Pergamon

Biochemical Pharmacology, Vol. 48, No. 7, pp. 1455–1458, 1994.

0006-2952/94 $7.00 + 0.00

0006-2952(94)00302-5

ENHANCED BIOAVAILABILITY OF PHOSPHONOFORMIC ACID BY DIETARY PHOSPHORUS RESTRICTION

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(*Received* 9 *March* 1994; *accepted* 17 *May* 1994)

Abstract—Phosphonoformic acid (PFA, foscarnet) is a potent inhibitor of Na^+-P_i cotransport in intestinal and renal brush border membranes (BBM). We have studied the effect of dietary phosphorus restriction on intestinal PFA absorption and bioavailability. Rats were placed on low (0.04% P_i, LPD) or normal (0.95% P_i, NPD) phosphorus diets for 5 days, followed by administration of an oral bolus of [^{14}C]PFA (100 mg/kg). Of the oral PFA dose, 60 ± 4% was absorbed in LPD rats, compared with 43 ± 3% in NPD rats ($P < 0.05$, $N = 5$). This was associated with higher plasma PFA concentrations in LPD compared with NPD rats (44.2 ± 2.0 and 17.9 ± 4.3 μg/mL, respectively). [^{14}C]PFA uptake, determined in intestinal BBM vesicles (BBMV), was Na^+ gradient ($Na^+_{out} > Na^+_{in}$) dependent. Dietary phosphorus restriction resulted in a 39.8% increase in the initial (1 min) Na^+-dependent [^{14}C]PFA uptake by intestinal BBMV. We conclude that PFA absorption is enhanced by dietary phosphorus restriction.

Key words: phosphate absorption; adaptation; biological transports; foscarnet; brush border membrane

P_i§ is transported across the renal and intestinal BBM via an active, carrier-mediated transport process [1]. Inhibitors of P_i transport may have a role as potential therapeutic agents to reduce phosphate accumulation in pathologic conditions. Phosphonocarboxylic acids have been shown to be specific inhibitors of Na^+-P_i cotransport in intestinal as well as renal proximal tubular BBM [2, 3]. Among these agents, the monophosphonate phosphonoformic acid was found to be one of the most potent [3, 4]. In addition to a direct inhibitory effect on the Na^+-dependent P_i transport by intestinal BBMV [2, 5], PFA can be absorbed by the BBM of the enterocyte, presumably via an active transport system that is similar to the Na^+-P_i cotransporter [6]. Recently, we found that both intraperitoneal and oral administration of PFA to rats resulted in marked phosphaturia [7]. In preliminary studies, we showed that dietary phosphate restriction resulted in an increased absorption of PFA. Since PFA and P_i presumably compete for the same binding sites on the Na^+-P_i cotransporters, we wondered if phosphate restriction could result in an increased uptake of PFA at the level of the intestinal BBM. To answer this question, we have explored the uptake of [^{14}C]PFA by intestinal BBMV from rats placed on NPD or LPD. The results demonstrated that both the intestinal PFA absorption and the initial Na^+ gradient-dependent BBMV uptake of PFA are enhanced in rats fed a low phosphorus diet.

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§ Abbreviations: P_i, inorganic phosphate; PFA, phosphonoformic acid (foscarnet); BBM, brush border membrane; BBMV, brush border membrane vesicles; LPD, low phosphorus diet; NPD, normal phosphorus diet; FE_{Pi}, fractional excretion of phosphate; V_{max}, maximal uptake velocity; and MES, 2-(*N*-morpholino)ethanesulfonic acid.

MATERIALS AND METHODS

For determination of intestinal absorption of PFA, Sprague–Dawley rats weighing 250–300 g were placed on low phosphorus diets (LPD: 0.04% P_i) or normal phosphorus diets (NPD: 0.95% P_i) for 5 days. They were transferred to metabolic cages 3 days before the beginning of the study. PFA was administered as a bolus by gastric gavage in a volume of 1 mL, along with tracer amounts of [^{14}C]PFA, followed by rinsing with 0.5 mL of saline. A dose of 100 mg/kg was chosen based on previous studies of pharmacokinetics of PFA in patients [8] and in other animal species [9], where an i.v. dose of 30 mg/kg was used, as well as our own preliminary studies and information from Astra Laboratories, suggesting that approximately 30–40% of oral PFA is absorbed. Urine was collected under mineral oil for 48 hr, and radioactivity was counted in 300-μL aliquots. [^{14}C]PFA absorption was estimated by the fraction of the administered dose recovered in urine over 48 hr. Preliminary studies showed that >98% of PFA absorbed is excreted within this time period. Because PFA is not metabolized [8, 10], the amount excreted in the urine is roughly equal to the amount absorbed. Approximately 10% of PFA is retained by bone [8]. Therefore, the results underestimate the absorption of PFA. In some experiments, rats were placed on LPD or NPD for 5–7 days; then

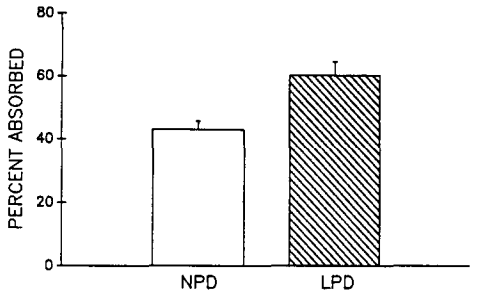

Fig. 1. Effect of dietary phosphorus on intestinal PFA absorption. Rats were placed on low (LPD) or normal (NPD) phosphorus diets for 5 days. PFA (100 mg/kg) was administered as an oral bolus and urine was collected for 48 hr. Results are means ± SEM of 5 experiments.

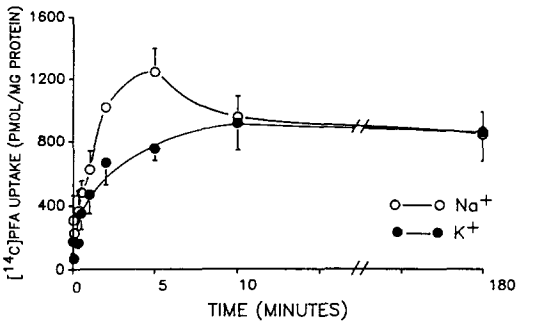

Fig. 2. Time course of [^{14}C]phosphonoformic acid uptake by intestinal BBMV. [^{14}C]PFA uptake was determined under 100 mM Na^+ gradient (in > out) or K^+ gradient as well as under H^+ gradient ($pH_{in} = 7.5$, $pH_{out} = 6.0$) conditions at different times indicated on the abscissa. The results are means ± SEM of 3–5 experiments.

PFA was administered in drinking water for 5 days while diets were continued. Urine was collected for 24 hr for measurement of P_i excretion. Blood samples were obtained at the completion of the collection period. FE_{Pi} was calculated by the following formula: $FE_{Pi} = (U_{Pi} \cdot P_{Cr}/U_{Cr} \cdot P_{Pi}) \cdot 100$, where U_{Pi} = urinary P_i, P_{Cr} = plasma creatinine; U_{Cr} = urinary creatinine, and P_{Pi} = plasma P_i concentrations.

Intestinal BBMV was prepared by a double Mg^{2+}-precipitation method as previously described [2, 11]. Jejunum, defined as the first half of the small intestine distal to the ligament of Treitz, was used for BBMV preparations. The final BBMV pellet was suspended in a buffer containing 300 mM mannitol, 5 mM Tris/HEPES, pH 7.5. The purity of the BBMV preparations was assessed by enrichment of BBM enzyme markers (alkaline phosphatase, gamma glutamyl transpeptidase, leucine aminopeptidase) and reduction of the activity of basolateral (Na^+-K^+ ATPase) and mitochondrial (succinate dehydrogenase) enzymes. Enzyme enrichments were similar to those reported in our previous publications [2, 5, 7].

Transport measurements were carried out at 30°, using a rapid filtration method as previously described [2, 6]. [^{14}C]PFA uptake was measured, under Na^+ gradient ($Na^+_{out} > Na^+_{in}$) and pH gradient ($pH_{out} = 6.0$, $pH_{in} = 7.5$) conditions. Transport medium consisted of 100 mM mannitol, 100 mM NaCl, 5 mM Tris/MES (pH 6.0), and 1.0 mM PFA. Uptake measurements were performed in quadruplicate and results expressed per milligram of membrane protein, determined by the method of Lowry *et al.* [12].

The results are means ± SEM of at least three separate experiments. Statistical analysis was performed using Student's *t*-test for group comparisons. A P value >0.05 was considered non-significant. [^{14}C]Phosphonoformic acid (sp. act. 30 mCi/mmol) was purchased from New England Nuclear (Boston, MA).

RESULTS

Effect of dietary phosphorus on PFA absorption. Rats were placed on either a low phosphorus diet (LPD) or a normal phosphorus diet (NPD) for 5 days. Following administration of PFA by gavage, urine was collected for 48 hr. Measurement of the PFA retained showed that 43 ± 3% of an oral dose of PFA was absorbed in NPD rats, compared with 60 ± 4% in LPD rats ($P < 0.05$, $N = 5$) (Fig. 1). The increased PFA absorption was accompanied by higher plasma PFA concentrations, determined 4 hr after the administration of a PFA bolus (17.9 ± 4.3 and 44.2 ± 2.0 μg/mL for NPD and LPD, respectively).

Time course of [^{14}C]PFA uptake by intestinal BBMV. Since it was shown previously that PFA uptake by intestinal BBMV is highest when measured under Na^+ gradient ($Na^+_{out} > Na^+_{in}$) as well as pH gradient ($pH_{in} > pH_{out}$) [6], [^{14}C]PFA uptake was measured under these conditions (see Materials and Methods). PFA uptake increased rapidly with time of incubation, reaching a maximum at about 2–5 min, and then decreased gradually toward an equilibrium (Fig. 2). Na^+-independent uptake (Na^+ replaced by equimolar K^+) was much lower, and did not show an overshoot. At 1 min of incubation, Na^+-dependent PFA uptake was 704 ± 106 pmol/mg protein and Na^+-independent uptake was 488 ± 145 pmol/mg protein, N = 5.

Effect of dietary phosphorus on [^{14}C]PFA uptake by intestinal BBMV. To determine the mechanism of increased PFA absorption in rats placed on LPD, intestinal BBMV were prepared from animals stabilized on LPD and NPD, and [^{14}C]PFA uptake was measured as above. There was a marked increase in the initial Na^+-dependent PFA uptake by intestinal BBMV prepared from rats placed on LPD, compared with uptake by BBMV from rats on NPD (Fig. 3). There was no significant difference in Na^+-independent uptakes between the two groups (281 ± 22 vs 289 ± 18 pmol/mg protein/min). Equilibrium uptake of PFA, measured at 180 min, was slightly higher in LPD but the difference was not significant (1071 ± 126 vs 880 ± 75 pmol/mg protein for LPD and NPD, respectively).

Effect of oral PFA on P_i absorption and renal P_i

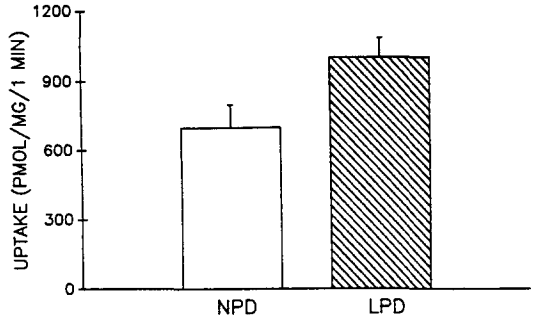

Fig. 3. Effect of dietary phosphorus on [^{14}C]PFA uptake by intestinal BBMV. Rats were placed on low (LPD) or normal (NPD) phosphorus diets for 5 days, followed by preparation of intestinal BBMV. [^{14}C]PFA uptake was determined under Na^+ and H^+ gradient conditions. Results are means ± SEM of 4 experiments.

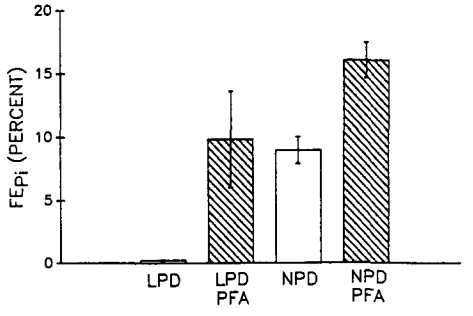

Fig. 4. Effect of oral PFA on fractional excretion of P_i. Rats were placed on oral PFA for 5 days and urine was collected for 24 hr. Blood was drawn at the end of urine collection. FE_{Pi}: fractional excretion of P_i. LPD: low phosphorus diet; NPD: normal phosphorus diet. Values are means ± SEM of results obtained in 8 rats.

excretion. PFA can competitively inhibit P_i transport when added *in vitro* to intestinal BBMV [2, 5]. To determine the interaction of oral PFA with intestinal P_i absorption, rats were placed on oral PFA for 5 days, fasted for 14 hr, and then given a bolus of phosphate (10 mg in 1 mL, containing trace amounts of $^{32}P_i$). The amount of P_i absorbed by the GI tract was not different between control and PFA-treated animals (15.5 ± 3.1 vs 18.2 ± 3.7%, respectively, N = 6). Despite no effect on the overall intestinal P_i absorption, PFA produced a significant increase in urinary P_i excretion in experiments where it was administered chronically for 5 days (Fig. 4). Furthermore, the phosphaturic effect of PFA (expressed as relative increase in FE_{Pi}) was much more pronounced in LPD than in NPD rats (+4004 vs +79%, respectively).

DISCUSSION

Phosphonocarboxylic acids, which were originally developed as antiviral agents [8], were found to be specific and competitive inhibitors of Na^+ gradient-dependent P_i transport in renal [3] and intestinal BBMV [2] as well as in cultured renal epithelial cells [13, 14]. The relative lack of toxicity of PFA [8] led us to explore the possibility of using this drug as a therapeutic agent to reduce phosphate retention.

PFA can be absorbed by the BBM of the enterocyte, presumably via an active transport system, which is similar to the Na^+-P_i cotransporter [6]. In the present study, we have confirmed the presence of a Na^+ gradient-dependent uptake mechanism for PFA in intestinal BBMV of rats.

The bioavailability of oral PFA varies greatly within the animal species studied [8]. However, no studies are published regarding PFA absorption in rats. About 50% of an orally administered PFA dose was absorbed by the gastrointestinal tract in rats. The percentage of PFA absorbed in rats was higher than that in mice but lower than that reported in rabbits [8, 9]. Furthermore, we showed that the absorption of PFA is increased in animals placed on a low-phosphorus diet. The increased absorption was associated with increased PFA uptake by intestinal microvilli as evidenced by uptake measurements in isolated BBMV. We speculate that the low luminal P_i concentration following LPD may favor the membrane transport and transcellular uptake of PFA, since little P_i would be available to compete with PFA binding and its subsequent uptake at the BBM of the enterocyte.

Increased PFA absorption in LPD rats was secondary to enhanced carrier-mediated PFA uptake at the intestinal BBM. The Na^+-independent, passive diffusional uptake was not affected. Since PFA is not protein-bound and is completely ultrafiltrable, oral doses should reach the proximal tubule lumen, resulting in inhibition of Na^+-P_i cotransport [10]. Increased PFA absorption in LPD animals resulted in higher plasma concentrations of PFA. This, in turn, may have led to a higher filtered load and higher concentrations of PFA in the lumen of the proximal tubule of rats on LPD. This sequence provides an explanation for the more pronounced *relative* increase in FE_{Pi}, following PFA administration in LPD rats compared with NPD animals.

Recently, we showed that chronic administration of PFA causes a reduction of the apparent V_{max} of Na^+-P_i cotransport in intestinal and renal BBMV prepared from rats previously stabilized on LPD [7]. No changes in P_i uptake were seen in BBMV from animals on NPD. The lower V_{max} for P_i uptake was the result of a lower density of Na^+-P_i cotransporters on the BBM, suggesting that chronic PFA administration results in down-regulation of both intestinal and renal Na^+-P_i cotransporters when combined with a low phosphorus diet. From these *in vitro* data we speculate the following sequence in rats on LPD, receiving oral PFA: The decreased Na^+-P_i cotransporter density in proximal tubule BBM may reduce the ability of the tubules to reabsorb P_i. This, combined with the presence of increased concentrations of PFA in the proximal tubular lumen, may further reduce tubular P_i reabsorption, leading to phosphaturia, thus overcoming the avid reabsorption of P_i.

The increased PFA absorption with dietary phosphorus restriction, along with the ability of chronic PFA administration to down-regulate the Na^+-P_i cotransporter density in animals on LPD, provides an interesting combination that makes this drug of particular interest as a potential therapeutic agent in states of phosphate retention.

Acknowledgements—This work was supported by a grant from the Bonneville Dialysis Center, Ogden, UT, and by DVA Medical Research Funds. We wish to thank Dr. Alf Larsson, Astra Research Centre, Sodertalje, Sweden, for a gift of foscarnet. The technical assistance of Neal A. Custer is appreciated.

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