Sodium phosphate cotransporter 2a inhibitors: potential therapeutic uses

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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 (P_i)-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 P_i and parathyroid hormone (PTH) levels regardless of CKD. However, plasma fibroblast growth factor 23 (FGF23), a master regulator of P_i homeostasis, shows inconsistent responses between these two inhibitors (no effect by PF-06869206 vs. reduction by BAY-767). In addition to the effects on P_i homeostasis, Npt2a inhibition also enhances urinary excretions of Na^+, Cl^-, and Ca^{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^{-1}) attenuated the increases in plasma P_i and Ca^{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 P_i excretion and lowers plasma P_i 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 (P_i) homeostasis is precisely regulated. In adults, normal plasma P_i ranges from 0.80 to 1.45 mmol l^{-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 P_i homeostasis; therefore, impaired renal function results in P_i imbalance, including hypophosphatemia (plasma P_i levels $<$ 0.8 mmol l^{-1}) and hyperphosphatemia (plasma P_i levels $>$1.4 mmol l^{-1}). Hyperphosphatemia is closely associated with chronic kidney disease (CKD) in later stages of the disease and cardiovascular diseases [2].

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

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KEY POINTS

- Pharmacological inhibition of sodium phosphate cotransporter 2a (Npt2a) provides a novel approach to treating hyperphosphatemia in chronic kidney disease.
- Npt2a inhibition results in phosphaturia and thus declines in plasma P_i and PTH levels irrespective of kidney function.
- In addition to phosphaturic and plasma P_i-lowering effects, Npt2a inhibition exhibits pleiotropic ''off-target'' effects, including natriuresis (regardless of the presence of Npt2a), chloruresis, and calciuresis.
- Npt2a inhibition shows positive effects on vascular calcification and holds promise for other cardiovascular benefits, which warrants future studies.

a review, see [3,4▪,5]). The estimated relative contribution of Npt2a for renal P_i 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 P_i excretion and decreased plasma P_i concentration [7▪▪]. The contribution of Npt2c shows species differences and depends on the development stage, with more contribution to P_i 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 P_i homeostasis [11▪], and Npt2a/c double knockout mice demonstrate a similar effect on urinary P_i excretion compared with $Npt2a^{-/-}$ mice [12], highlighting the small contribution of Npt2c for renal P_i reabsorption. Similarly, both Pit1 and Pit2 appear to have a small contribution to renal P_i 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 P_i [15] and further studies are needed to address the importance of this finding.

FGF23 and PTH are primary regulators for systemic P_i homeostasis, and both reduce renal P_i reabsorption via retrieval of Npt2a/c from the luminal membrane [16]. Additionally, FGF23 downregulates vitamin D, leading to reduced P_i absorption from the intestine, whereas PTH exerts the opposite effects: increasing vitamin D and intestinal P_i absorption [17]. On the other hand, plasma Ca^{2+} and P_i 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 P_i at the transcription, translation, and posttranslational levels [19]. Elevated plasma P_i 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 P_i [21▪], implying that FGF23 is the principal P_i 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 P_i homeostasis in CKD animal models [22,23]. High plasma FGF23 and P_i 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 P_i 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^+/P_i 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 P_i homeostasis. Currently available treatment options include dietary P_i restriction, oral P_i binders, as well as niacin/nicotinamide [31–33]; however, all show severe limitations. Dietary P_i restriction and oral P_i

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 ESO789 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▪,44▪,45]. 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^+-dependent $^{32}P_i$ uptake (half maximal inhibitory concentration, $IC_{50} \sim 1$ mmol L^{-1}) by PF-06869206, with a maximum inhibitory effect of ~70% at 100 mmol L^{-1} [7▪▪]. In wild type (WT) (C57Bl/6J) 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%), $GALNT3^{-/-}$ (−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 [53]. 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 P_i 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 P_i (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 P_i (~2.5-fold) and lower plasma P_i (−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.

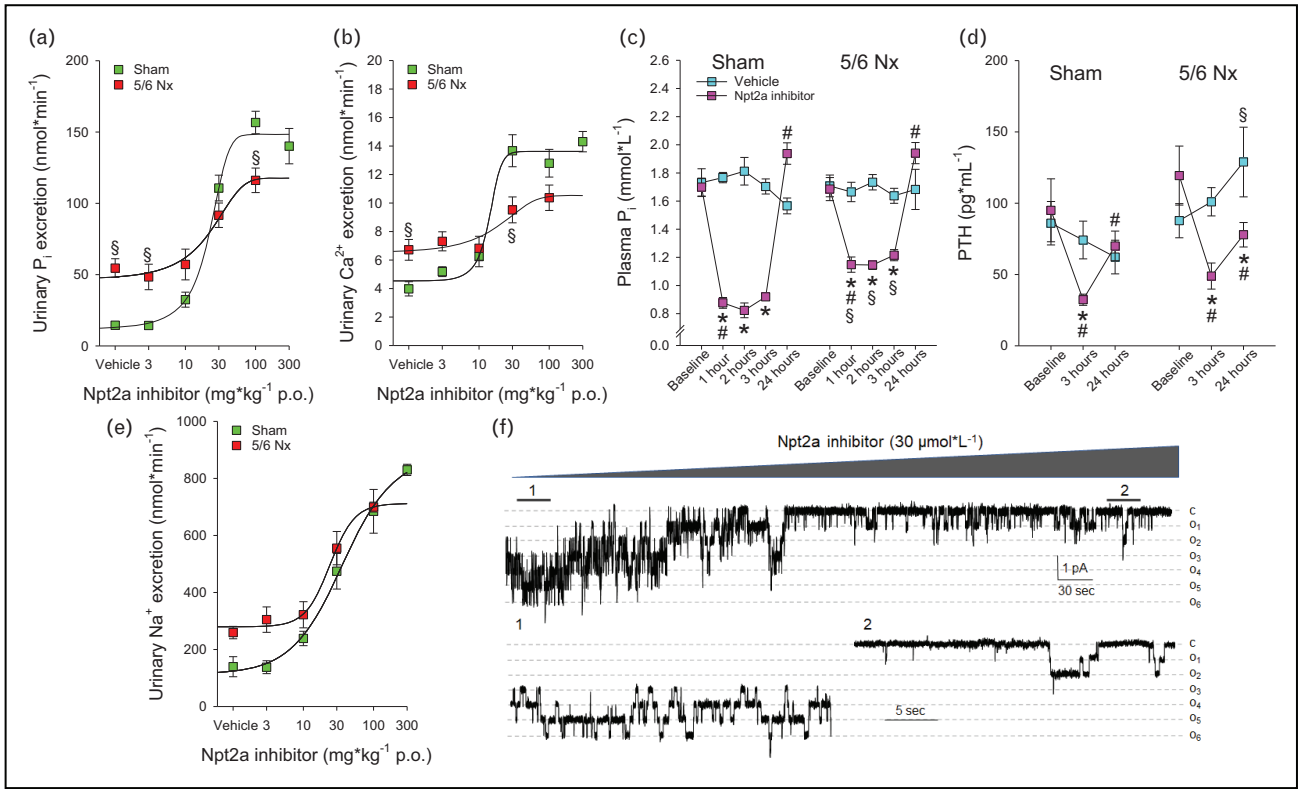

FIGURE 1. Inhibition of Npt2a with PF-06869206 affects urine and plasma parameters in mice with normal and reduced kidney function (5/6 Nx). An acute inhibition (3 h) of Npt2a with PF-06869206 resulted in a dose-dependent increase in urinary P_i (a) and Ca^{2+} (b) excretion in both sham and 5/6 Nx mice. 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, $^{\ddagger}P < 0.05$ 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 calciuria 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▪▪].

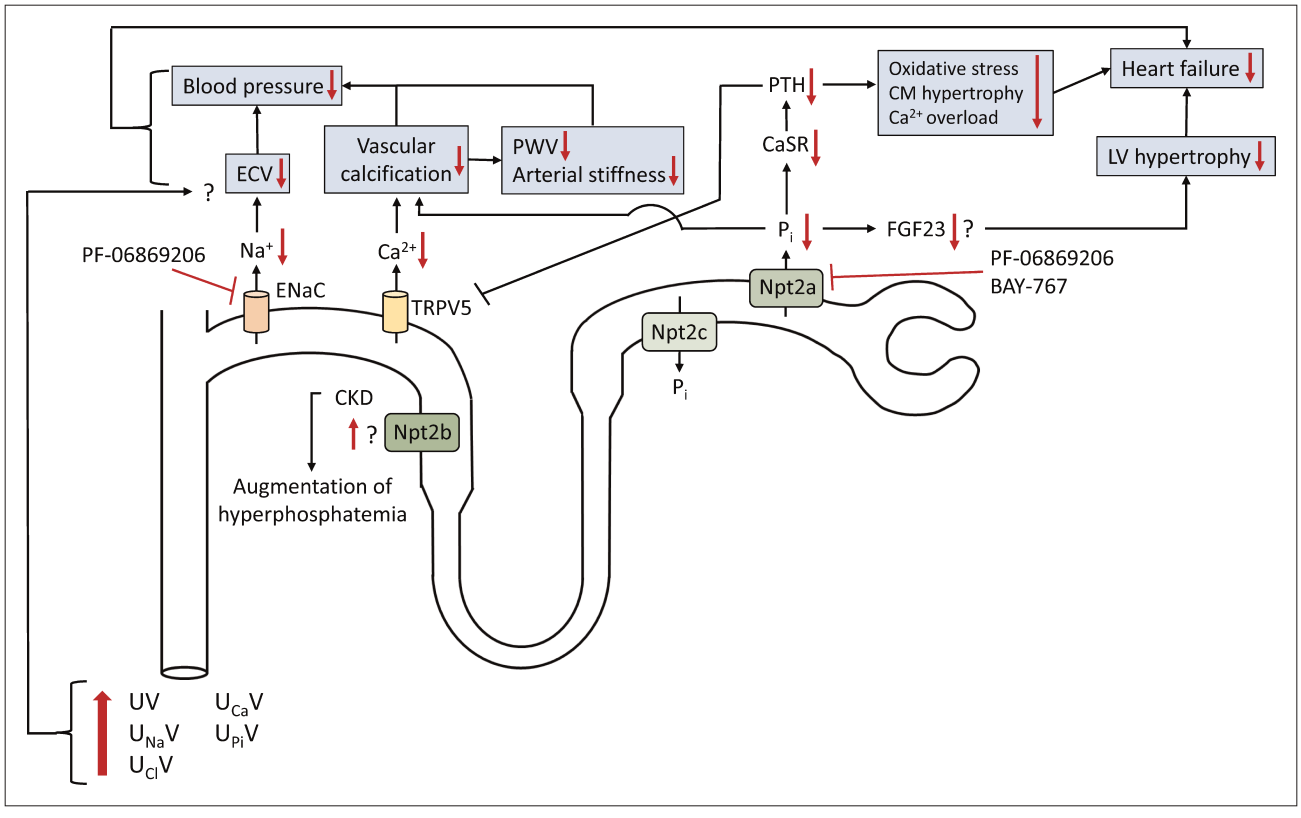

FIGURE 2. Overall effects of Npt2a inhibition on renal excretion of minerals/electrolytes and the proposed cardioprotection. Npt2a inhibition by either PF-06869206 or BAY-767 enhances the urinary P_i excretion, resulting in reduced plasma P_i and PTH levels. The PTH secretion is regulated by calcium-sensing 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

Calciuria is one of the critical pleiotropic effects caused by PF-06869206 and BAY-767. The imbalance of hormones and minerals (P_i and Ca^{2+}) 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 P_i levels (~1.4-fold) and aortic calcium content (~75%). In contrast, the oral P_i binder lanthanum carbonate (2.2%, administered via diet) did not reduce aortic Ca^{2+} content [50▪▪].

CONCLUSION

Npt2a inhibitors reduce renal P_i 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 Ca^{2+} 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 P_i 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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