMAI

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Intestinal first-pass metabolism of phenacetin in rabbits pretreated orally and intraperitoneally with 3,4-benzpyrene

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Phenacetin (PHT), used as an analgesic and an antipyretic, is metabolized extensively, the major metabolic route being O-de-ethylation to acetaminophen (NAPA). NAPA is subsequently conjugated to form a glucuronide (NAPA glucuronide, NAPAG) and a sulfate (NAPA sulfate, NAPAS).

It has been reported that an enzyme system present in the mucosal lining of the small intestine of rats is capable of metabolizing PHT and that the enzyme activity increases in rats pretreated with 3,4-benzpyrene [1, 2] and 3-methylcholanthrene [3]. The activity of this intestinal enzyme system is also increased in rats that have been exposed to cigarette smoke [1, 3, 4] or have been fed charcoal-broiled beef [5], rat chow [5] or vegetables [6]. Klippert *et al.* [7] found that 3-methylcholanthrene pretreatment results in enhanced PHT disposition as was shown from decreased plasma half-life time, decreased oral availability, increased clearance, and an increase in metabolite levels. In rats pretreated with 3-methylcholanthrene, the intestine contributes significantly, and predominantly over the liver, to PHT first-pass metabolism. In contrast, gut wall metabolism in control rats could not be demonstrated [7].

In a previous report [8], we demonstrated enhanced PHT metabolism after oral pretreatment of rabbits with 3,4-benzpyrene as shown by decreased levels of PHT and increased levels of NAPA and NAPAG in mesenteric