Significant Species Differences in Intestinal Phosphate Absorption between Dogs, Rats, and Monkeys

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Summary A treatment for hyperphosphatemia would be expected to reduce mortality rates for CKD and dialysis patients. Although rodent studies have suggested sodium-dependent phosphate transporter type IIb (NaPi-IIb) as a potential target for hyperphosphatemia, NaPi-IIb selective inhibitors failed to achieve efficacy in human clinical trials. In this study, we analyzed phosphate metabolism in rats, dogs, and monkeys to confirm the species differences. Factors related to phosphate metabolism were measured and intestinal phosphate absorption rate was calculated from fecal excretion in each species. Phosphate uptake by intestinal brush border membrane vesicles (BBMV) and the mRNA expression of NaPi-IIb, PiT-1, and PiT-2 were analyzed. In addition, alkaline phosphatase (ALP) activity was evaluated. The intestinal phosphate absorption rate, including phosphate uptake by BBMV and NaPi-IIb expression, was the highest in dogs. Notably, urinary phosphate excretion was the lowest in monkeys, and their intestinal phosphate absorption rate was far lower. Dogs and rats showed positive correlations between Vmax/Km of phosphate uptake in BBMV and NaPi-IIb expression. Although phosphate uptake was observed in the BBMV of monkeys, NaPi-IIb expression was not detected and ALP activity was low. This study revealed significant species differences in intestinal phosphate absorption. NaPi-IIb contributes to intestinal phosphate uptake in rats and dogs. However, in monkeys, phosphate is poorly absorbed due to the slight degradation of organic phosphate in the intestine.

Key Words NaPi-IIb, PiT-1, PiT-2, BBMV, uptake

Blood phosphate levels are regulated by absorption in the intestines, release, and utilization by bones, and excretion from kidneys. Among these, the kidney plays an important role in regulating blood phosphate concentration, which it maintains by adjusting the urinary excretion of phosphate (1). Phosphate excretion into urine is performed via reabsorption by sodium-dependent phosphate transporter type IIa (NaPi-IIa) and sodium-dependent phosphate transporter type IIc (NaPi-IIc) in kidneys, and FGF23 negatively regulates the translocation of NaPi-IIa and NaPi-IIc into the renal tubular epithelium (2). However, in patients with chronic kidney disease (CKD) or/and dialysis, urinary phosphate excretion decreases remarkably as kidneys fail to remove dietary phosphate from the body, leading to hyperphosphatemia (3). Hyperphosphatemia causes secondary hyperparathyroidism and vascular calcification, which are known risk factors for death or a decline in the quality of life (4, 5). Phosphate binders are used in patients with hyperphosphatemia to suppress phosphate absorption in the gastrointestinal tract (6–8). In patients with CKD or/and dialysis, blood phosphate levels are strongly influenced by uptake in the small intestine (9, 10).

Dietary phosphate is composed of organic phosphates and phosphoric acid, an inorganic phosphate. Organic phosphate is metabolized into phosphoric acid by alkaline phosphatase (ALP) in the digestive tract (11). In the gastrointestinal tract this phosphate is absorbed. There are two pathways of phosphate absorption in the small intestine, active transport via intestinal epithelial cells and passive transport via the tight junction between cells. Little is known about the contribution between passive flow from tight junction and active transport, but active transport has been thought to play an important role in maintaining phosphate absorption in the small intestine (12–14). In humans and rats, the active transport of phosphate mainly happens in the upper part of the small intestine where NaPi-IIb, a sodium-dependent phosphate transporter, is thought to have an important function (15–18). However, it was reported that the efficacy of a NaPi-IIb specific inhibitor was different between rats and humans (19). And NaPi-IIb is also active in mice, but mainly in the lower part of the small intestine (15, 16, 20). These reports indicate that there are species differences in the intestinal phosphate
absorption system between rats, mice, and humans.

NaPi-IIb which has a strong affinity for phosphate is highly expressed in the small intestine, and it is well known that NaPi-IIb is regulated by 1.25(OH)2D3 in rodents e.g., 1.25(OH)2D3 administration or 1.25(OH)2D3 receptor deletion causes the increase or decrease of NaPi-IIb protein expression, respectively (21, 22). However, the expression of PIT-1 and PIT-2, together known as NaPi-III, in addition to NaPi-IIb have also been confirmed in the small intestine. As PIT-1 and PIT-2 are thought to be low affinity transporters for phosphate, on the other hands NaPi-IIb is high affinity, the characteristic difference of these transporters might be physiological meaning (12, 23).

Rats, dogs, and monkeys are widely used for the prediction of drug efficacy and toxicity in humans. In choosing an animal model to predict the effect of an inhibitor for phosphate absorption in the human small intestine, it is important to confirm the species difference. In this study, urinary and fecal phosphate excretion in vivo, phosphate uptake in the small intestine by brush border membrane vesicles (BBMV), and the expression levels of three intestinal sodium-dependent phosphate transporters were examined in rats, dogs and monkeys to confirm the species difference in phosphate absorption.

**MATERIALS AND METHODS**

**Animals.** Animal procedures and protocols were in accordance with the Guidelines for the Care and Use of Laboratory Animals at Chugai Pharmaceutical Co. Ltd. and approved by the Institutional Animal Care and Use Committee (approval number: 17-073, 17-160, 17-264). Nine weeks old rats (Wistar), 2 y old dogs (Beagle) and 5 y old monkeys (Cynomolgus monkey) were included in the study. Rats were fed a 10 g pellet diet (CE-2, CLEA Japan, Inc., Japan), dogs were fed a 200 g pellet diet (CD-5M, CLEA Japan, Inc.) and monkeys were fed a 100 g pellet diet (Certified Primate Diet 5048, LabDiet, MO, USA) per day. Phosphate, calcium, and vitamins in these diets were derived from soybeans and whitefish. The contents are as follows; CE-2: phosphate 1.1 g, calcium 1.1 g, vitamin D3 275 IU/100 g, CD-5M: phosphate 1.2 g, calcium 1.5 g, vitamin D3 265 IU/100 g. Certified Primate Diet 5048: phosphate 0.6 g, calcium 1.0 g, vitamin D3 670 IU/100 g. The animals were euthanized by exsanguination under isoflurane or ketamine anesthesia.

**Biochemical analysis.** For each animal species, urine was collected for 24 h and feces was stored for 48 h. Feces samples were suspended in 0.5 mol/L hydrochloric acid (HCl) and centrifuged to collect the supernatant (15 min, 1,630 ×g, 4°C). Urine samples were diluted with an equal amount of 1 mol/L HCl and centrifuged to collect the supernatant (15 min, 1,630 ×g, 4°C). Blood samples were centrifuged to collect serum (15 min, 1,630 ×g, 4°C). Concentrations of phosphate (IATRO LQ IP II, LSI Medical Corporation, Japan), calcium (IATROFINE Ca II, LSI Medical Co.), creatinine (L type Wako creatinine, FUJIFILM Wako Pure Chemical Corporation, Japan) and urea nitrogen (L type Wako UN, FUJIFILM Wako Pure Chemical Corporation) were measured by an automatic analyzer (TOSHIBA Co., Japan). Serum were stored and measured for 1.25(OH)2D3 (FR, FUJIREBIO Inc., Japan) and FGF23 (FGF-23 ELISA Kit, KAINOS Laboratories, Inc., Japan).

Preparation of BBMV and analysis of intestinal phosphate absorption, determination of protein concentration and the activity of alkaline phosphatase. The duodenum was sampled from stomach pylorus to Treitz’s ligament, jejunum was 10 or 15 cm descending from Treitz’s ligament, and ileum was 10 or 15 cm ascending from the cecum. These samples were washed with saline, and mucosal tissue was scraped with a slide glass to acquire material for mRNA measurement. BBMV purification, kinetic analysis and ALP analysis. BBMV purification was performed using small intestinal mucosa collected from each individual. BBMV was prepared by partially modifying the previous method (24). The purification of BBMV was confirmed by enrichment of ALP activity, which were more than 7 fold compared with the homogenized tissue. The isolated BBMV were suspended in an experimental buffer to give a final protein concentration of about 1 mg/mL. The final experimental buffer consisted of either 60 mmol/L mannitol, 110 mmol/L NaCl, 10 mmol/L HEPES-Tris, pH 7.5 (Na buffer). Uptake in the absence of Na+ was determined by substituting NaCl with KCl (K buffer). Protein concentration of homogenized suspension was measured using the Bradford method. The ALP activity of the homogenized suspension was measured (L type Wako ALP J2, FUJIFILM Wako Pure Chemical Corporation) with an automatic analyzer. KH2PO4 was added to the Na buffer for a final concentration of 1. 3. 10, 25, 50, 100 mmol/L. H313PO4 (PerkinElmer Co., Ltd., Waltham, MA, USA) was further added to prepare a Na buffer or K buffer. BBMV suspension was added to above solution and incubated at room temperature for 1 min. Immediately after the incubation, an ice-cold stop solution consisting of either 1.0 mmol/L KH2PO4, 150 mmol/L NaCl, and 10 mmol/L HEPES-Tris (pH 7.5) was added to terminate the reaction, and the whole amount was added to a 0.45 μm membrane filter (Merck Millipore, Burlington, MA, USA). Radioactivity was measured using a liquid scintillation counter (PerkinElmer Co., Ltd.) and sodium dependent uptake was calculated by deducting the uptake with K buffer.

**Gene expression.** Using the small intestinal mucosa of each collected individual, mRNA was purified using RNeasy kit (RNeasy kit, QIAGEN, Venio, Netherlands). cDNA was prepared using SuperScript IV (Life technology Japan, Ltd., Japan). Expression of mRNA was measured by real-time PCR (TaqMan Universal PCR Mix, Life technology Japan, Ltd., Advanced Universal Probes Supermix, Bio-Rad Laboratories, Inc., Hercules, CA, USA). The cDNA fragments of the target sequences were generated by RT-PCR with specific primers from the total intestinal RNA of each species. Each cDNA fragment was ligated into the pcDNA3.1 (Life technology Japan, Ltd.). Reference sequence and gene localization of each cDNA fragment are shown in Table 1. The con-
centrations of the purified plasmid DNA were measured by spectrophotometry, and corresponding copy numbers were calculated. Serial dilutions of the respective plasmid DNA were used as standards to make calibration curves. At that time, the plasmids containing the mRNA sequence corresponding to the real-time PCR probe (Life technology Japan, Ltd., Bio-Rad Laboratories, Inc.) of NaPi-IIb, PiT-1, PiT-2, and Villin-1 were used as a standard, and the absolute amount of mRNA of each gene was calculated from a calibration curve.

**Statistical analysis.** Statistical analysis was performed using Microsoft Office Excel (Microsoft Corporation, Redmond, WA, USA). The Michaelis-Menten curve was generated based on substrate concentration ($s$) ($s=1, 3, 10, 25, 50, 100 \mu\text{mol/L}$) and calculated velocity ($v$), and the apparent Michaelis constant ($K_m$) and maximum velocity ($V_{\text{max}}$) of phosphate uptake were calculated nonlinearly with JMP 11.2.1 (SAS Institute Japan Ltd., Japan). For biochemical and gene expression, statistical analysis was performed using one-way

| Table 1. Gene localization in the plasmid standards used for real-time PCR. |
|--------------------------|-----------------|--------------------------|
| cDNA target | Reference sequence | Gene localization |
| Rat | NaPi-IIb | NM_053380 | 601–730 |
| | PiT-1 | NM_031148 | 511–740 |
| | PiT-2 | NM_017223 | 601–810 |
| | Villin-1 | NM_001108224 | 391–540 |
| Dog | NaPi-IIb | XM_022417261 | 387–478 |
| | PiT-1 | XM_540181 | 862–957 |
| | PiT-2 | XM_025993676 | 485–550 |
| | Villin-1 | XM_026002027 | 80–106 |
| Monkey and human | NaPi-IIb | NM_006424 | 1006–1175 |
| | PiT-1 | NM_005415 | 2279–2408 |
| | PiT-2 | NM_001257180 | 982–1171 |
| | Villin-1 | NM_007127 | 2331–2540 |

| Table 2. Serum phosphate, calcium, urea nitrogen, creatinine, FGF23, and 1,25(OH)$_2$D$_3$ levels in each species. |
|---------------------|---------------------|---------------------|---------------------|
| Rat | Dog | Monkey | Human |
| Phosphate (mg/dL) | 8.0±0.1 | 3.3±0.2$^a$ | 4.7±0.5$^{a,b}$ | 3.4±0.5 |
| Calcium (mg/dL) | 10.2±0.1 | 10.1±0.1 | 10.0±0.2 | 9.3±0.4 |
| BUN (mg/dL) | 10.1±1.0 | 10.8±0.6 | 24.0±2.0$^{a,b}$ | |
| Creatinine (mg/dL) | 0.23±0.01 | 0.52±0.01$^a$ | 0.64±0.06$^a$ | |
| FGF23 (pg/mL) | 167.7±9.6 | 553.8±17.3$^a$ | 67.4±14.7$^{a,b}$ | 69.7 (56.8–92.7) |
| 1,25(OH)$_2$D$_3$ (pg/L) | 94.2±18.1 | 28.0±4.7 | 218.8±23.2 | 39.9 (30.2–49.6) |

| Table 3. Urinary phosphate, calcium, urea nitrogen excretion, and the fractional excretion of phosphate in each species. |
|---------------------|---------------------|---------------------|---------------------|
| Rat | Dog | Monkey | Human |
| Phosphate (mg/mg cre) | 3.0±0.1 | 1.8±0.1$^a$ | 0.01±0.00$^{a,b}$ | |
| Calcium (mg/mg cre) | 0.04±0.00 | 0.08±0.01$^a$ | 0.65±0.09$^{a,b}$ | |
| BUN (mg/mg cre) | 11.5±1.3 | 13.3±0.3 | 9.9±0.9$^b$ | |
| FEPi$^1$ (%) | 8.6±0.5 | 29.1±1.3$^a$ | 0.1±0.1$^{a,b}$ | 14.5 (11.9–20.1) |

Values are expressed as means±SE, $n=4$.

$^a$ 0.05, significant different from rat, $^b$ 0.05, significant different from dog by one-way ANOVA and followed by Tukey–Kramer multiple comparison tests.

Human data is referenced from a previous study (29).
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Table 4. Fecal phosphate and calcium excretion and phosphate absorption rate in each species.

|                          | Rat          | Dog          | Monkey       | Human       |
|--------------------------|--------------|--------------|--------------|-------------|
| Phosphate excretion 1 (mg/kg/d) | 170±4        | 128±11<sup>a</sup> | 120±14<sup>a</sup> |             |
| Calcium excretion 1 (mg/kg/d)   | 303±29       | 335±33       | 58±9<sup>ab</sup>   |             |
| Phosphate intake 2 (mg/kg/d)   | 322±6        | 271±3<sup>a</sup> | 145±20<sup>ab</sup> |             |
| Phosphate absorption rate 2 (%) | 47.1±0.9     | 52.7±4.3     | 13.2±14.0<sup>ab</sup> | 60–70       |

Values are expressed as means±SE, n=4.

1 Phosphate excretion, calcium excretion and phosphate intake were corrected by body weight.
2 Phosphate absorption rate was calculated from phosphate in food and feces, as follows (phosphate intake−phosphate excretion)/phosphate intake×100 (%).

<sup>a</sup>p<0.05, significant different from rat, <sup>b</sup>p<0.05, significant different from dog by one-way ANOVA and followed by Tukey–Kramer multiple comparison tests.

Human data is referenced from a previous study (30).

Fig. 1. Michaelis-Menten curves of phosphate uptake in rats intestinal BBMV. Values at each concentration are expressed as means±SE, n=4. Phosphate uptake (v) was measured in the duodenum (A), jejunum (B), and ileum (C). BBMV treated with phosphate concentration (s) ranging from 1 to 100 μM for 1 min. Michaelis-Menten curves were analysed by JMP 11.2.1 using single kinetics model.

Fig. 2. Michaelis-Menten curves of phosphate uptake in dogs intestinal BBMV. Values at each concentration are expressed as means±SE, n=4. Phosphate uptake (v) was measured in the duodenum (A), jejunum (B), and ileum (C). BBMV treated with phosphate concentration (s) ranging from 1 to 100 μM for 1 min. Michaelis-Menten curves were analysed by JMP 11.2.1 using single kinetics model.
ANOVA. Tukey–Kramer multiple comparison tests were used to compare data, with \( p<0.05 \) being considered statistically significant with JMP 11.2.1.

**RESULTS**

**Biochemical analysis of phosphate metabolisms in blood, urine, and feces**

Serum biochemical data are shown in Table 2. Serum phosphate concentration was the highest in rats and lowest in dogs. There was no species difference in calcium concentration. Serum FGF23 concentration in dogs was markedly high, followed by rats. Serum 1,25(OH)\(_2\)D\(_3\) concentration was highest in monkeys. Urinary biochemical data are shown in Table 3. Compared to rats and dogs, urinary phosphate excretion was remarkably low in monkeys and calcium excretion was markedly high. Fecal biochemical data are shown in Table 4. The intestinal phosphate absorption rate, calculated based on the ratio of dietary to fecal phosphate was remarkably low in monkeys and the highest in dogs. Fecal calcium excretion was also lowest in monkeys. It was comparable in rats and dogs.

**Kinetics analysis of phosphate uptake in intestinal BBMV**

Figures 1–3 show the Na\(^+\)-dependent phosphate uptake concentration dependence curve in each vesicle prepared from duodenum, jejunum, and ileum of three species. Kinetic parameters are shown in Table 5. The apparent \( K_m \) for phosphate uptake in duodenum, jejunum, and ileum was 39.9, 78.9, 367.4 \( \mu \)mol/L in rats, 46.9, 14.4, 8.9 \( \mu \)mol/L in dogs and 102.3, 44.9 \( \mu \)mol/L in monkeys respectively. Dogs showed high \( V_{max} \) at any site in small intestine. The relative contribution rate (\( V_{max}/K_m \)) at each site was the highest in the ileum in dogs and monkeys, and in the duodenum in the rats.

**mRNA expression of NaPi-IIb, PiT-1 and PiT-2 in intestine**

NaPi-IIb mRNA was expressed most highly in the ileum in dogs, but in the duodenum in rats (Fig. 4). In rats, PiT-1 mRNA was highly expressed in ileum, but PiT-2 mRNA was poorly expressed in all segments. In dogs, the mRNA expression of PiT-1 and PiT-2 was similar in all tissues. NaPi-IIb mRNA expression in monkeys was barely detectable in every part of the intestine, but conversely mRNA expression of PiT-1 and PiT-2 was confirmed (Fig. 4). In humans, expression of NaPi-IIb, PiT-1, and PiT-2 were evaluated using the Human Digestive System MTC™ Panel (Clontech Laboratories, Inc., Mountain View, CA, USA). NaPi-IIb was higher in the duodenum than in the rest of the human small intestine, but expression levels of PiT-1 and PiT-2 were similar throughout (Supplemental Online Material. Fig. S1).

**Intestinal ALP activity**

ALP activity in monkeys was lower than in dogs and rats (Fig. 5). In rats and dogs, organic phosphate is decomposed in the upper part of intestine, and thus ALP activity is high in the duodenum and jejunum.

**DISCUSSION**

In this study, we confirmed significant differences between rats, dogs, and monkeys in the absorption of phosphate. NaPi-IIb would contribute largely to phosphate uptake by BBMV in the small intestine of dogs and rats, whereas PiT-1 or PiT-2 would play that role in monkeys. Although monkey intestine is at least capable of the absorption of phosphate, the in vivo absorption and ALP activity was surprisingly low compared with dogs and rats, suggesting that the use of phosphate is much lower in monkeys.

BBMV analysis confirmed sodium-dependent phosphate uptake capacity in dogs, monkeys, and rats. In rats, NaPi-IIb was highly expressed in the duodenum where the \( V_{max}/K_m \) ratio in BBMV was also the highest. These results suggest that the uptake of phosphate by NaPi-IIb in rats is most common in the duodenum.
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Whereas in dogs, the $V_{\text{max}}/K_m$ ratio and high expression of NaPi-IIb mRNA suggests more uptake in the ileum. These results suggest that phosphate uptake mainly occurs in ileum and that NaPi-IIb primarily contributes to this in dogs. In monkeys, the ileum was considered to be most involved in phosphate uptake based on the value of $V_{\text{max}}/K_m$. However, unlike in dogs and rats, not NaPi-IIb but PiT-1 and PiT-2 are mainly expressed in intestine in monkeys. This suggests that in monkeys, PiT-1 or PiT-2 contributes to phosphate uptake in the ileum.

It is known that NaPi-IIb in the small intestine is controlled by 1,25(OH)$_2$D$_3$, and it has been reported that the expression of NaPi-IIb is increased by administration of 1,25(OH)$_2$D$_3$ or ED-71 (21, 25, 26). However, in monkeys, despite having higher serum 1,25(OH)$_2$D$_3$ than rats or dogs. Although, PiT-1 and PiT-2 were highly expressed in each intestinal segment of monkeys, there is no correlation between BBMV phosphate uptake and the absolute mRNA amount of PiT-1 and PiT-2 in duodenum, jejunum and ileum. Another sodium-dependent phosphate transporter identified by Candeal et al. (27) may also be involved in monkeys. The verification of protein expression level and membrane localization of PiT-1 or PiT-2 will be required to confirm this in future studies.

In monkeys, the phosphate absorption rate calculated from diet and fecal excretion was significantly lower than in dogs and rats. ALP activity in the small intestine was also lower. When ALP activity is low, organic phosphate cannot be decomposed into phosphoric acid in sufficient amounts. This suggests that while monkeys are capable of phosphate uptake in the small intestine, only small amounts of phosphate can be absorbed in the physiological condition. It remains unknown if systemic phosphate is supplied from intestine in an inorganic or organic form.

In addition to species differences in intestinal phosphate absorption, differences in the fractional urinary excretion of phosphate were also observed. Urinary phosphate excretion is mediated through reabsorption by renal NaPi-IIa and NaPi-IIc, and FGF23 negatively regulates the internalization and degradation of NaPi-IIa and NaPi-IIc in the renal tubular epithelium (2). In dogs, urinary excretion of phosphate would be accelerated to regulate the large amount of phosphate absorbed in the small intestine as a result of high FGF23 concentration in the blood. In monkeys, the absorption of phosphate in the small intestine was low, and it was confirmed that serum FGF23 was also low and that reabsorption in kidneys was enhanced.

In humans, phosphate is reported to be mainly absorbed in upper intestine (28). High phosphate uptake in the duodenal BBMV of rats suggests that, compared with monkeys and dogs, rats should be the first choice for predicting phosphate metabolism in humans. However, we confirmed differences between humans and rats in the mRNA expression of PiT-1 and PiT-2 in the small intestine. The contribution of PiT-1 and PiT-2 to phosphate absorption in the small intestine remains

Table 5. Michaelis-Menten kinetics parameters of intestinal phosphate uptake in BBMV in rats, dogs, and monkeys.

|       | Duodenum | Jejunum | Ileum |
|-------|----------|---------|-------|
| A Rats |          |         |       |
| $K_m$ (μM) | 39.9     | 78.9    | 367.4 |
| $V_{\text{max}}$ (pmol/min/μg protein) | 0.57     | 0.27    | 0.56  |
| $V_{\text{max}}/K_m$ (pmol/min/μg protein/μM) | 0.014    | 0.003   | 0.002 |
| B Dogs |          |         |       |
| $K_m$ (μM) | 46.9     | 14.4    | 8.9   |
| $V_{\text{max}}$ (pmol/min/μg protein) | 2.2      | 3.8     | 6.0   |
| $V_{\text{max}}/K_m$ (pmol/min/μg protein/μM) | 0.047    | 0.26    | 0.67  |
| C Monkeys |         |         |       |
| $K_m$ (μM) | 31.6     | 102.3   | 44.9  |
| $V_{\text{max}}$ (pmol/min/μg protein) | 0.49     | 1.4     | 5.9   |
| $V_{\text{max}}/K_m$ (pmol/min/μg protein/μM) | 0.015    | 0.013   | 0.13  |

Data was analyzed by JMP 11.2.1 using velocity and phosphate concentration in each species. $V_{\text{max}}$ represents the maximum absorption velocity of phosphate. $K_m$ is the Michaelis-Menten constant and they were obtained by fitting the experimental data into nonlinear least squares regression.
unknown. NaPi-IIb specific inhibitor ASP3325 ameliorates the hyperphosphatemia in rats but is not effective in ESRD patients (19). It was suggested that not only NaPi-IIb but also PiT-1 and PiT-2 might relate with intestinal phosphate absorption. More research is necessary to confirm the validity of the rat model in predicting human responses.

In conclusion, dogs and monkeys absorb phosphate mainly in the lower intestine and rats in the upper part of the small intestine. NaPi-IIb mainly promotes uptake in dogs and rats, but other transporters besides NaPi-IIb do so in monkeys. Of the three species, dogs appear to absorb phosphate the most efficiently, and blood phosphate is maintained by high excretion in urine. Although monkeys possess active phosphate uptake mechanisms in the ileum, they could not utilize phosphate as much as the other species due to low ALP activity in the intestine. This study suggests that there are significant species differences between rats, dogs, and monkeys in the absorption of phosphate in the small intestine.

Disclosure of state of COI
YI, NH, SO, and NH are employees of Chugai Pharmaceutical Co., Ltd. RT, TK, TN, MH, HA, YM, and HA are employees of Chugai Research Institute for Medical Science, Inc.

Supporting information
Supplemental Online Material is available on J-STAGE.

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