Sodium phosphate cotransporter 2a inhibitors: potential therapeutic uses

Jianxiang Xue\textsuperscript{a,\+}, Linto Thomas\textsuperscript{a,\+}, Jessica A. Dominguez Rieg\textsuperscript{a,b}
Timo Rieg\textsuperscript{a,b,c}

Purpose of review
Targeting sodium phosphate cotransporter 2a (Npt2a) offers a novel strategy for treating hyperphosphatemia in chronic kidney disease (CKD). Here we review recent studies on the efficacy of Npt2a inhibition, its plasma phosphate (Pi)-lowering effects, as well as potential “off-target” beneficial effects on cardiovascular consequences.

Recent findings
Two novel Npt2a-selective inhibitors (PF-06869206 and BAY-767) have been developed. Pharmacological Npt2a inhibition shows a significant phosphaturic effect and consequently lowers plasma Pi and parathyroid hormone (PTH) levels regardless of CKD. However, plasma fibroblast growth factor 23 (FGF23), a master regulator of Pi homeostasis, shows inconsistent responses between these two inhibitors (no effect by PF-06869206 vs. reduction by BAY-767). In addition to the effects on Pi homeostasis, Npt2a inhibition also enhances urinary excretions of Na\textsuperscript{+}, Cl\textsuperscript{−}, and Ca\textsuperscript{2+}, which is recapitulated in animal models with reduced kidney function. The effect of Npt2a inhibition by BAY-767 on vascular calcification has been studied, with positive results showing that oral treatment with BAY-767 (10 mg kg\textsuperscript{−1}) attenuated the increases in plasma Pi and Ca\textsuperscript{2+} content in the aorta under the setting of vascular calcification induced by a pan-FGF receptor inhibitor. Together, Npt2a inhibition offers a promising therapeutic approach for treating hyperphosphatemia and reducing cardiovascular complications in CKD.

Summary
Npt2a inhibition significantly increases urinary Pi excretion and lowers plasma Pi and PTH levels; moreover, it exerts pleiotropic “off-target” effects, providing a novel treatment for hyperphosphatemia and exhibiting beneficial potential for cardiovascular complications in CKD.

Keywords
chronic kidney disease, FGF23, hyperphosphatemia, PTH, sodium phosphate cotransporter

INTRODUCTION
Phosphate (Pi) homeostasis is precisely regulated. In adults, normal plasma Pi ranges from 0.80 to 1.45 mmol l\textsuperscript{−1} [1], which is maintained by inter-organ interplay, including intestinal absorption, bone (de) mineralization, and renal excretion. The kidney is the key organ for fine-tuning Pi homeostasis; therefore, impaired renal function results in Pi imbalance, including hypophosphatemia (plasma Pi levels < 0.8 mmol l\textsuperscript{−1}) and hyperphosphatemia (plasma Pi levels > 1.4 mmol l\textsuperscript{−1}). Hyperphosphatemia is closely associated with chronic kidney disease (CKD) in later stages of the disease and cardiovascular diseases [2].

Several Pi transporters have been identified in the renal proximal tubule, including sodium-phosphate cotransporters Npt2a (SLC34A1), Npt2c (SLC34A3), Pit1 (SLC20A1), and Pit2 (SLC20A2) (for

*Department of Molecular Pharmacology and Physiology, \textsuperscript{b}Center for Hypertension and Kidney Research, University of South Florida and \textsuperscript{c}James A. Haley Veterans’ Hospital, Tampa, Florida, USA

Correspondence to Timo Rieg, MD, Department of Molecular Pharmacology and Physiology, Morsani College of Medicine, University of South Florida, 12901 Bruce B. Downs Blvd, MDC8, Tampa, FL 33612, USA.

E-mail: trieg@usf.edu

\textsuperscript{+}These authors contributed equally to this work.

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a review, see [3,4,5]). The estimated relative contribution of Npt2a for renal Pi reabsorption is ~70%, as evidenced by studies using brush border membrane vesicles isolated from Npt2a−/− mice [6]. Pathologically these mice are characterized by increased urinary Pi excretion and decreased plasma Pi concentration [7**]. The contribution of Npt2c shows species differences and depends on the developmental stage, with more contribution to Pi reabsorption in juveniles compared to adults [8]. In humans, SLC34A3 mutations cause hereditary hypophosphatemic rickets with hypercalciuria (HHRH) [9,10]. In contrast, in mice, loss of renal Npt2c shows no prominent impact on Pi homeostasis [11*], and Npt2a/c double knockout mice demonstrate a similar effect on urinary Pi excretion compared with Npt2a−/− mice [12], highlighting the small contribution of Npt2c for renal Pi reabsorption. Similarly, both Pit1 and Pit2 appear to have a small contribution to renal Pi transport [13,14]. Interestingly, a recent study localized Npt2b to the thick ascending limb of the kidney; however, its abundance was not regulated by dietary Pi [15] and further studies are needed to address the importance of this finding.

FGF23 and PTH are primary regulators for systemic Pi homeostasis, and both reduce renal Pi reabsorption via retrieval of Npt2a/c from the luminal membrane [16]. Additionally, FGF23 downregulates vitamin D, leading to reduced Pi absorption from the intestine, whereas PTH exerts the opposite effects: increasing vitamin D and intestinal Pi absorption [17]. On the other hand, plasma Ca2+ and Pi levels regulate PTH release from the parathyroid gland via the calcium-sensing receptor (CaSR) [18]. Although the exact mechanism for regulating FGF23 release is incompletely understood, it has been suggested that FGF23 production by bone is regulated in response to dietary Pi at the transcription, translation, and posttranslational levels [19]. Elevated plasma Pi level upregulate both, FGF23 and GALNT3 production, the latter an enzyme protecting FGF23 from proteolytic cleavage via posttranslational glycosylation [20]. The Chronic Renal Insufficiency Cohort study showed that FGF23 is an early biomarker during CKD progression, with elevated plasma FGF23 preceding the increase in PTH and Pi [21*], implying that FGF23 is the principal Pi homeostasis regulator compared to PTH, at least in the early stages of CKD. The increased plasma FGF23 in the early stages of CKD, followed by an increase in PTH, is sufficient to prevent hyperphosphatemia until stage 4–5 CKD. This hypothesis is supported by animal studies showing that targeting FGF23 production impairs Pi homeostasis in CKD animal models [22,23]. High plasma FGF23 and Pi levels are independently associated with poor cardiovascular outcomes in patients with CKD, but the primary physiological function of FGF23 is to protect the body from hyperphosphatemia, which subsequently can cause detrimental cardio-renal consequences [24–26].

ADAPTATION OF RENAL Pi TRANSPORTERS IN CHRONIC KIDNEY DISEASE

In CKD, renal function declines gradually with age, showing a reduction in nephron numbers and elevated FGF23 and PTH levels. These changes can potentially downregulate Npt2a/c expression. Supporting this theory, adenine-induced CKD animal models show markedly reduced Npt2a expression at the protein and mRNA levels [27–29]. Consistently, in rats and mice with reduced kidney function (5/6 Nx), a substantial reduction in Npt2a mRNA expression is also observed [30]. Additionally, Npt2a activity is affected by urine pH: more alkaline urine increases Npt2a activity. Notably, CKD is characterized by low urine pH, possibly associated with lower Npt2a activity. Therefore, these observations need to be considered when targeting renal Na+/Pi cotransporters as therapeutic strategies.

THERAPEUTIC STRATEGIES FOR LOWERING HYPERPHOSPHATEMIA IN CHRONIC KIDNEY DISEASE

Treating hyperphosphatemia in CKD is challenging due to sophisticated regulatory mechanisms for Pi homeostasis. Currently available treatment options include dietary Pi restriction, oral Pi binders, as well as niacin/nicotinamide [31–33]; however, all show severe limitations. Dietary Pi restriction and oral Pi,
binders are supposed to reduce $P_i$ entry; however, the maladaptive upregulation of $P_i$ uptake from the gastrointestinal tract limits their efficacy [34,35]. An alternative approach to lower intestinal $P_i$ absorption is targeting Npt2b, responsible for >90% of active $P_i$ uptake in the intestine [36]. Unfortunately, results from clinical trials indicate the limited efficacy of two newly developed Npt2b inhibitors (ASP3325; DS-2330b) for lowering plasma $P_i$ in healthy volunteers and patients on hemodialysis [37*,38*]. Similarly, downregulation of Npt2b by niacin/nicotinamide also shows unsatisfactory results [39,40]. Compared to the Npt2b-selective inhibitor, a novel pan-phosphate transporter inhibitor (EOS789, targeting Npt2b, Pit1/2) shows a more potent serum $P_i$ lowering effect with decreased FGF23 and PTH in rats with adenine-induced hyperphosphatemia [41**]. Although a recent phase 1b clinical trial supports the safety of EOS789 in patients on hemodialysis [42], its efficacy needs to be confirmed by further studies. Inhibition of intestinal Na$^+$/H$^+$ exchanger isoform 3 (NHE3) by a non-absorbable inhibitor, tenapanor, shows a serum $P_i$-lowering effect in patients on hemodialysis [43*]. Mechanistically, it was proposed that tenapanor inhibits paracellular rather than transcellular $P_i$ absorption. In order to unravel such a mechanism we studied inducible intestinal epithelia cell-specific NHE3 knockout mice [46]. Of note, genetic deletion of intestinal NHE3 resulted in enhanced rather than reduced intestinal $P_i$ uptake [47**], implying that different mechanisms/conditions are causing the differences between pharmacological inhibition vs. genetic deletion. Last year the United States Food and Drug Administration denied the approval of tenapanor due to small effect of unclear clinical significance [48]; however, this decision has been appealed by Ardelyx, Inc.

**PHOSPHATURIC EFFECT OF SODIUM PHOSPHATE COTRANSPORTER 2a INHIBITION**

In addition to reducing intestinal $P_i$ absorption, promoting renal $P_i$ excretion is another strategy for lowering plasma $P_i$. Until recently, two Npt2a-selective inhibitors (PF-06869206 from Pfizer; BAY-767 from Bayer) have been developed. The selectivity of these inhibitors for Npt2a has been confirmed in vitro [49*,50**] and in vivo [7**,51**]. Using opossum kidney (OK) cells, we showed a dose-dependent inhibition of Na$^+$/H$^+$ exchanger isoform 3 (NHE3)/2P$_i$ uptake (half maximal inhibitory concentration, $I_C_{50} \sim 1$ mmol L$^{-1}$) by PF-06869206, with a maximum inhibitory effect of ~70% at 100 mmol L$^{-1}$ [7**]. In wild type (WT) (C57Bl/6) mice, the dose of 100 mg kg$^{-1}$ PF-06869206 increases urinary $P_i$ excretion by ~6-fold (3 h period) compared to vehicle (median effective dose, ED$_{50} \sim 21$ mg kg$^{-1}$) [52**]. Another study reported a similar phosphaturic effect of PF-06869206 where a dose of 500 mg kg$^{-1}$ caused a ~17-fold increase in the fractional excretion index (FEI) of $P_i$ (4 h period) in WT mice [51**]. Of note, this phosphaturic effect is consistently observed in different hyperphosphatemic animal models (FGF23$^{-/-}$ and GALNT3$^{-/-}$ mice), with a ~9-fold and ~2-fold increase in FEI of $P_i$ (4 h time frame), respectively, in response to PF-06869206 at a dose of 300 mg kg$^{-1}$ compared with vehicle [51**]. Another Npt2a inhibitor, BAY-767, also showed a profound phosphaturic effect and a dose of 10 mg kg$^{-1}$ resulted in a ~1.7-fold increase in fractional urinary $P_i$ excretion (16 h time frame) [50**].

**RESPONSES OF PLASMA $P_i$, PTH, AND FGF23 TO SODIUM PHOSPHATE COTRANSPORTER 2a INHIBITION**

Will the phosphaturic effect of Npt2a inhibition reduce plasma $P_i$ levels? Our studies employing PF-06869206 at a dose of 30 mg kg$^{-1}$ showed a reduction in plasma $P_i$ levels starting 30 min after oral administration in WT mice, with a maximum reduction at 2 h (~35%) and complete recovery after 24 h [7**,52**]. Another study in WT mice by Clerin et al. [51**] showed that 300 mg kg$^{-1}$ of PF-06869206 significantly reduced plasma $P_i$ levels. Importantly, PF-06869206 showed similar plasma $P_i$-lowering effects in FGF23$^{-/-}$ (~20%), GALANT3$^{-/-}$ (~20%), and Npt2c$^{-/-}$ (~33%) mice 2–4 h after administration, the former two mouse models are hyperphosphatemic. In contrast, no effect was observed in Npt2a$^{-/-}$ mice, supporting the selectivity of PF-06869206 for Npt2a [7**,51**]. Another Npt2a inhibitor, BAY-767, at a dose of 10 mg kg$^{-1}$ reduced plasma $P_i$ levels (~20%) in rats after 3 days of treatment [53].

As two predominant regulators of Npt2a membrane abundance, PTH and FGF23 [54,55] demonstrated distinct responses to PF-06869206. We found that PF-06869206 at a dose of 30 mg kg$^{-1}$ reduced plasma PTH levels by ~50% in mice after 3 h of administration [52**]. Clerin et al. [51**] also observed a reduction of PTH levels in mice (~65%) at a dose of 300 mg kg$^{-1}$ 2–4 h after administration. In both studies, the reduction of PTH levels was fully recovered after 24 h. Three days of treatment with BAY-767 (10 mg kg$^{-1}$) in rats reduced the PTH levels by 50% [53]. How do PTH levels decline upon Npt2a inhibition? The CaSR in the parathyroid gland acts as a plasma $P_i$ sensor and thus modulates PTH secretion [18]. In contrast, plasma FGF23 levels were not affected by PF-06869206 [51**,**52**]. Of note,
BAY-767 reduced plasma FGF23 levels (~25%) compared to the vehicle [33]. The inconsistency between PF-06869206 and BAY-767 in FGF23 responses is unknown and needs further study.

**EFFICACY OF SODIUM PHOSPHATE COTRANSPORTER 2a INHIBITION IN CHRONIC KIDNEY DISEASE/REDUCED KIDNEY FUNCTION**

In 5/6 Nx mice, a model of reduced kidney function, acute administration of PF-06869206 increased urinary Pi excretion dose-dependently (Fig. 1a); however, the maximum phosphaturic effect at the dose of 100 mg kg\(^{-1}\) (compared to vehicle) was lower compared to sham mice (~2-fold vs. ~10-fold, respectively). After 3 h of administration of 100 mg kg\(^{-1}\) PF-06869206 significantly reduced plasma Pi (Fig. 1c) and PTH levels (Fig. 1d) in both 5/6 Nx and sham mice. Clerin et al. [51**] treated 5/6 Nx rats with 300 mg kg\(^{-1}\) PF-06869206 for 8 weeks and observed higher FEI of Pi (~2.5-fold) and lower plasma Pi (~15%) compared to vehicle-treated rats. However, 5/6 Nx rats lacked hyperphosphatemia, whereas elevated PTH and FGF23 levels were observed compared to sham rats. Surprisingly, long-term treatment with PF-06869206 did not affect plasma PTH or FGF23 level. So far, the efficacy of BAY-767 has not been investigated in CKD models.

**FIGURE 1.** Inhibition of Npt2a with PF-06869206 affects urine and plasma parameters in mice with normal and reduced kidney function (5/6 Nx). An acute inhibition (3 h) of Npt2a with PF-06869206 resulted in a dose-dependent increase in urinary P\(_i\) (a) and Ca\(^{2+}\) (b) excretion in both sham and 5/6 Nx mice. The enhanced phosphaturia was associated with the acute reductions in plasma P\(_i\) (c) and PTH (d). PF-06869206 also induced a dose-dependent natriuresis (e) in both groups. Because of the absence of kaliuresis and persistence of dose-dependent natriuresis in Npt2a--/-- mice (both data not shown), we assumed that the cause of natriuresis could be from the aldosterone sensitive segment of the distal nephron, where the epithelial sodium channel (ENaC) is located. In the further electrophysiological studies in the acutely split-open cortical collecting ducts of C57BL/6 mice, ENaC open probability was acutely inhibited (~85%) by PF-06869206, possibly explaining the cause of natriuresis. A trace of continuous current is shown in (f), where dashed lines are the respective levels of current, with “o” denoting the open state and ‘c’ denoting the closed state. Areas 1 and 2 under the bars over the continuous traces are shown below at expanded timescales. Figure 1a–e reused with permission from [52**]. Figure 1f reused with permission from [7**]. \(P < 0.05\) vs. vehicle, \(P < 0.05\) vs. sham, \#P < 0.05 vs. previous time point. Figure 1 reprinted with permission from Biochem Soc Trans. 2022;50(1):439–446. CKD, chronic kidney disease; Npt2a, sodium phosphate cotransporter 2a.
"OFF-TARGET" EFFECTS OF SODIUM PHOSPHATE COTRANSPORTER 2a INHIBITION

In addition to the dose-dependent phosphaturic effect, PF-06869206 also increased urinary Na\(^+\), Cl\(^-\), and Ca\(^{2+}\) excretion dose-dependently without affecting their plasma levels [52**]. At a dose of 300 mg kg\(^{-1}\), PF-06869206 increased urinary Ca\(^{2+}\) excretion three-fold compared to vehicle in our studies (Fig. 1b), and Clerin et al. [51**] observed a five-fold increase with the same dose. The possible mechanism for increased calciauria by PF-06869206 is the inhibition of Ca\(^{2+}\) reabsorption either in the proximal tubule (via the paracellular pathway) or the distal convoluted tubule (via TRPV5, transcellular). The latter may be affected by the decreased PTH levels observed after PF-06869206 treatment. On the other hand, PF-06869206 did not alter urinary excretion of K\(^+\), glucose, amino acids, or pH [52**]. This implies that PF-06869206 does not lead to a generalized proximal tubular dysfunction, as seen with Fanconi syndrome. Like C57BL/6 mice, the urinary excretion of Na\(^+\) (Fig. 1e), Cl\(^-\) and Ca\(^{2+}\) (Fig. 1b) was dose-dependently increased in response to PF-06869206 in mice with 5/6 Nx, whereas urinary excretion of K\(^+\), glucose, and pH were unaffected [52**].

As a selective Npt2a inhibitor, we thought PF-06869206-induced natriuresis would be absent in Npt2a\(^{-/-}\) mice; however, natriuresis was unaffected in Npt2a\(^{-/-}\) mice in response to PF-06869206 [7**].

![Diagram](image)

**FIGURE 2.** Overall effects of Npt2a inhibition on renal excretion of minerals/electrolytes and the proposed cardioprotection. Npt2a inhibition by either PF-06869206 or BAY-767 enhances the urinary P\(_{i}\) excretion, resulting in reduced plasma P\(_{i}\) and PTH levels. The PTH secretion is regulated by calcium-sensing receptor (CaSR) expressed in the parathyroid gland. The reduction in PTH may have cardioprotective effects because of the PTH-induced hypertrophy of cardiomyocytes, Ca\(^{2+}\) overload in heart tissues, and oxidative stress. Inhibition of Npt2a with BAY-767 resulted in decreased FGF-23 levels (a potent stimulator for developing left ventricular hypertrophy (LV) in CKD). Therefore, reducing the FGF-23 level might benefit LV and possibly other heart diseases. The natriuretic and diuretic effects of Npt2a inhibition might be beneficial for lowering blood pressure and effective circulating volume (ECV). The increase in urinary Ca\(^{2+}\) excretion upon Npt2a inhibition is either by the direct inhibition of Ca\(^{2+}\) reabsorption in the proximal tubule or the indirect inhibition of PTH mediated Ca\(^{2+}\) transport (via transient receptor potential cation channel 5, TRPV5). Together, phosphaturic and calciuric effects of Npt2a inhibition might decrease the vascular calcification, arterial stiffness, and pulse wave velocity (PWV). A new study observed the increased expression of renal Npt2b in CKD; however, further studies are needed to confirm and determine its (patho)physiological importance. Reprinted with permission from Biochem Soc Trans. 2022;50(1):439–446. CKD, chronic kidney disease; Npt2a, sodium phosphate cotransporter 2a.
Due to the lack of kaliuresis, we hypothesized that the observed natriuresis could be an off-target effect of PF-06869206 via inhibiting ENaC in the aldosterone-sensitive distal nephron. In the subsequent electrophysiological studies in acutely isolated and split-open cortical collecting ducts, the open probability of ENaC was ~85% inhibited (Fig. 1f) in the presence of PF-06869206, giving a possible explanation for a natriuresis observed in Npt2a−/− mice. Blood pressure and total body Na⁺ levels are closely interconnected. However, Clerin et al. [51**] observed no change in systolic blood pressure upon long-term treatment with PF-06869206 in 5/6 Nx rats, despite the presence of acute natriuresis and diuresis in 5/6 Nx mice [52**]. Further studies are needed to determine the reason(s) for these differences.

**POTENTIAL CARDIOVASCULAR BENEFITS BY SODIUM PHOSPHATE COTRANSPORTER 2a INHIBITION**

Calcium is one of the critical pleiotropic effects caused by PF-06869206 and BAY-767. The imbalance of hormones and minerals (P, and Ca²⁺) commonly seen in CKD, provide the perfect environment for the acceleration of vascular calcification. Vascular calcification causes reduced arterial elasticity and increased blood pressure and pulse wave velocity (Fig. 2). In conjunction with increased FGF23 levels, all of these pathological phenotypes lead to the development of left ventricular hypertrophy and consequently heart dysfunction (Fig. 2). In hemodialysis patients, heart failure with left ventricular hypertrophy is commonly observed and is associated with cardiovascular mortality [56]. The effect of BAY-767 on vascular calcification was studied in rats with vascular calcification induced by a pan-FGF receptor inhibitor [53]. In this study, oral treatment with BAY-767 at the dose of 10 mg·kg⁻¹ reduced plasma Pₐ levels (~1.4-fold) and aortic calcium content (~75%). In contrast, the oral Pₐ binder lanthanum carbonate (2.2%, administered via diet) did not reduce aortic Ca²⁺ content [50**].

**CONCLUSION**

Npt2a inhibitors reduce renal Pₐ reabsorption in the proximal tubule. We are beginning to recognize that in addition to phosphaturia there are several accessory effects that might be indirectly related to inhibition of Npt2a. Notably, the renal handling of Na⁺, Cl⁻, and Ca²⁺ are affected. It remains to be seen if Npt2a inhibition is still efficacious in conditions where hyperphosphatemia is present, for example, in severe CKD, hemolysis, acute tumor lysis syndrome, rhabdomyolysis, etc. Clearly, further studies are needed to determine if long-term treatment with Npt2a inhibitors will, via a feedback mechanism, increase intestinal Pₐ uptake and/or result in changes in bone mineralization.

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**Conflicts of interest**

Dr Rieg had consultancy agreements with Ardelyx and Akros Pharmaceuticals.

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