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Original Article

Effects of the novel sodium-dependent phosphate cotransporter 2b inhibitor DZ1462 on hyperphosphatemia in chronic kidney disease

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Abstract: Background: Serum phosphate levels remain insufficiently controlled in chronic kidney disease (CKD) patients, and novel therapeutic strategies are needed. Blocking intestinal phosphate absorption mediated by sodium-dependent phosphate cotransporter type 2b (NPT2b) holds promise as one such strategy. Methods: The in vitro cellular potency of DZ1462 was evaluated using a radioactive Pi uptake assay on stable Chinese hamster ovary (CHO) cell clones transfected with human NPT2b (hNPT2b) or rat NPT2b (rNPT2b). The ability of DZ1462 to inhibit phosphate absorption was studied in vivo in an acute model after oral bolus challenge with $^{33}PO_4$ and in an adenine-induced chronic hyperphosphatemia rat model. PK and minitox was also evaluated. Results: The cellular assays with the hNPT2b-CHO and rNPT2b-CHO clones showed that DZ1462 significantly and potently inhibited phosphate uptake. In vivo, in a chronic Pi-fed rat model, DZ1462 effectively inhibited intestinal Pi uptake. In a hyperphosphatemia rat model, DZ1462 significantly inhibited Pi uptake, and DZ1462 in combination with sevelamer had a synergistic effect. The pharmacokinetics (PK) study confirmed that DZ1462 is a gastrointestinal (GI)-restricted compound that can remain in the intestine for a sufficient duration. In addition, DZ1462 also reduced cardiovascular events and ameliorated osteoporosis in a CKD animal model. Conclusions: This study revealed that a GI-restricted NPT2b inhibitor DZ1462 potently inhibits NPT2b in vitro and blocks intestinal phosphate uptake in multiple animal models with potential to reduce various cardiovascular events in CKD models. Therefore, DZ1462 may be useful to treat renal disease patients who have shown an unsatisfactory response to phosphate binders.

Keywords: Sodium-dependent phosphate cotransporter type 2b (NPT2b), hyperphosphatemia, chronic kidney disease (CKD), DZ1462, rats, phosphate uptake, efficacy

Introduction

In healthy individuals, serum phosphate concentrations are maintained primarily by the major organs, including the intestines, kidneys, and bones [1-3]. Chronic kidney disease (CKD) often presents with elevated serum phosphorus levels and end-stage renal failure because the ability to excrete phosphate through the kidney is compromised, leading to hyperphosphatemia. Hyperphosphatemia is a serious clinical problem in patients with end-stage renal disease (ESRD) and is significantly associated with patient mortality [4, 5]. Current clinical strategies for treating ESRD patients include dietary phosphate restriction, hemodialysis, and the use of phosphate binders to reduce the serum phosphate concentration [6-8]. However, poor patient compliance, a heavy economic burden, and gastrointestinal (GI) side effects lead to these treatments having limited effect [9-11]. Thus, novel therapeutic strategies are needed.

The mechanism of intestinal phosphate absorption in humans has not been fully elucidated [12]. Conceptually, phosphate is absorbed mainly in the upper small intestine by both passive and active mechanisms. More than 95% of patients are prescribed phosphate binders to assist in the management of hyperphosphatemia in patients undergoing dialysis [13]. Among phosphate binders, sevelamer is a nonabsorbable synthetic ion-exchange polymer that was

the first member of its class to be marketed [14, 15]. However, the high price and pill burden as well as GI side effects are the main drawbacks for the use of sevelamer in the management of hyperphosphatemia in CKD patients [16, 17].

Sodium-dependent phosphate cotransporter type 2b (NPT2b or SLC34A2) mediates active phosphate absorption in the small intestine, and in rodents, it may account for up to 50% of overall phosphate absorption [18, 19]. NPT2b deletion attenuates hyperphosphatemia associated with CKD, and the phosphate binder sevelamer carbonate further reduces serum phosphate levels in NPT2b-deficient CKD models [20, 21]. These findings suggest that NPT2b may be a candidate for targeted hyperphosphatemia treatment. Previous studies have shown that nicotinamide inhibits sodium/phosphorous transport (NPT2b) in both renal and intestinal brush borders [22-24], and several clinical trials have shown that nicotinamide could lower serum phosphorus levels in patients with ESRD undergoing hemodialysis [24, 25]. However, the use of nicotinamide in the clinic is limited by its toxicity to platelets and, more importantly, pulmonary alveolar microlithiasis due to systemic inhibition of NPT2b family members [26-28]. Therefore, we generated a GI-restricted NPT2b inhibitor to overcome this issue.

In this study, we screened NPT2b inhibitors from a small molecule library and selected DZ1462. We then described the characterization of DZ1462. By inhibiting NPT2b in the GI tract only, DZ1462 potently inhibited phosphate adsorbed from the diet and rescued the decrease in efficacy after sevelamer treatment in animal models of hyperphosphatemia.

Materials and methods

Cells and chemicals

The Flp-In-CHO cell line (#P758-07) was obtained from Invitrogen and cultured with Ham's F12 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 100 μg/mL Zeocin. DZ1462 was synthesized by Dizal Pharmaceutical Co., Ltd. (Shanghai, China). A low-Pi diet (0.9%) was customized and supplied by Beijing Keao Xieli Feed Co., Ltd. (Beijing, China). Adenine (0.75%) was obtained from Sigma-Aldrich Co. Phosphoric acid-^{33}P (Cat. No. NEZ080001MC) was purchased from PerkinElmer (USA).

Establishment of stable NPT2b-CHO clones

A Flp-In system (#K6010-01, Invitrogen) was utilized to generate stable isogenic clones expressing human NPT2b (hNPT2b) or rat NPT2b (rNPT2b). The hNPT2b or rNPT2b gene was cloned and inserted into the pcDNA/FRT expression vector. Then, the pcDNA5/FRT expression vector containing hNPT2b or rNPT2b and the pOG44 Flp-Recombinase Expression Vector (V600520, Invitrogen) were cotransfected into host Flp-In-CHO cells at a ratio of 1:9 using diluted Lipofectamine™ 2000 (#11668-019, Invitrogen, USA) according to the manufacturer's instructions. The transfected cells were cultured in selective medium (Ham's F-12, 10% FBS, 1% penicillin-streptomycin, and 600 μg/mL hygromycin), and stable clones were selected by the limiting dilution method.

In vitro phosphate (Pi) uptake assay

Validation of the sodium-dependent Pi uptake capability of the NPT2b-CHO clones: Stable hNPT2b-CHO or rNPT2b-CHO cell clones were seeded in an Isoplate-96 TC plate (PerkinElmer, USA) at 3×10^4 cells/well in complete medium (F12 medium supplemented with 600 μg/mL hygromycin and 10% FBS) for 36 hours. Then, the cells were washed twice with assay buffer Na+ (137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 14 mM Tris HCl) [sodium group] or assay buffer Na- (137 mM $C_5H_{14}ONCl$, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgSO_4$, 14 mM Tris HCl) [sodium-free group]. Then, the cells in the sodium and sodium-free groups were challenged with 1 μCi/mL phosphoric acid-^{33}P/0.1 mM KH_2PO_4 (100 μL/well) in assay buffer Na+ and in assay buffer Na-, respectively, for different durations (5, 15, 30, 45, and 60 min). At each timepoint, the sampled cells were washed with precooled washing buffer (137 mM NaCl, 14 mM Tris HCl) 4 times and then lysed with 0.5% Triton X-100 (20 μL/well) for 2 min before being mixed with MicroscintTM-20 liquid scintillation cocktail (100 μL/well, #87-13391, PerkinElmer, USA) for 5 min to detect radioactivity (counts per minute, CPM) using a Microbeta 2450 microplate counter (PerkinElmer, USA).

DZ1462-mediated inhibition of Pi uptake by the NPT2b-CHO clones: hNPT2b-CHO or rNPT2b-CHO clones were seeded and washed as described above and then preincubated with 80 μL/well serial dilutions of compounds (10,000 nM to 1.3 nM) or 0.1% DMSO in assay

buffer Na+ for 15 min. Then, the clones were challenged with 1 μCi/mL phosphoric acid-^{33}P/0.1 mM KH_2PO_4 in assay buffer Na+ for 30 min (20 μL/well or 100 μL/well). Then, the cells were subjected to scintillation counting as described above. In parallel, the background signal of a Min well was measured, which used assay buffer Na- in the washing step and ^{33}P in the challenge step.

The percent inhibition of each well was calculated according to the following formula.

$$\text{Pi uptake inhibition}(\%) = \left(1 - \frac{CPM_{compound} - CPM_{Min}}{CPM_{DMSO} - CPM_{Min}}\right) \times 100\%$$

In vivo study conditions

Male SPF Wistar rats were purchased from Beijing Vital River Co., Ltd. (Beijing, China). All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and conducted in compliance with the standard and local regulatory requirements of Dizal Pharmaceuticals.

Phosphate uptake inhibition in rats

Male SPF Wistar rats aged 5-7 weeks were fed a low-Pi diet for 9 days and fasted overnight before compound dosing. To evaluate the effect of the time interval between compound dosing and Pi feeding on the ability of DZ1462 to inhibit intestinal Pi uptake at different timepoints (0.25, 0.5, 1, and 2 hours) after 30 mg/kg DZ1462 or vehicle treatment (n=3 rats/timepoint/group), the rats were orally challenged with a bolus of radioactive phosphate solution (1 mL of 8.3 mM NaH_2PO_4 with 5 μCi of phosphoric acid-^{33}P). Fifteen minutes later, blood samples were collected, and the radioactivity in the serum was determined by scintillation counting (counts per minute, CPM) on a PerkinElmer 1450 LSC counter. The percentage of Pi uptake inhibition was calculated according to the following formula.

$$\text{Pi uptake inhibition}(\%) = \frac{\text{mean of } CMP_{vehicle} - CMP_{compound}}{\text{mean of } CMP_{vehicle}} \times 100\%$$

DZ1462 efficacy in an adenine-induced chronic hyperphosphatemia rat model

Male SPF Wistar rats aged 5-7 weeks were fed a low-Pi diet supplemented with 0.75% adenine. Adenine was not given to the rats in the normal control group. Four weeks after the initiation of adenine treatment, the rats were randomized to receive oral treatment with vehicle (0.5% HPMC/0.5% Tween 80) or 30 mg/kg DZ1462 3 times a day for 7 days (n=6 rats/group). On the day of randomization (defined as baseline) or day 7, blood samples were collected to detect the serum phosphate concentration with an inorganic phosphorus determination kit (catalog #: 990-40091, Shaoxing Chuangye Biotechnology Co., Ltd., China) following the manufacturer's instructions.

Determination of drug concentrations in the plasma and intestine

Rats were orally treated with a single dose of 10 mg/kg or 30 mg/kg DZ1462 (n=3 rats/group). Blood samples were collected from the tail vein at different time points (0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, and 24 hours) posttreatment (n=3/time point) to collect plasma. Then, the drug concentration in the plasma was detected by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Mucus samples from the duodenum, jejunum and ileum were weighed separately and homogenized with 3 volumes of diH_2O containing 5% formic acid (FA) on wet ice (w:v=1:3). The samples were kept on wet ice and immediately and directly quenched with acetonitrile (ACN) (with 5% FA and IS, v:v=1:4). After centrifugation at 10,000×g for 15 min, the supernatant was transferred to a labeled tube and stored at -80°C until analysis. All samples were analyzed by using LC-MS/MS.

Statistical analysis

The data are presented as the mean ± SEM. Statistical comparisons were performed by two way ANOVA or Ordinary one-way ANOVA with Tukey's multiple comparisons test using GraphPad Prism 8.0.

Results

DZ1462 potently inhibits phosphate uptake mediated by NPT2b

To evaluate the ability of DZ1462 to inhibit hNPT2b, a stable isogenic cell line expressing hNPT2b was generated (hNPT2b-CHO). In addition, to investigate whether rats could be used for subsequent in vivo animal studies, a stable

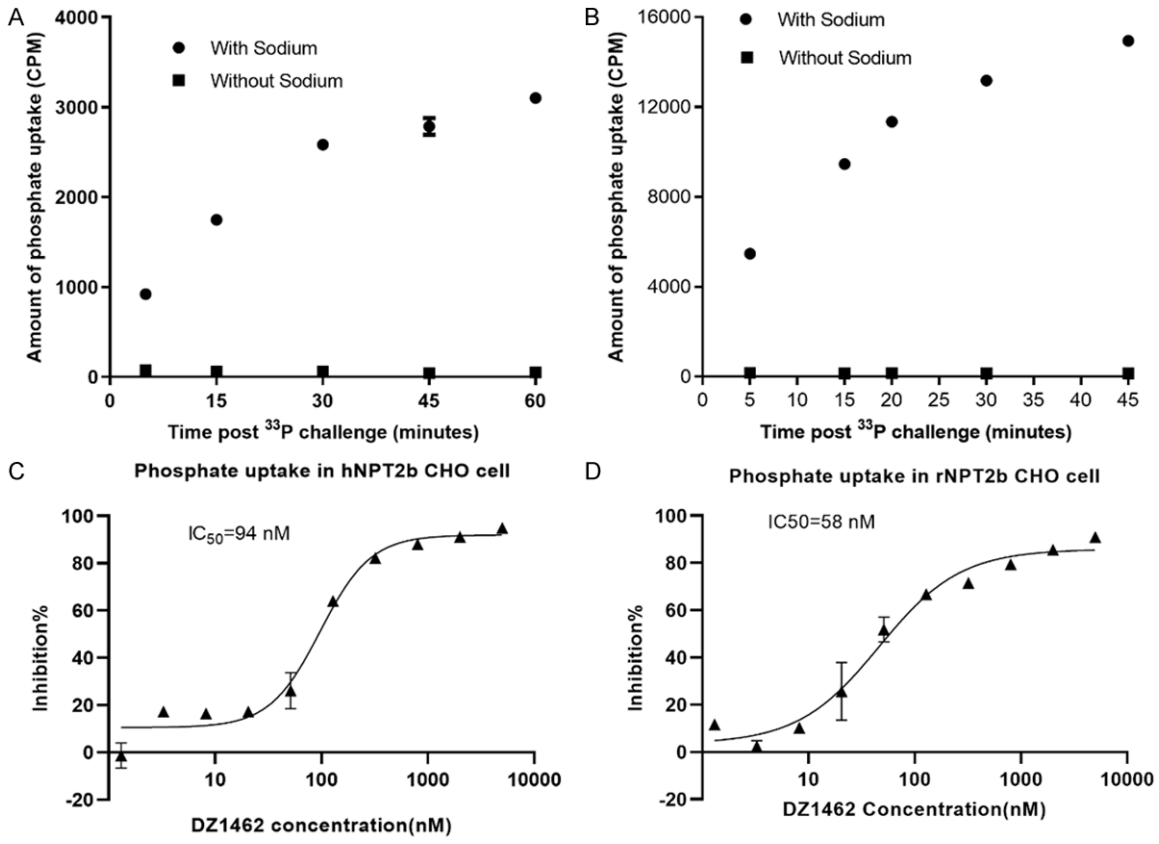

Figure 1. Phosphate uptake by NPT2b-transfected CHO cells. (A) Stable hNPT2b-CHO and (B) rNPT2b-CHO cell clones were preincubated in a 96-well plate for 36 hours and then challenged with 1 μCi/mL phosphoric acid-^{33}P/0.1 mM KH_2PO_4 (100 μL/well) in assay buffer with or without sodium for different durations. Then, the cells were washed, and the amount of phosphate absorbed by the cells was determined by scintillation counting. Dose-dependent inhibition of phosphate uptake by DZ1462 in hNPT2b CHO (C) and rNPT2b CHO (D) cells. Cells were seeded in 96-well plates, treated with a series of compound dilutions for 15 min, and then challenged with ^{33}P for 30 min before detection by scintillation counting. The data are presented as the mean ± SEM. N=6.

isogenic cell line expressing rat NPT2b was generated (rNPT2b-CHO) to evaluate the cross-species effects of DZ1462 on rNPT2b.

We first evaluated Pi absorption by stable NPT2b-CHO clones to verify that isogenically expressed NPT2b function properly. The in vitro Pi uptake assay results were analyzed at 5 min, 15 min, 30 min, 45 min and 60 min after ^{33}P challenge. The results showed that ^{33}P absorption increased rapidly from 0-30 min and reached a plateau afterward (**Figure 1A**, circle). However, the uptake of ^{33}P was very low, and there was almost no uptake in the absence of sodium ion, which is essential for the function of NPT2b (**Figure 1A**, square). The trend was basically the same for both the hNPT2b-CHO and rNPT2b-CHO cell lines (**Figure 1A** and **1B**). Thus, the construction of the hNPT2b-CHO and rNPT2b-CHO cell lines was successful, and these cell lines could be used for subsequent studies.

The ability of DZ1462 to inhibit Pi uptake was investigated in NPT2b-transfected CHO cells. The cells were preincubated with serial dilutions of compound for 15 min and then challenged with ^{33}P for 30 min. As shown in **Figure 1C** and **1D**, the inhibition of Pi uptake by DZ1462 was concentration dependent in both hNPT2b and rNPT2b CHO cells (IC_{50}=94 nM and 58 nM, respectively).

DZ1462 has high drug exposure in the intestine and low systemic exposure

To evaluate the effect of DZ1462 in vivo, we first evaluated the intestinal and systemic exposure of DZ1462 in the rats. After a single oral dose (30 mg/kg), the drug exposure in the duodenal mucus was the highest, followed by the

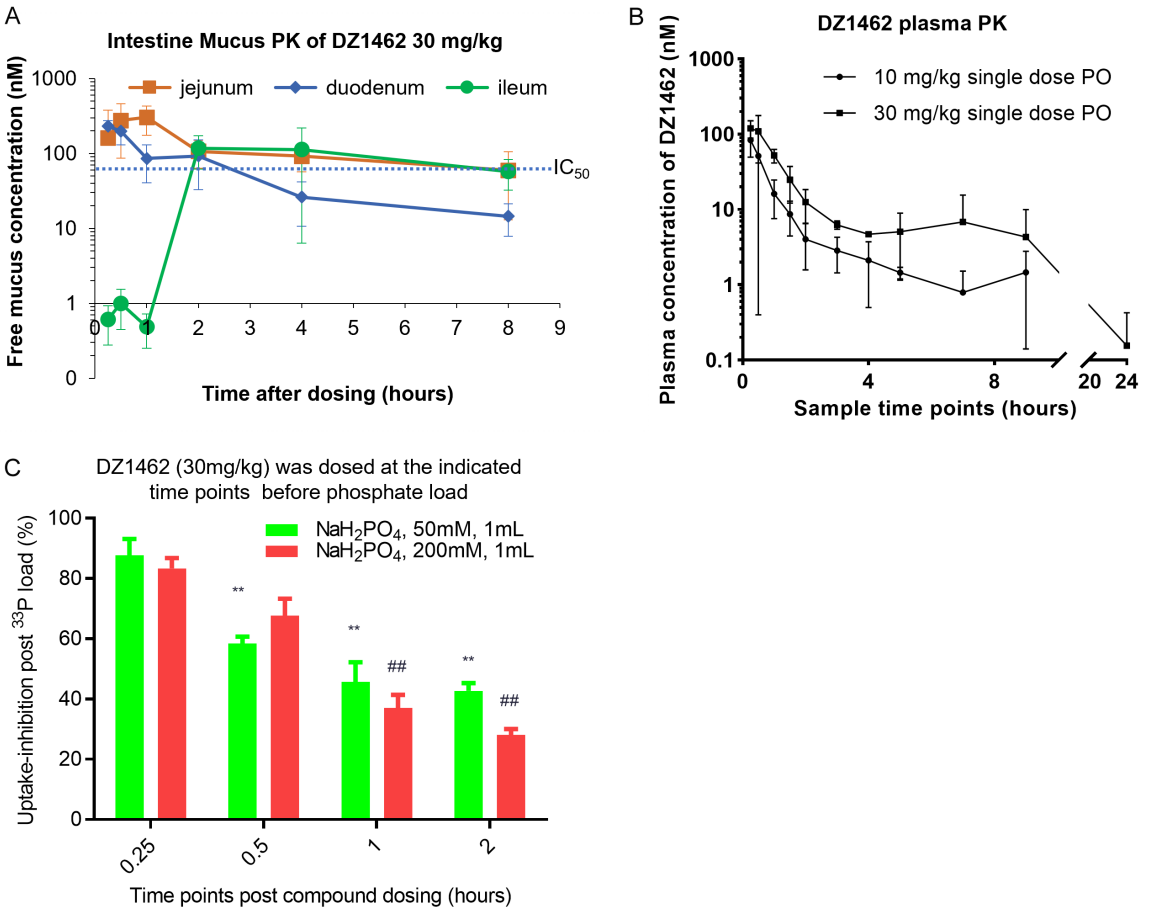

Figure 2. Determination of DZ1462 concentration and inhibition of Pi uptake after loading in vivo. A. Mucus concentration of DZ1462 after the administration of 30 mg/kg DZ1462 determined at different time points post-dosing. B. Plasma concentrations of DZ1462 after the administration of 10 and 30 mg/kg DZ1462. Rats were dosed with 10 mg/kg or 30 mg/kg DZ1462, and blood was collected at various time points after dosing to determine the systemic compound concentration. C. Ability of DZ1462 to inhibit phosphate uptake in an in vivo chronic rat model. Male rats were fed low-Pi diet for 9 days. Then, the rats were dosed with 30 mg/kg DZ1462 or vehicle. At different timepoints (0.25, 1, 2, and 4 hours) after DZ1462 or vehicle treatment (n=3 rats/time point), the rats were given a bolus oral challenge of radioactive phosphate solution. Fifteen minutes later, blood was collected, and the radioactivity in the serum was determined by scintillation counting to calculate the percent phosphate uptake inhibition. Two-way ANOVA. **, P<0.01, compared to 0.25 hr of NaH_2PO_4, 50 mM, 1 mL group. ##, P<0.01, compared to 0.25 hr of NaH_2PO_4, 200 mM, 1 mL group.

jejunum and ileum (**Figure 2A**). Free DZ1462 at this concentration was above the rNPT2b IC_{50} for more than 8 hours in the rat small intestine, supporting the use of twice per day dosing (BID) or three times per day dosing (TID) for further chronic treatment. Furthermore, to understand the toxicity of DZ1462, 10 mg/kg and 30 mg/kg DZ1462 were orally administered to the rats. Then, plasma was collected at different time points postdose, and the drug concentration in plasma was measured. As shown in **Figure 2B**, the maximum serum concentrations (C_{max} values) of 10 mg/kg and 30 mg/kg DZ1462 were 84 nM and 120 nM, respectively. Because the percentage of the unbound plasma protein fraction of DZ1462 was <0.1% (data not shown), the plasma free drug concentrations were less than 0.08 and 0.1 nM, respectively. The C_{max} increased quickly after dosing and then decreased rapidly. These results suggested that DZ1462 has a low systemic exposure, which could limit its toxicity.

DZ1462 decreases the serum phosphorus concentration in various animal models

Then, we measured the Pi concentration in serum to evaluate the inhibitory effect of DZ1462 on Pi absorption in the GI tract. As shown in **Figure 2C**, in acute phosphate-pulsed

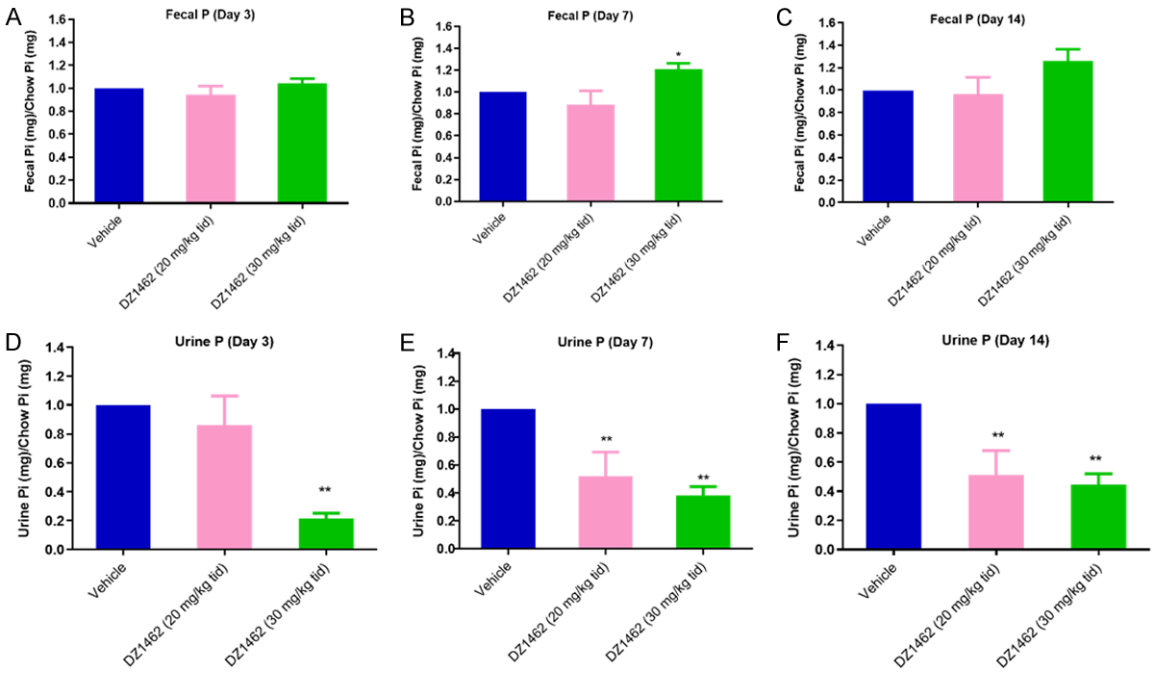

Figure 3. Fecal and urine Pi excretion after DZ1462 treatment. Normal rats were acclimated for 5 days, followed by DZ1462 treatment with a normal Pi diet. Fecal samples (A-C) and 24-h urine samples (D-F) were collected on days 3, 7 and 14 for Pi analysis. Excretion was calibrated to chow Pi (urine or fecal Pi/chow Pi). *, P<0.05, **, P<0.01, compared to vehicle treatment group.

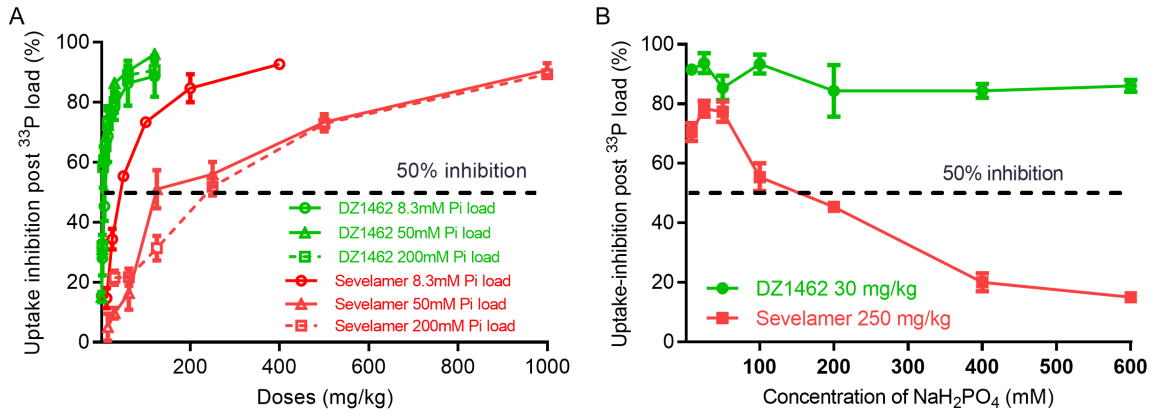

Figure 4. DZ1462 potency in vivo. DZ1462 was more potent and effective than sevelamer in inhibiting ^{33}P uptake by normal rats. A. Male SD rats (7-8 weeks old) were fed a low-P (0.15%) diet for 5 days, and an acute phosphate load (NaH_2PO_4 at 8.3, 50, or 200 mM in 1 mL) was administered after treatment with different doses of compound. Uptake inhibition was measured 15 min after phosphate loading. B. Sevelamer (250 mg/kg) and DZ1462 (30 mg/kg) were administered, and different doses of acute phosphate were given. The 250 mg/kg sevelamer dose is equivalent to the highest clinically used dose, which has >70% compliance. The 30 mg/kg DZ1462 dose is equivalent to 1.5-fold the predicted clinically efficacious dose.

rats, nearly 80% of the inhibitory effect on ^{33}P uptake occurred approximately 15 min after dosing, while ^{33}P inhibition decreased to 20% at 2 hours, which was correlated with dynamic changes in the intestinal mucus concentration of DZ1462 (**Figure 2A**).

Furthermore, normal rats were fed a normal Pi diet, and fecal and urine Pi excretion were detected. The amount of Pi excreted in the urine significantly decreased from day 3 after DZ1462 treatment while the amount of Pi excreted in the feces increased from day 7

Table 1. Comparison of the in vivo activities of DZ1462 and sevelamer

Acute phosphate load (NaH_2PO_4, mM, 1 mL)	In vivo EC_{50} of ^{33}P uptake inhibition	
	DZ1462 (mg/kg)	Sevelamer (mg/kg)
8.3	8	44
50	6	180
200	5	213

Table 2. Comparison of in vivo ^{33}P uptake inhibition after treatment with human equivalent doses of DZ1462 and sevelamer

Acute phosphate load (NaH_2PO_4, mM, 1 mL)	In vivo ^{33}P uptake inhibition (%)	
	DZ1462 (30 mg/kg)	Sevelamer (250 mg/kg)
8.3	91	71
25	94	78
50	85	77
100 (Equivalent to a normal physiological Pi load)	94	55
200	84	45
400	85	20
600	86	15

(**Figure 3A-F**). This inhibition was highly significant at the 30 mg/kg TID dosage (**Figure 3D**). These data suggested that DZ1462 inhibited Pi uptake through intestine blockage.

Sevelamer was the first Pi binder to be approved. DZ1462 is more potent and effective than sevelamer in inhibiting ^{33}P uptake (**Figure 4A**). The EC_{50} values of DZ1462 and sevelamer were 5~8 mg/kg and 44~213 mg/kg, respectively (**Figure 4A** and **Table 1**). In addition, at a human equivalent dose, the efficacy of sevelamer decreased rapidly with increasing Pi load, while DZ1462 remained effective even under a high Pi load (**Figure 4B** and **Table 2**).

In the adenine-induced chronic hyperphosphatemia model, the serum phosphorus concentrations in hyperphosphatemic rats (vehicle and compound groups) were significantly greater than that of normal control rats, suggesting that this model can be used to mimic hyperphosphatemia in CKD (**Figure 5A**). After 7 days of treatment, DZ1462 at or above a dose of 10 mg/kg significantly decreased the serum phosphonate concentration (**Figure 5A**), and this result was more significant than those with 250 mg/kg sevelamer. No significant changes in the serum creatinine, Na, K, Cl, PTH or Ca levels were observed in the adenine-induced hyperphosphatemia model after DZ1462 treatment (**Figure 5A** and **5B**). These data indicated the potential of using DZ1462 to inhibit Pi uptake in CKD patients.

DZ1462 decreases cardiovascular events and improves osteoporosis in a CKD model

FGF23 is strongly and independently correlated with mortality and cardiovascular events in CKD patients. Therefore, we next detected the effect of DZ1462 on FGF23 in vivo. DZ1462 had a greater effect on decreasing serum FGF23 levels than sevelamer did (**Figure 6A**), which was correlated with a decrease in β-MHC expression in the heart. CKD patients may benefit from treatment with DZ1462 in the future to prevent mortality from cardiovascular disease.

Moreover, DZ1462 improved osteoporosis in the CKD model. The tibias of adenine-induced hyperphosphatemic rats were collected for pathological examination. As shown in **Figure 6**, compared to bone content of normal rats, the adenine model group exhibited a decrease in bone content, bone trabeculae thinning, and interval widening (**Figure 6B**). Treatment with 10 or 30 mg/kg DZ1462 tended to increase both the bone content and the thickening of bone trabeculae, while treatment with sevelamer did not (**Figure 6C**). These findings suggested that DZ1462 likely has the potential to improve osteoporosis in CKD patients.

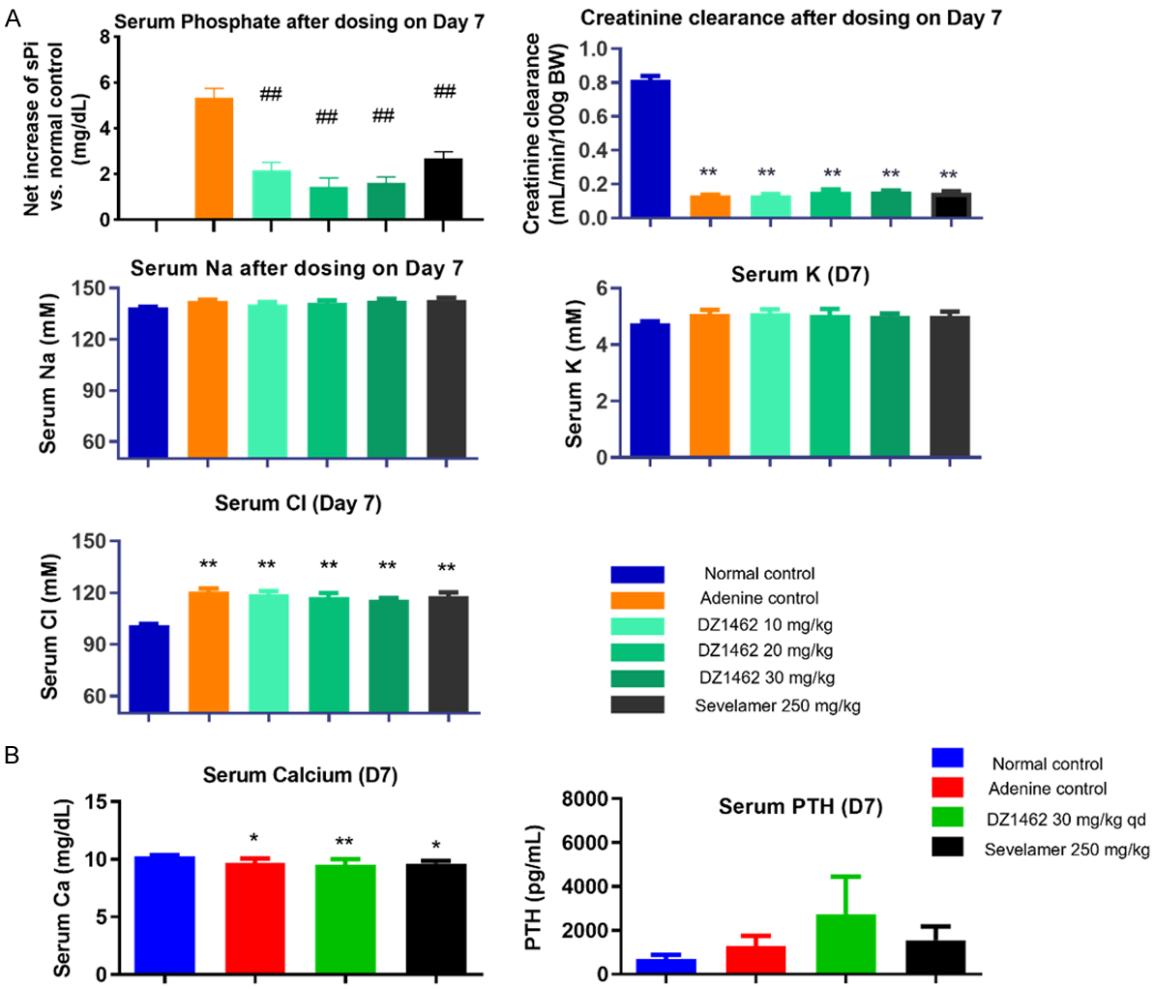

Figure 5. Changes in serum ion levels after DZ1462 treatment. In vivo serum phosphate, Na, K, Cl, and creatinine levels (A) and PTH and Ca levels after 7 days of treatment with different doses of DZ1462 and sevelamer in an adenine-induced hyperphosphatemia rat model. (B) One-way ANOVA. *, P<0.05, **, P<0.01, compared to normal control group. ##, P<0.01, compared to adenine control group.

DZ1462 rescues resistance to sevelamer in a CKD model

In the adenine-induced chronic hyperphosphatemia model, upregulation of NPT2b in the GI tract was detected after 7 days of treatment with sevelamer and DZ1462 (**Figure 7A**). Phosphate was then added to measure the inhibitory potencies of DZ1462 and the sevelamer. As illustrated in **Figure 7A**, although the expression of NPT2b was upregulated in both groups, the efficacy of DZ1462 was retained, but the efficacy of sevelamer decreased. Furthermore, the decreased efficacy of sevelamer was rescued by DZ1462 treatment (**Figure 7B**). These data demonstrated that the sevelamer-induced upregulation of NPT2b expression may be a mechanism of Pi uptake relapse and can be reversed by sequential DZ1462 treatment through NPT2b inhibition.

To evaluate the clinical relevance of DZ1462, we treated adenine-induced CKD models with DZ1462 in combination with or without sevelamer. Compared to monotherapy, combination therapy with sevelamer and DZ1462 induced profound synergistic effects (**Figure 7C**) without influencing creatinine clearance (**Figure 7D**).

Discussion

Chronic kidney disease (CKD) is an important disease that affects human health [5, 29]. In patients with CKD, metabolic waste (such as urea nitrogen and phosphate) and excessive

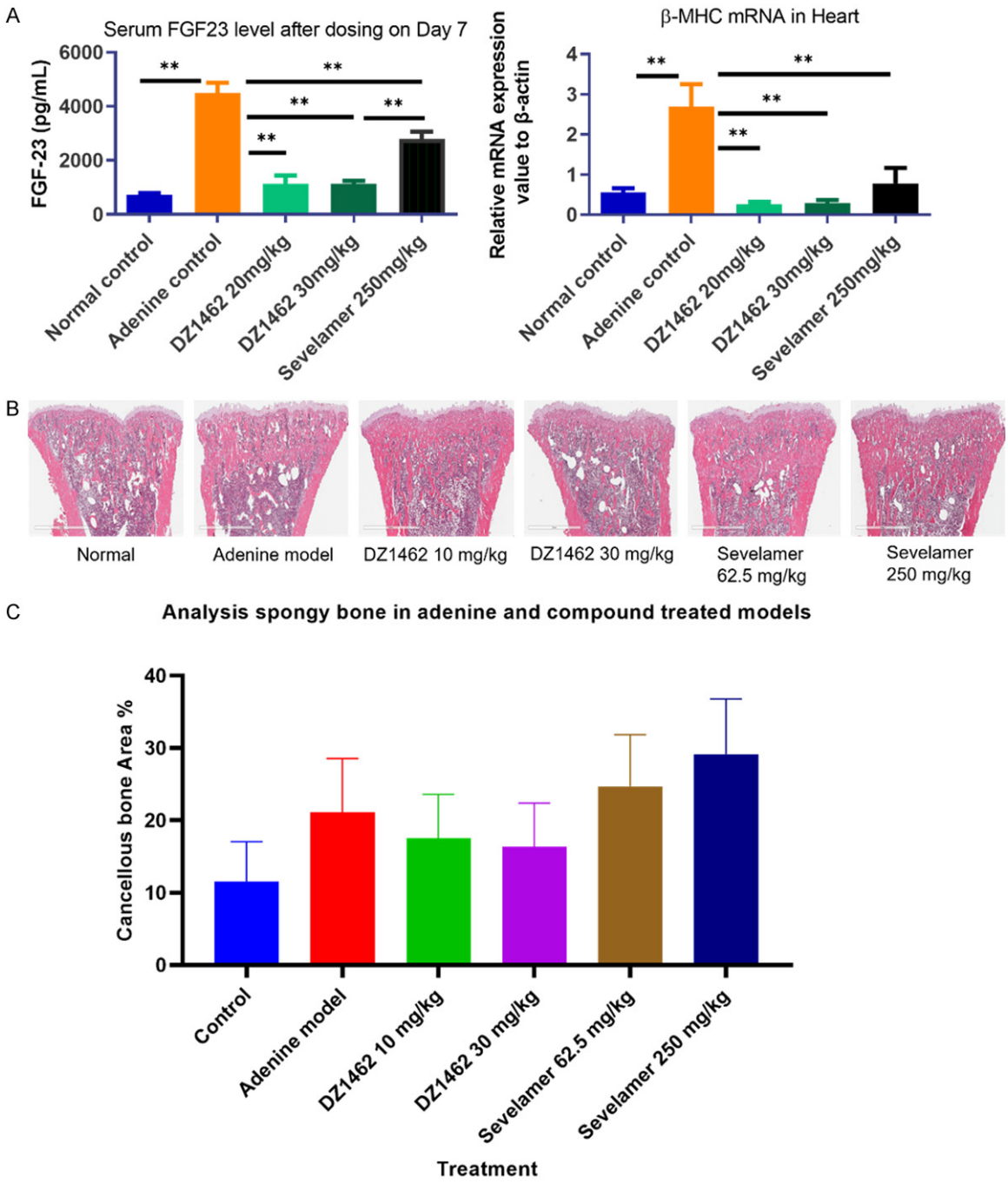

Figure 6. Changes in FGF-23 and β-MHC levels and cancellous bone area in adenine-induced hyperphosphatemia model rats after treatment. (A) H&E-stained images (scale bar 2 mm) of tibia after DZ1462, sevelamer, and DZ1462 plus sevelamer combination therapy. In the control group, the cancellous bone area was dense, the trabecular structure was clear, and high bone density was observed. The model group showed a decrease in bone content, bony trabeculae thinning, and interval widening. There was an increase in bone content and thickening of the bony trabeculae in the treatment group. (B) Cancellous bone area measurements from the data in (C). n=6. One-way ANOVA. *, P<0.05, **, P<0.01.

inorganic salts ingested through the diet cannot be excreted through the urinary system, leading to an electrolyte imbalance [30]. Phosphate ions are important electrolytes in the body. When the intake of phosphate ions exceeds the amount excreted, ions accumulate, and hyperphosphatemia occurs, followed by various symptoms, such as osteoporosis,

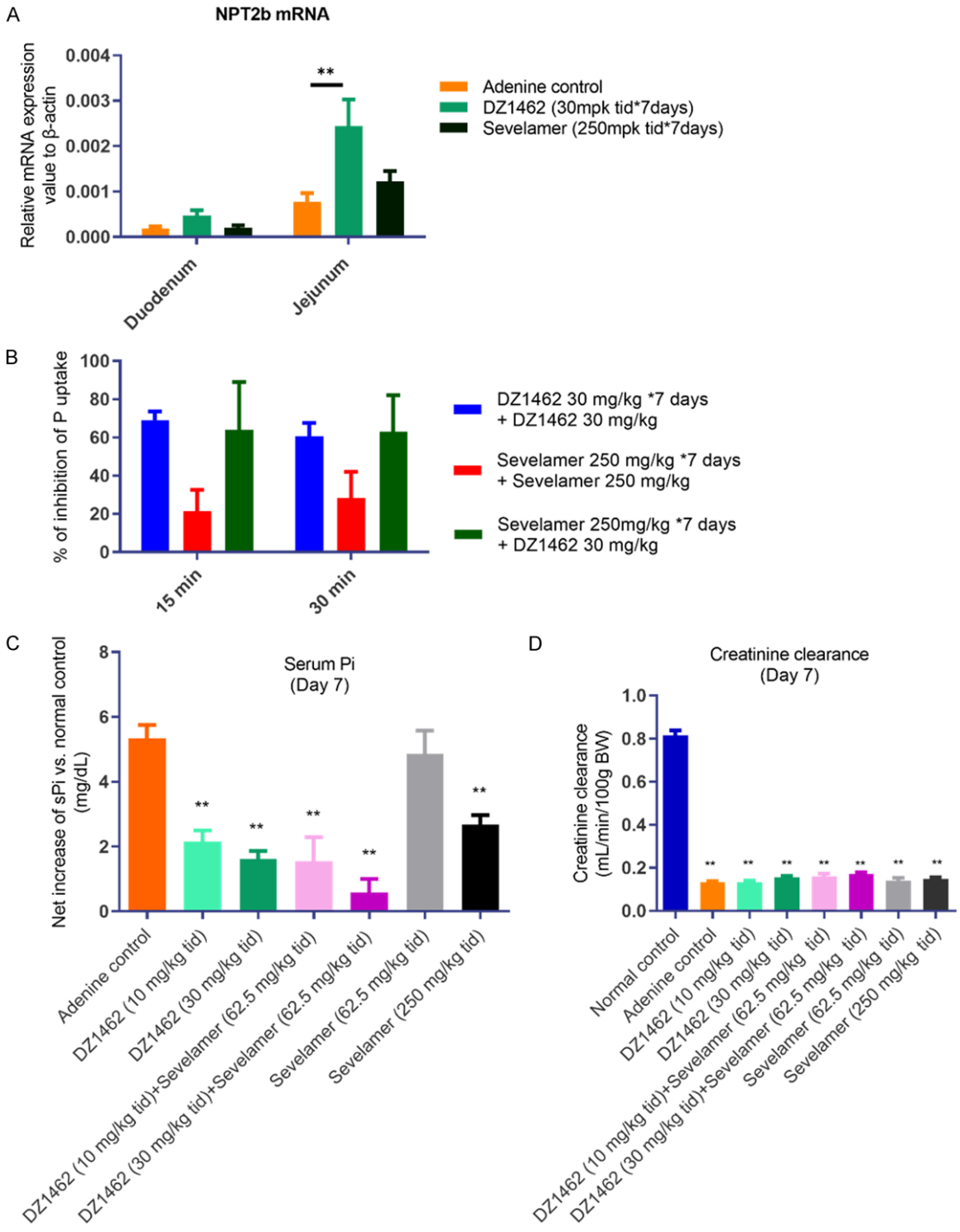

Figure 7. Effect of DZ1462 and sevelamer combination treatment in adenine-induced chronic hyperphosphatemia model. Chronic treatment with sevelamer led to increased expression of NPT2b (A) and decreased efficacy, which was reversed by DZ1462 (B). Detection of serum Pi levels (C) and creatinine clearance (D) after DZ1462 monotherapy, sevelamer monotherapy, and combination therapy. One-way ANOVA. *, P<0.05, **, P<0.01.

soft tissue calcification, coronary artery calcification, and anemia; the most vital adverse effects are cardiovascular disorder-associated morbidity and mortality [4, 10]. These symptoms cause great pain and economic pressure for patients with hyperphosphatemia.

The intestine is the most important organ for the absorption of phosphate ions (accounting for more than 70% of phosphate absorption), and more than 90% of the active transport of phosphorus occurs through NPT2b [31]. The development of new small molecule intestinal NPT2b inhibitors has become an important direction for controlling blood phosphate levels [10]. However, because NPT2b is also expressed in renal and other tissues, inhibition of NPT2b in humans may cause fetal toxicity, such as pulmonary alveolar microlithiasis (PAM). Although several small molecule inhibitors of NPT2b have been developed, most of them have dose-limiting toxicity due to systemic inhibition of multiple NPT2 family members [10].

ASP3325 (a small molecule NPT2b inhibitor) has been demonstrated to alleviate hyperphosphatemia induced by CKD in several rat models. However, subsequent clinical studies failed to show that ASP3325 affected the excretion of urinary phosphate or fecal phosphate in healthy subjects or reduced the serum phosphate level in ESRD patients [18, 32]. These ineffective treatment results were attributed to the chemical structural defects of the compound, which may cause severe toxicity due to high systemic exposure, the small sample size, and the short treatment duration [18, 29].

Thus, we generated a GI-restricted NPT2b inhibitor to overcome this issue. DZ1462 inhibited Pi uptake, increased fecal Pi excretion and decreased urine Pi excretion. Systemic exposure of DZ1462 was very low, the plasma C_{max} of 10 mg/kg DZ1462 was 78.4 ng/mL, and the dose-normalized area under the curve (DNAUC) of DZ1462 in the portal vein after 0-7 hours after dosing was 14 (ng·h/mL)/(mg/kg). The plasma C_{max} of ASP3325 at 10 mg/kg was 1730 ng/mL, and the DNAUC of ASP3325 was 360 (ng·h/mL)/(mg/kg) (data not shown). Thus, lower systemic exposure may significantly reduce the occurrence of PAM [32, 33]. Based on another mode test of DZ1462, a series of parallel lines were identified on the Eadie-Hofstee plot, indicating that DZ1462 is a non-competitive inhibitor of phosphate absorption, which sugggests there will be fewer side effects for patients. In addition, the drug-drug interactions caused by drug metabolism in vivo (data not shown) were more effectively prevented with DZ1462 treatment than with ASP3325 treatment.

An in vitro phosphate uptake study on the cell clones demonstrated that the inhibitory effect of DZ1462 on NPT2b was potent. However, DZ1462 displays controlled systemic toxicity due to its low systemic exposure. Furthermore, DZ1462 potently decreased serum FGF23 levels, which suggested that DZ1462 has cardioprotective potential because FGF23 is strongly and independently correlated with mortality and cardiovascular events in CKD patients [34]. DZ1462 treatment improved osteoporosis, decreased kidney Pi burden, and did not damage renal function.

DZ1462 also had an advantage over phosphate binders. The in vivo efficacy studies in rats fed a Pi diet or adenine-induced hyperphosphatemia model, DZ1462 showed better efficacy than sevelamer did and significantly inhibited the level of blood phosphorus in a dose-dependent manner. DZ1462 rescued the Pi uptake inhibition effects induced by sevelamer, which suggested that a combination strategy could benefit CKD patients.

Conclusion

Our data demonstrate that DZ1462 is a novel and potent GI-restricted NPT2b inhibitor. DZ1462 overcomes the systemic toxicity issue of NPT2B inhibitors and provides a potential synergistic benefit for hyperphosphatemia treatment.

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Disclosure of conflict of interest

None.

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