HYPERTENSION

Intestinal Inhibition of the Na^+/H^+ Exchanger 3 Prevents Cardiorenal Damage in Rats and Inhibits Na^+ Uptake in Humans

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The management of sodium intake is clinically important in many disease states including heart failure, kidney disease, and hypertension. Tenapanor is an inhibitor of the sodium-proton (Na^+/H^+) exchanger NHE3, which plays a prominent role in sodium handling in the gastrointestinal tract and kidney. When administered orally to rats, tenapanor acted exclusively in the gastrointestinal tract to inhibit sodium uptake. We showed that the systemic availability of tenapanor was negligible through plasma pharmacokinetic studies, as well as autoradiography and mass balance studies performed with ^{14}C-tenapanor. In humans, tenapanor reduced urinary sodium excretion by 20 to 50 mmol/day and led to an increase of similar magnitude in stool sodium. In salt-fed nephrectomized rats exhibiting hypervolemia, cardiac hypertrophy, and arterial stiffening, tenapanor reduced extracellular fluid volume, left ventricular hypertrophy, albuminuria, and blood pressure in a dose-dependent fashion. We observed these effects whether tenapanor was administered prophylactically or after disease was established. In addition, the combination of tenapanor and the blood pressure medication enalapril improved cardiac diastolic dysfunction and arterial pulse wave velocity relative to enalapril monotherapy in this animal model. Tenapanor prevented increases in glomerular area and urinary KIM-1, a marker of renal injury. The results suggest that therapeutic alteration of sodium transport in the gastrointestinal tract instead of the kidney—the target of current drugs—could lead to improved sodium management in renal disease.

INTRODUCTION

The mammalian gastrointestinal (GI) tract plays an important role in maintaining salt and fluid balance (*1*). Humans reabsorb about 7 to 9 liters of GI fluid daily, a process driven largely by sodium-dependent nutrient cotransporters and sodium reuptake (*2*, *3*). Expressed in the apical regions of enterocytes, electroneutral Na^+/H^+ exchangers such as NHE2, NHE3, and NHE8 transport sodium from the intestinal lumen into enterocytes, whereas the electrogenic sodium channel ENaC acts in the colon (*1*, *4*, *5*). NHE3 (SLC9A3) is particularly important for intestinal sodium transport (*6*–*8*). NHE3 null mice exhibit perturbed sodium-fluid balance, underscoring NHE3's role in preserving volume homeostasis (*9*, *10*). Although apical NHEs can compensate for one another (*11*, *12*), defects in intestinal sodium absorption are most severe in NHE3 null mice (*10*–*13*), indicating that NHE3 makes the major contribution to intestinal sodium uptake (*14*).

Sodium-fluid imbalances can underlie clinical sequelae in disease states such as hypertension, heart failure (HF), end-stage renal disease (ESRD), and chronic kidney disease (CKD). Clinical guidelines for HF and ESRD recommend restricting dietary sodium and point to noncompliance with such restrictions as a common factor in rehospitalization, fluid overload, and other adverse outcomes (*15*–*20*). In ESRD, large shifts in volume during dialysis can cause myocardial stunning and intradialytic hypotension and potentially increased left ventricular mass (*21*–*24*); tools that allow for more effective management of sodium and volume in these patients are therefore desired. Recent studies suggest that sodium can directly contribute to disease pathogenesis through the vascular endothelial growth factor C pathway, autoimmune activation via pathogenic T helper 17 (T_H17) cells, and Rac1/mineralocorticoid receptor signaling (*25*–*28*). In CKD and preclinical models thereof, excess dietary sodium exacerbates hypertension and accelerates cardiac and renal dysfunction (*29*–*32*).

Available methods for managing sodium and fluid balance are inadequate for many patients. Patients in recent large kidney trials ingested 9 to 11 g of salt per day, well above the recommended 5 to 6 g (*18*). Moreover, in HF patients, the cornerstone treatment of fluid overload (diuretics) becomes rapidly inefficient as patients develop resistance to diuretics and/or develop concomitant renal failure. High-dose loop diuretics in congestive HF patients treated for acute symptoms produce hypotonic urine with a modest amount of sodium and high levels of potassium. Because volume overload is driven by extracellular sodium, the therapeutic value of loop diuretics with their modest natriuretic effect and urinary potassium loss can be short term (*15*, *33*, *34*).

Here, in both rodents and humans, we describe the pharmacokinetic (PK) and pharmacological properties of tenapanor, an inhibitor of NHE3 activity designed to act locally in the intestine to reduce sodium uptake.

RESULTS

Inhibitory properties and PK of tenapanor

The ability of tenapanor to inhibit sodium channels relevant to GI Na^+ uptake was evaluated in cell-based assays of intracellular pH recovery (pH_i recovery) that measured Na^+/H^+ exchange. Tenapanor exhibited IC_{50} (half-maximal inhibitory concentration) values of 5 and 10 nM

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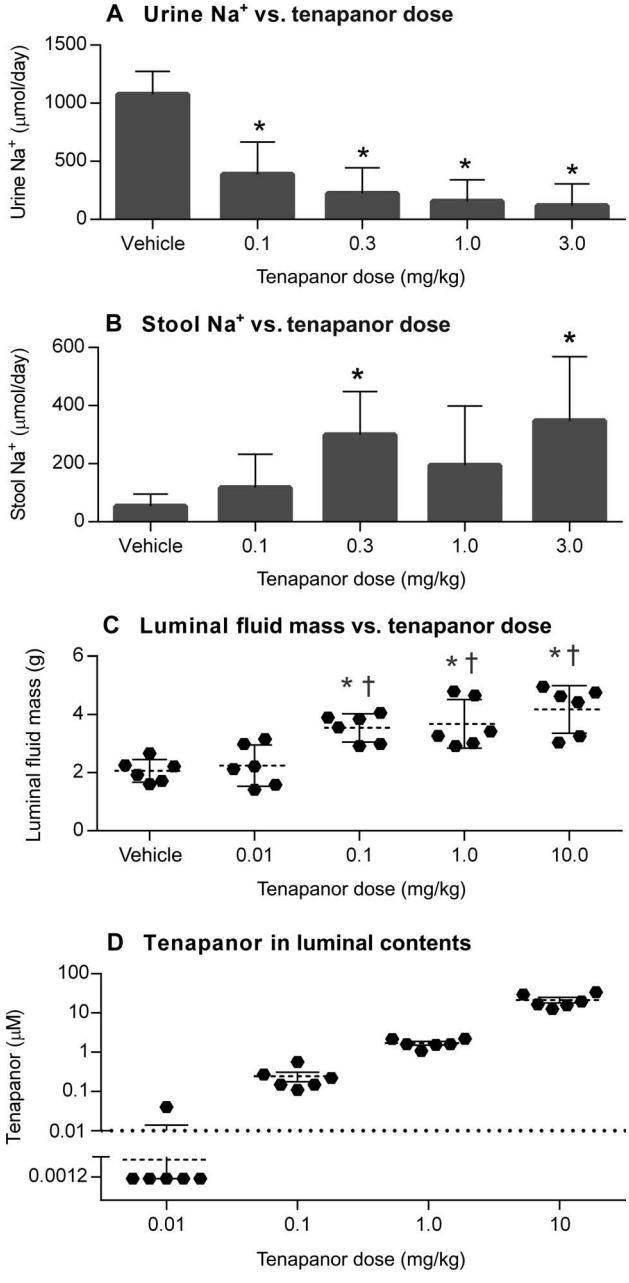

Fig. 1. Effects of single doses of tenapanor on Na^+ in rats. (**A** and **B**) In single-dose studies of Sprague-Dawley rats, sodium was determined in urine (A) and stool (B). (**C** and **D**) The mass of luminal fluid (C) and tenapanor concentrations therein (D) were measured in luminal contents of the ileum collected 1 hour after administration of tenapanor at doses ranging from 0.1 to 10 mg/kg. *P < 0.05 from vehicle; †P < 0.05 from 0.01 mg/kg. Data are means ± SEM (n = 6 rats per group).

against human and rat NHE3, respectively (table S1). Human intestinal transporters NHE1 (SLC9A1), NHE2 (SLC9A2), TGR5 (GPBAR1), ASBT (SLC10A2), and Pit-1 (SLC20A1) and the sodium-dependent phosphate transporter NaPiIIb (SLC34A2) were not inhibited by tenapanor at concentrations up to 10 to 30 μM (table S1).

When orally administered to rats, dogs, or humans in standard PK studies, tenapanor exhibited minimal systemic exposure. Average plasma C_{max} values of tenapanor in rats and humans were <1 ng/ml with negligible area under the curve at doses of up to 30 mg/kg in rats, 10 mg/kg in dogs, and 900 mg in humans (table S2). This result was consistent with tenapanor's limited permeability across cell monolayers (table S3) and was confirmed in mass balance and quantitative whole-body autoradiography (QWBA) studies of ^{14}C-tenapanor in rats (tables S4 to S6 and fig. S1). In the latter studies, all administered radioactivity was recovered from rats within 24 hours of dosing. More than 99% of the collected radioactivity was present in feces as unchanged ^{14}C-tenapanor. Negligible (0.34 ± 0.1%) radioactivity was recovered in urine. In bile duct–cannulated rats, radioactivity representing 98.2 ± 1.8% and 2.3 ± 0.2% of recovered material was found in feces and bile, respectively. The radioactive material recovered from bile did not coelute with tenapanor upon high-performance liquid chromatography analysis, likely reflecting the absorption of impurities associated with the radiosynthesis or metabolites thereof. In humans, quantifiable levels of tenapanor were observed in only 13 of 895 (1.45%) plasma samples from 50 subjects. In all cases, plasma tenapanor concentrations were ≤1.4 ng/ml (table S2). Tenapanor was highly protein-bound (>98%), and the maximum observed free drug concentration in human plasma (<0.015 nM) was well below its in vitro potency of 5 nM (table S2).

In vivo effects of tenapanor in normal rats and healthy human volunteers

Upon administering single doses of tenapanor to rats, we observed dose-dependent reductions in urinary sodium and increases in fecal sodium and luminal fluid mass, and these values were associated with increasing tenapanor concentration in the intestinal lumen (Fig. 1). Stool form score increased with tenapanor dose from a baseline of 1.2 to 2.2 (0.3 mg/kg), 3.3 (1 mg/kg), and 4.5 (3 mg/kg) (fig. S2). Qualitatively similar effects on urinary and fecal sodium were observed in fasted rats fed a sodium-deficient test meal (fig. S2), suggesting that tenapanor can affect sodium reabsorption from endogenous intestinal secretions. The acute negative sodium balance observed in the absence of exogenous sodium diminished during prolonged feeding of a sodium-free diet with tenapanor administration (fig. S2), as expected under these conditions (*35*, *36*).

Chronic administration of tenapanor to rats fed with standard chow (0.49% NaCl) caused a sustained reduction of urinary sodium and increase in fecal sodium, both of which normalized upon tenapanor withdrawal (Fig. 2). Urinary and fecal potassium were transiently affected (Fig. 2, C and D). No significant trends were observed in urine NH_4^+ or urine Cl^- (Fig. 2, E and F). The changes in urinary sodium and potassium suggest that there may be another anion(s) that one would expect to be exchanged to maintain electric neutrality across the membrane; such exchange was not characterized in this work. Serum bicarbonate was unaffected by tenapanor administration (23.5 to 26.3 mEq/liter), indicating that the animals' acid-base chemistry was not substantially affected by the treatment. Repeated administration of tenapanor at 1, 3, or 10 mg/kg per day increased day 3 to 4 plasma aldosterone concentrations from a baseline of <0.1 ng/ml to 0.6 ± 0.4, 1.1 ± 0.3, and 2.3 ± 0.6 ng/ml (±SEM), respectively, and returned to baseline upon withdrawal. Taken in sum, these data from normal rats support a mechanism of action in which tenapanor acts to reduce intestinal sodium uptake.

In a phase 1 multiple ascending dose study in healthy human subjects, administration of 15 to 60 mg of tenapanor twice daily produced

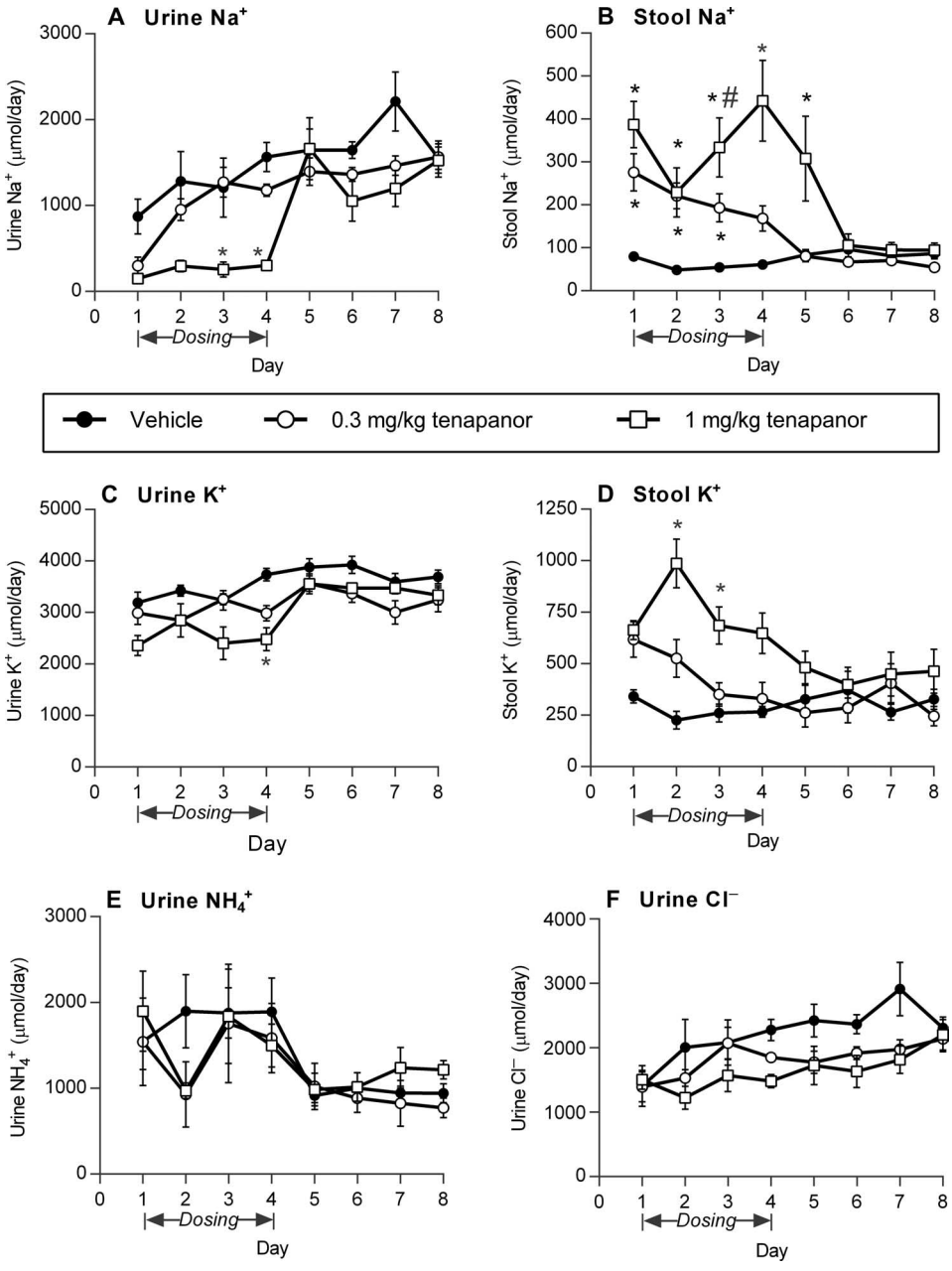

Fig. 2. Effect of 4-day repeat dosing of tenapanor on fecal and urine electrolytes in rats. (**A** to **F**) Two doses of tenapanor (0.3 and 1.0 mg/kg) were administered for 4 days, and the sodium in urine (A) and feces (B) along with urinary (C) and fecal (D) potassium, urine ammonium (E), and urine chloride (F) were determined. *$P < 0.05$ from vehicle; #$P < 0.05$ from 0.3 mg/kg. Data are means ± SEM ($n = 6$ rats per group).

an increase in fecal sodium of 20 to 50 mmol/day and decreases of similar magnitude in urinary sodium (Fig. 3). A dose-dependent effect of tenapanor on stool sodium content was demonstrated in studies in which 30 mg of tenapanor was administered once, twice, or three times daily (Fig. 4). A similar relationship between tenapanor dose and stool sodium was observed when subjects were administered various doses once daily (Fig. 4). A significant correlation was observed between the reduction in urinary sodium and the increase in stool sodium ($r^2 = 0.91$, $P = 0.003$; Fig. 4). Tenapanor also produced an increase in Bristol Stool Form Scale scores (fig. S3).

Neither urinary nor fecal potassium levels were affected by tenapanor administration in humans (Fig. 3). Similarly, no changes in serum bicarbonate, serum chloride, urinary calcium, or urine pH were observed in 7-day repeat-dose studies of tenapanor (figs. S3 and S4). Tenapanor was well tolerated, with no drug-related serious adverse events reported. All adverse events were mild or moderate in severity.

Effects of tenapanor in a rat model of CKD with hypervolemia

Tenapanor was evaluated in a 5/6th nephrectomized (NPX) rat model of sodium-driven volume expansion and hypertension. Vehicle-treated animals fed a high-salt diet (4% NaCl) exhibited an increase in the ratio of extracellular fluid volume (ECFV) to total body water (Fig. 5A). Initiation of tenapanor administration concurrently with introduction of the high-salt diet (prophylactic treatment) prevented volume expansion in a dose-dependent manner (Fig. 5A). Initiation of tenapanor administration 2 weeks after introduction of the high-salt diet (interventional treatment) reversed ECFV expansion (Fig. 5A).

Salt-fed NPX rats recapitulated the elevated blood pressure, albuminuria, cardiac and renal hypertrophy associated with human CKD, and hypervolemia; administration of tenapanor reduced these adverse outcomes in a dose-dependent manner. In vehicle-treated animals, salt feeding increased systolic blood pressure (SBP) by ~60 mmHg and diastolic blood pressure (DBP) by ~50 mmHg. Prophylactic tenapanor administration reduced the SBP increase by 31 to 92% ($P \leq 0.05$) (Fig. 5B) and the DBP increase by 38 to 91% ($P \leq 0.05$). Interventional treatment with tenapanor (3 mg/kg) reduced elevated blood pressure to values similar to those seen in the prophylactic group (3 mg/kg). Prophylactic tenapanor treatment attenuated the 20-fold increase in albuminuria seen in control animals by 45 to 88% ($P \leq 0.05$), whereas interventional tenapanor treatment reduced albuminuria by 71% ($P \leq 0.05$) (Fig. 5C). Both prophylactic and interventional tenapanor administration diminished left ventricular hypertrophy (LVH) relative to vehicle controls (Fig. 5). Urinary sodium and chloride excretion trended lower in tenapanor-treated NPX animals and rose over time (Fig. 5), whereas potassium was not substantially affected (not shown). The increases in urinary salt excretion and diuresis were consistent with a pressure natriuresis response to arterial

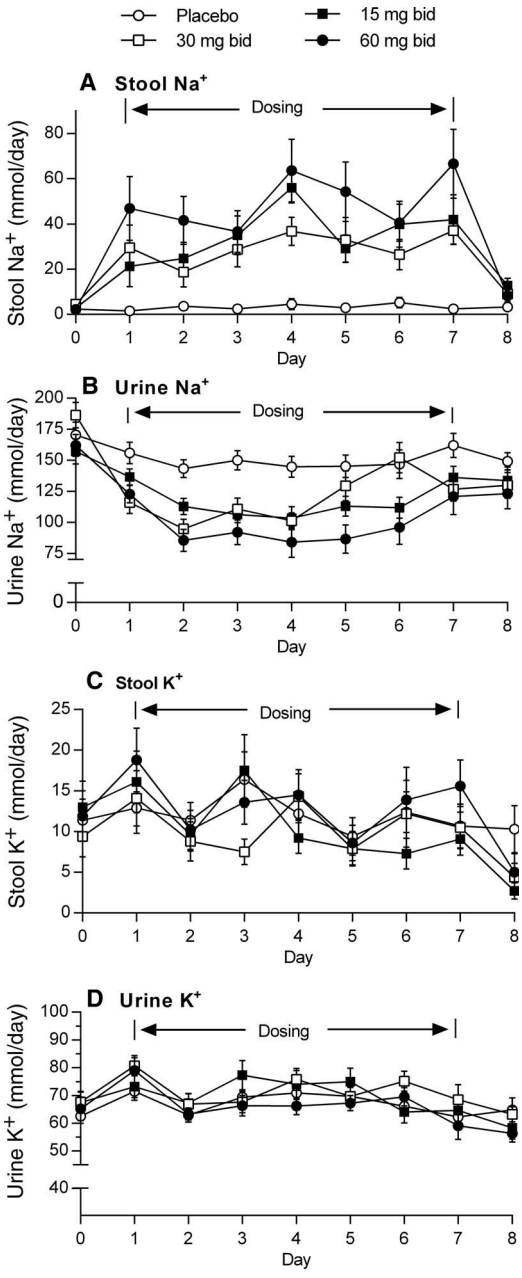

Fig. 3. Effect of 7-day administration and withdrawal of tenapanor on fecal and urinary electrolytes in healthy human volunteers. Tenapanor was administered twice daily to healthy subjects ($n = 12$ per group, $n = 15$ for placebo) at strengths of 15, 30, or 60 mg. (**A** to **D**) Sodium and potassium were determined in feces (A and C) and urine (B and D). Baseline values (day 0) are expressed as the average of the 4 days of collections (day −4 to day −1) before initiation of treatment with tenapanor or placebo. Data are means ± SEM.

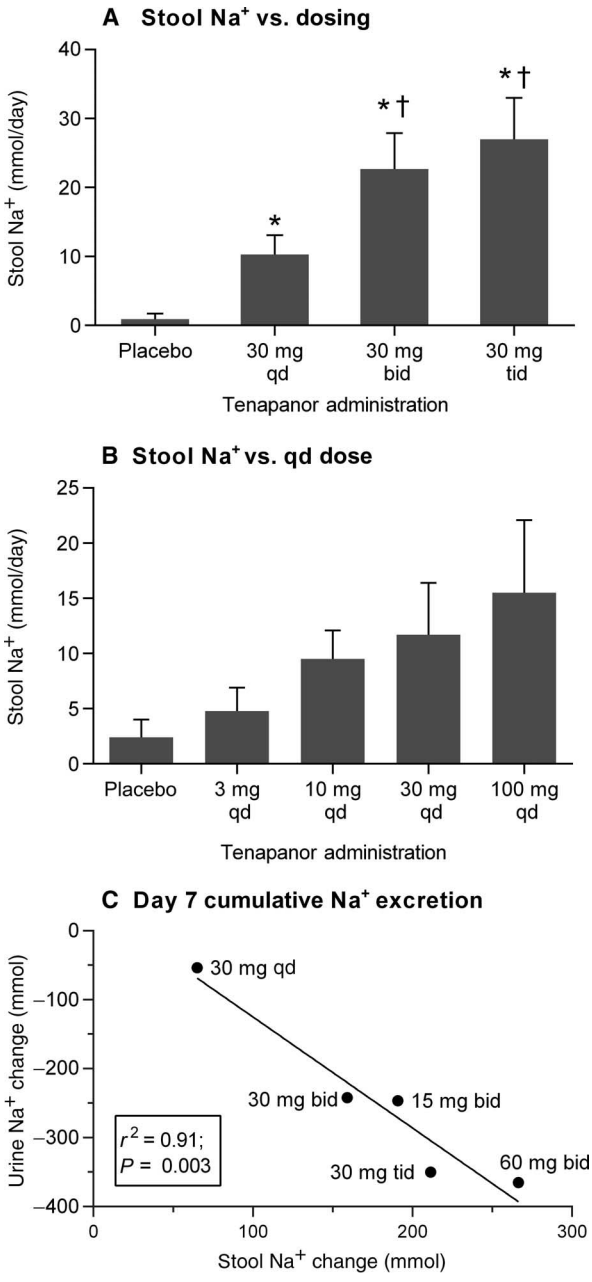

Fig. 4. Effect of a single dose of tenapanor on fecal and urinary electrolytes in healthy human volunteers. (**A**) Tenapanor (30 mg) was given to healthy subjects once (qd) ($n = 6$), twice (bid) ($n = 10$), or three times (tid) ($n = 10$) daily for 7 days, and average daily fecal sodium was determined ($n = 10$ for placebo). (**B**) Once daily doses of tenapanor of increasing strength (3 to 100 mg) were given to healthy volunteers ($n = 6$ per group, $n = 10$ for placebo), and average daily fecal sodium was determined. (**C**) Correlation between stool and urine sodium content in response to tenapanor in healthy subjects. *$P < 0.05$ versus placebo, †$P < 0.05$ versus 30 mg once daily. Data are means ± SEM.

hypertension (*29*). Although rats appear to be more sensitive to tenapanor than humans (see Discussion), particularly on a high-salt diet, serum bicarbonate levels were lower in animals given tenapanor (3 mg/kg), suggesting that acid-base balance can be affected in NPX rats at this supratherapeutic dose (fig. S5).

Tenapanor combined with angiotensin-converting enzyme inhibition in salt-fed, NPX rats

Angiotensin-converting enzyme (ACE) inhibitors are widely used in the treatment of hypertension and HF. The potential utility of coadministration

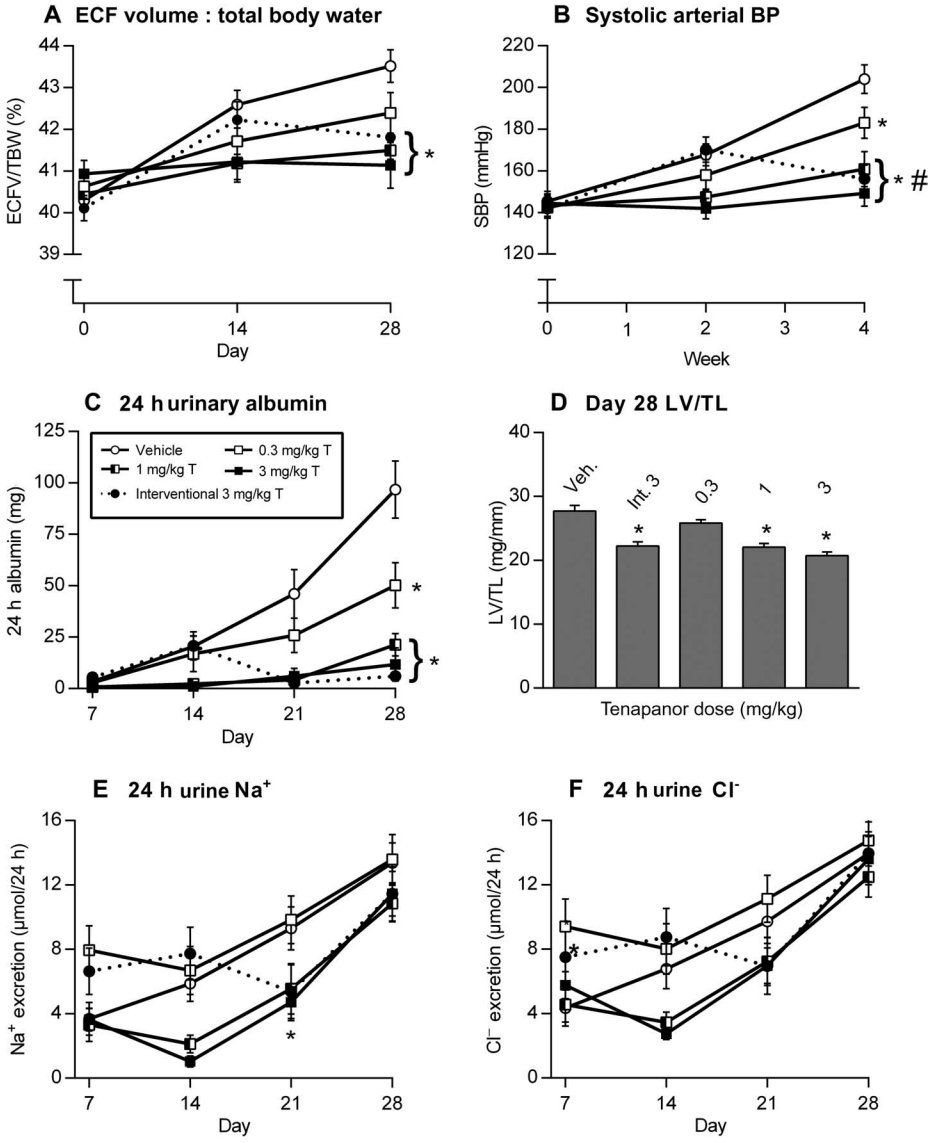

Fig. 5. Effects of tenapanor in salt-fed NPX rats. (**A** to **F**) Tenapanor was administered to a salt-fed rat model of renal insufficiency (5/6th nephrectomy) either preventively from study inception or interventionally beginning at week 2 (dotted lines; see methods), and its effects on (A) ECFV, (B) SBP, (C) albuminuria, (D) endpoint cardiac hypertrophy as measured by LVH relative to tibia length (LV/TL), and (E) urinary sodium and (F) urinary chloride excretion were determined. Significance by one-way analysis of variance (ANOVA): *$P < 0.05$ versus vehicle. #$P < 0.05$ versus 0.3 mg/kg tenapanor group. Data are means ± SEM ($n = 12$ rats per group).

of tenapanor with an ACE inhibitor was evaluated in studies of tenapanor and enalapril (hereafter, "combination") in salt-fed (4% NaCl) NPX rats, which exhibited ~35 to 50 mmHg increases in SBP (Fig. 6). Prophylactic tenapanor administration prevented this increase, maintaining SBP values that were indistinguishable from non-NPX naïve controls, which consumed standard chow (0.49% NaCl; "naïve controls") (Fig. 6). Enalapril prevented 45% ($P \leq 0.05$) of the increase in SBP observed in vehicle controls (Fig. 6), in agreement with previous studies by others (*37*). The increase in albuminuria observed in vehicle-treated NPX animals relative to naïve controls was reduced 83% ($P \leq 0.05$) by tenapanor and 93% ($P \leq 0.05$) by the combination (Fig. 6). The extent of albuminuria in tenapanor-treated animals was similar to that in non-NPX, naïve controls (Fig. 6B). The extent of LVH observed in NPX animals compared to naïve controls was reduced ~67 to 75% ($P \leq 0.05$) in groups receiving tenapanor or the combination (Fig. 6C). Enalapril alone did not reduce LVH despite its antihypertensive activity. Remnant kidney weights in tenapanor-treated groups were lower than those in untreated NPX animals (Fig. 6D). The combination provided further protection against cardiac and renal hypertrophy when compared to the enalapril group.

Increases in concentrations of the type I membrane protein urinary KIM-1, which correlate with kidney damage (*38*, *39*), were prevented in tenapanor-treated rats (Fig. 7A). Glomerular size expanded 67% ($P \leq 0.05$) in salt-fed NPX animals relative to naïve controls, an increase that was inhibited by 83% ($P \leq 0.05$), 37% ($P \leq 0.05$), and 90% ($P \leq 0.05$) in groups treated with tenapanor, enalapril, or the combination, respectively (Fig. 7C). Total collagen staining in remnant kidneys varied, with the combination group exhibiting higher values than treatment with enalapril alone (Fig. 7D). Reduced glomerular size, but not total collagen, was associated with reductions in remnant kidney weight, LVH, KIM-1, creatinine clearance, and the extent of albuminuria observed in NPX groups (Figs. 6 and 7).

As measured by trans-mitral E/A—the ratio between early and late ventricular filling velocity for which a reduction correlates with diastolic dysfunction—the deterioration of diastolic function observed in vehicle-treated NPX rats relative to naïve controls was improved 33% ($P \leq 0.05$) in the combination group (Fig. 7E). Pulse wave velocity (PWV) increased 44% ($P \leq 0.05$) in vehicle-treated NPX rats relative to naïve controls; the combination prevented 81% ($P \leq 0.05$) of this increase (Fig. 7B). Clinical chemistry data were in the normal range for NPX animals and similar to vehicle controls (fig. S5).

DISCUSSION

Sodium has a well-established role in the etiology of renal and cardiovascular disease and in activating the RAAS (*40*, *41*), and the Na^+/H^+ antiporter NHE3 is central in facilitating GI absorption of sodium. We therefore undertook the design, synthesis, and evaluation of tenapanor, an inhibitor of NHE3 that has minimal access to the systemic system because its action is largely confined to the GI tract. The present report describes the effects of tenapanor in normal rats, in healthy human volunteers, as monotherapy, and, in combination with enalapril, in a salt-fed NPX rat model of CKD.

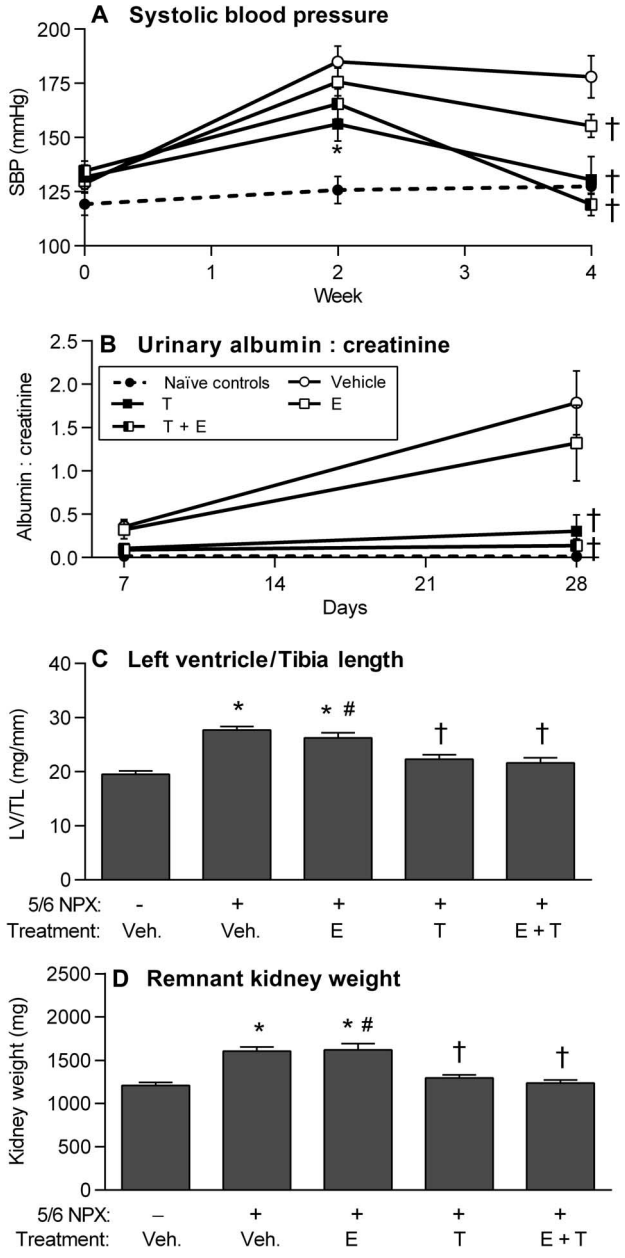

Fig. 6. Effect of tenapanor and enalapril on blood pressure, albuminuria, and organ hypertrophy in NPX rats. Tenapanor (1 mg/kg), enalapril (10 mg/kg), or the combination (tenapanor + enalapril) were administered in a salt-fed (4% NaCl) model of renal insufficiency (5/6th nephrectomy). (**A** to **D**) In treated groups, vehicle controls, or non-NPX (naïve) controls on a normal salt diet (0.49% NaCl), the effects on (A) SBP, (B) albuminuria, (C) endpoint cardiac hypertrophy as measured by LVH relative to tibia length (LV/TL), and (D) renal hypertrophy were determined. Significance by one-way ANOVA: *$P < 0.05$ versus naïve controls; †$P < 0.05$ versus NPX vehicle controls; ‡$P < 0.05$ versus NPX + enalapril; #$P < 0.05$ versus NPX + enalapril + tenapanor. Data are means ± SEM ($n = 12$ rats per group).

PK, autoradiography, and radiolabel recovery experiments confirm the very low systemic availability of tenapanor in rats and humans predicted by in vitro permeability experiments. Mass balance and quantitative whole-body radiography studies in rats with ^{14}C-tenapanor confirmed that the drug is restricted to the GI tract with minimal absorption or metabolism. These results are consistent with the results of PK experiments conducted in rodents, dogs, and humans in which tenapanor exhibits properties of a nonabsorbed drug (*42*). The major route of tenapanor clearance in rodents is via feces, and it exhibits similar PK properties in normal and NPX rats (table S2).

In normal rats, the pharmacological effects of tenapanor include reduced urinary sodium and increased fecal sodium, activities consistent with reduced intestinal sodium absorption and increased sodium reabsorption in the kidney (*43*). These effects in rats were dose-dependent, reversible, and maintained during repeat dosing (including 90-day studies). Effects on stool and urine Na^+ levels varied with the concentration of tenapanor in the intestinal lumen, providing further support for a GI site of action. Other phenotypic effects of tenapanor administration to normal rats include loosened stool form, increased luminal fluid, and higher serum aldosterone. Salt-fed rats were protected from hypertension by tenapanor. These observations are consistent with the phenotype of NHE3 null mice (*10*) and the activity of another intestinal NHE3 inhibitor (*44*) published after our initial reports (*45*–*48*). Tenapanor induced acute but not chronic negative sodium balance in rats fed sodium-deficient chow, suggesting that it inhibits reabsorption of sodium from intestinal secretions until other sodium reclaiming mechanisms (for example, ENaC) are activated (*35*, *36*).

In healthy human volunteers, the effects of tenapanor on stool and urine sodium are qualitatively similar to those in rats but quantitatively smaller. Urinary sodium reductions in man were ~50% compared to ≥90% in rats at the doses tested. In healthy volunteers, tenapanor diverted 20 to 50 mmol of sodium per day (up to ~3 g of dietary salt)—quantities we predict will provide a significant clinical benefit to patients with CKD, HF, or ESRD.

Increased plasma aldosterone associated with tenapanor dosing provides a hormonal link—perhaps indirect—between drug administration and reduced sodium uptake, and is consistent with studies correlating sodium intake with RAAS activity (*41*, *49*). Tenapanor exhibited protective effects on renal function in salt-fed NPX rats, including the prevention and reversal of increasing ECFV. The prevention of albuminuria by tenapanor along with reduced urinary KIM-1 and remnant kidney hypertrophy reflect reduced kidney damage in tenapanor-treated animals. Tenapanor also prevented increases in glomerular size, an important predictor of CKD progression (*50*–*52*). Most important, we observed the benefits of tenapanor administration in this model with both interventional and prophylactic treatment. Many renal endpoints in the combination study of tenapanor and the ACE inhibitor enalapril in salt-fed (4% NaCl) rats—blood pressure, albuminuria, urinary KIM-1, and glomerular area, for example—were improved to values similar to those in naïve control animals consuming standard chow (0.49% NaCl). Given its mechanism of action, we speculate that tenapanor has the potential to mitigate direct pathogenic effects of sodium (*25*–*28*).

In CKD patients with hypertension, LVH is common and correlates strongly with morbidity and mortality (*53*, *54*). Tenapanor provided dose-dependent reductions in LVH in NPX rats, which correlated with the compound's antihypertensive activity. Because tenapanor acts in the intestine, we reasoned that cardiac and renal protective effects of the drug in NPX rats may be additive with those provided by systemic enalapril. Indeed, the combination of tenapanor and enalapril improved measures of cardiac and vascular function, E/A ratio, and PWV. Increased PWV is an independent predictor of cardiovascular risk in hypertensive patients and is associated with mortality in ESRD and CKD

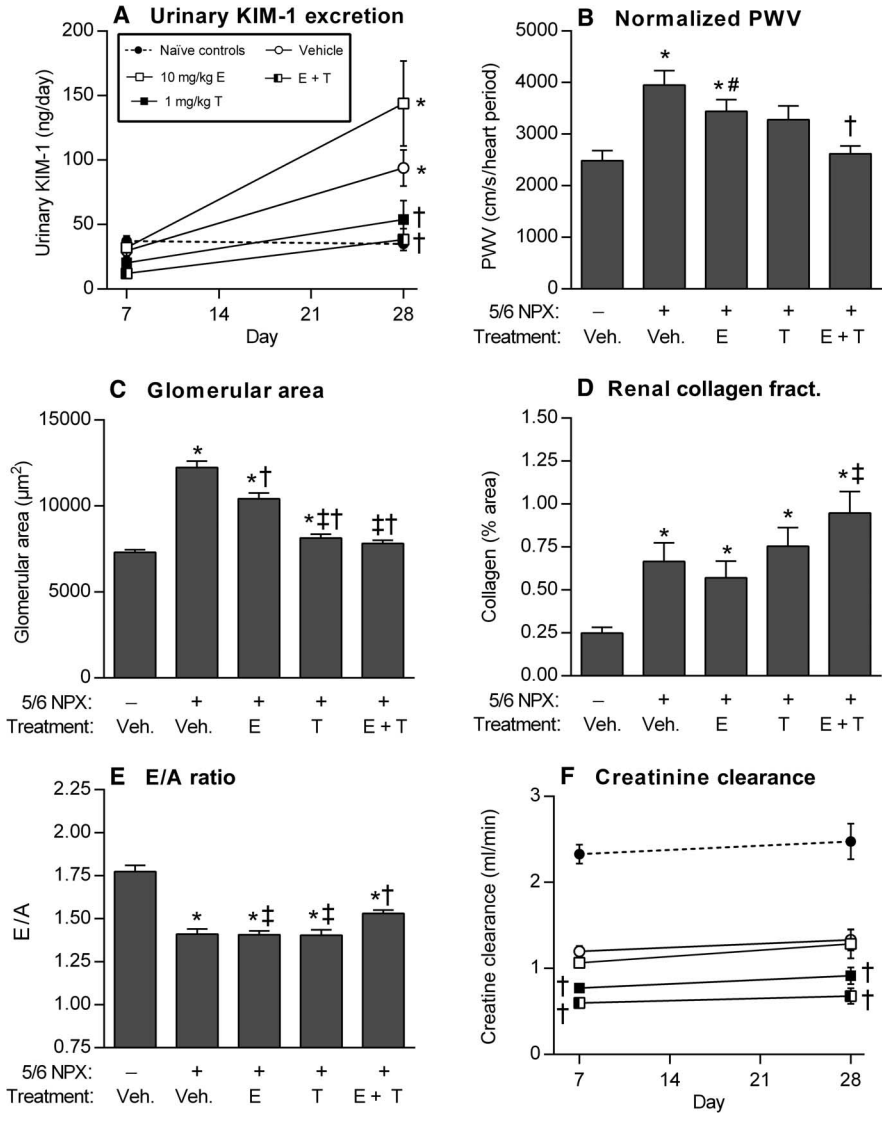

Fig. 7. Effect of tenapanor and enalapril on cardiac and renal outputs in NPX rats. Tenapanor (1 mg/kg), enalapril (10 mg/kg), or the combination (tenapanor + enalapril) were administered in a salt-fed (4% NaCl) model of renal insufficiency (5/6th nephrectomy). (**A** to **D**) Urinary KIM-1 was determined (A) along with PWV (B), glomerular area (C), and collagen fraction (D). (**E** and **F**) Endpoint cardiac E/A ratio (E) and creatinine clearance (F) were also determined. Significance by one-way ANOVA: *$P < 0.05$ versus naïve controls; †$P < 0.05$ versus NPX vehicle controls; ‡$P < 0.05$ versus NPX + enalapril; #$P < 0.05$ versus NPX + enalapril + tenapanor. Data are means ± SEM ($n = 12$ rats per group).

(*55*–*57*). That the combination of enalapril and tenapanor—but neither agent alone—normalized PWV highlights the potential for combining such agents to mitigate the effects of angiotensin II and sodium on end-organ damage. It will be of interest to determine whether such functional outcomes translate to patients because small clinical studies have reported both E/A and PWV to be sensitive to salt intake (*58*–*60*).

Mice lacking NHE3 exhibit defective proximal tubule bicarbonate reabsorption but are only mildly acidotic, likely due to compensation by renal NHE2 (*10*, *13*, *61*). In normal rats and healthy human volunteers, defects in acid-base balance are not observed with tenapanor administration. In salt-fed NPX rats, efficacious doses of tenapanor (1 mg/kg) do not chronically affect serum Cl^-, and urinary Cl^- levels mirror the moderate rise in urinary sodium indicative of pressure natriuresis. Serum bicarbonate is reduced in NPX rats only at supratherapeutic doses of tenapanor. This finding is consistent with the elevation of serum chloride observed in NPX animals (fig. S5) and a reduced extrusion of luminal protons, which may diminish the neutralization and reuptake of bicarbonate. Overall, our observations suggest that acid-base balance is not profoundly affected by gut-restricted tenapanor, and data from renal patients will emerge from ongoing clinical studies. Because NHE3 is thought to be coupled to Cl^-/HCO_3^- exchangers like SLC26A3 or A6 (*1*), its inhibition may have a blunted impact on GI bicarbonate losses. The decrease in creatinine clearance observed in tenapanor-treated NPX rats was likely the result of reduced hyperfiltration relative to vehicle controls, which is corroborated by the enlarged glomeruli observed in the latter group. In addition, significant reductions in volume and removal of salt and water are known to cause reversible reductions in glomerular filtration rate (*43*, *62*).

Our central finding is that intestinal inhibition of sodium uptake with tenapanor results in modulation of ECFV and cardiorenal protective effects in salt-fed NPX rats. The PK and pharmacological properties of tenapanor translate well to human subjects. Modulating intestinal sodium uptake instead of sodium excretion in the kidney may provide an attractive option to clinicians managing sodium-fluid balance in renal patients. Evaluations of tenapanor's efficacy in patients with chronic sodium overload disease such as CKD and ESRD are ongoing in prospective, randomized, placebo-controlled trials (reference numbers NCT01764854 and NCT01847092). The magnitude of the effect on sodium and the tolerability observed in our clinical studies are promising attributes of tenapanor in the context of treating fluid and salt overload.

MATERIALS AND METHODS

Study design

The studies were designed to test the hypothesis that NHE3 inhibition with tenapanor protected against disease in an animal model of kidney disease, and that the pharmacological and PK properties of tenapanor translated well to humans. Tenapanor was prepared as described in patent WO 2010/078449 assigned to Ardelyx Inc. (publication date 8 July 2010) (*63*). The structure is available at http://www.who.int/medicines/services/inn/en/. Normal or diseased rats (5/6th NPX with salt feeding,

see below) were used to evaluate the pharmacological effects of tenapanor, and electrolyte analyses (for example, ion chromatography), stool form evaluations, clinical chemistry, and functional evaluations of cardiac performance were performed. Phase 1 clinical studies were conducted in healthy volunteers (and so were not designed to meet efficacy endpoints). Clinical and animal studies were randomized and controlled with placebo or vehicle (water) groups, respectively. Clinical studies were double-blinded; rigorous maintenance of blinding in animal studies with tenapanor was precluded because of clear pharmacological effects of tenapanor on stool form in rats. Sample sizes in rat studies were determined through extensive experience in the animal models used. No formal calculations were performed to determine sample size for this study. Clinically, the sample sizes of 8 to 10 subjects per group are typical for phase 1 studies and were expected to provide sufficient numbers of observations to meet the study objectives. All data are representative of multiple experiments. Results from normal rats were repeated at least three times each (compound concentration experiments two times) (Figs. 1 and 2). Human data (Figs. 3 and 4) are from two separate clinical trials that showed similar results for once-daily 30-mg dosing in each trial; the data are similar to subsequently collected data from similarly designed phase 1 clinical trials not reported here. A total of three studies in 5/6th NPX rats were performed (Figs. 5 to 7); different designs were used, but common study endpoints showed consistent results from study to study (for example, blood pressure, albuminuria, urine electrolytes, LVH, and kidney weight).

Determination of tenapanor inhibition of NHE3 and other transporters

Genes encoding rat (SLC9A3, GenBank M85300) and human (SLC9A3, GenBank NM_004174.1) NHE3 were synthesized and expressed transiently in opossum kidney cells (American Type Culture Collection). Cells were loaded with pH-sensitive 2′,7′-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) and subjected to NH_4^+-induced acidification. Ethyl isopropyl amiloride, an inhibitor of NHE1 with minimal activity against NHE3, was used to block endogenous NHE1, whereas sodium-dependent, NHE3-mediated recovery of intracellular pH was monitored by following time-dependent changes in BCECF fluorescence. The concentration dependence of inhibition of pH recovery was fit to a logistic equation to determine IC_{50} values. Similar methods were used to measure the activities of NHE2 and NHE1. The activities of other transporters and receptors were measured with $^{33}PO_4$ uptake (NaPiIIb, Pit-1), adenosine 3′,5′-monophosphate production (TGR5), or [^{3}H]taurocolate uptake (ASBT).

PK–absorption, distribution, metabolism, and excretion studies in rats and dogs

PK studies were performed in 6- to 8-week-old male Sprague-Dawley rats (n = 3) or 5/6th NPX Sprague-Dawley rats (n = 3), which were administered tenapanor (1 to 30 mg/kg) in water by oral gavage. Retro-orbital blood (K_2EDTA) taken 0.5, 1, 2, and 4 hours after treatment was isolated as plasma and stored at −20 to −80°C until analysis by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The in-life phase of PK studies in beagle dogs was performed at Calvert Laboratories. The MDCK (Madin-Darby canine kidney) permeability assay was performed as described (*64*). Radiolabeled ADME (absorption, distribution, metabolism, and excretion) and QWBA studies (see table S4) were performed at Covance Inc. with normal or bile duct–cannulated rats. A metabolically stable ^{14}C radiolabel was incorporated into tenapanor (Ricerca Biosciences). Radiochemical purity of ^{14}C-tenapanor was 96.8%.

In vivo studies of tenapanor activity

Enteropooling (*65*) was used to assess compound effects on fluid in rat ileum. The concentration of tenapanor was determined in the 5000*g* supernatant of intestinal contents. For urinary and fecal sodium assessments, 8-week-old Sprague-Dawley rats (Charles River) were randomized into groups (six rats per sex per group) before oral administration of vehicle or tenapanor (10 ml/kg). After 16 to 24 hours, collected excreta were analyzed for electrolytes by ion chromatography. Fecal form was scored with a rodent stool form scale modeled after the Bristol Stool Form Scale. In some studies, tenapanor was administered on consecutive days and excreta were collected through a washout period to allow assessment of effects during administration and drug withdrawal. In normal rats, tenapanor doses ranged from 0.1 to 10 mg/kg. Higher doses within this range (1 to 10 mg/kg) were used to evaluate aldosterone levels and serum bicarbonate; lower doses (0.1 to 3 mg/kg) were used to evaluate urine electrolytes as well as other electrolytes. In some experiments, we used two cohorts to preclude effects on blood volume as a result of blood collection.

Clinical studies of tenapanor

Single-center (ICON plc), randomized, double-blind, placebo-controlled studies in healthy male and female subjects were performed to evaluate the safety, tolerability, pharmacodynamics, and PKs of tenapanor after single and repeated dosing. The protocol, amendments, and subject informed consent form for this study were reviewed and approved by IntegReview, an appropriately constituted Institutional Review Board. Subjects were screened within 3 weeks before checking into the clinical pharmacology unit (CPU). Each cohort was composed of either 8 (6 tenapanor, 2 placebo) or 15 (12 tenapanor, 3 placebo) subjects who checked into the CPU on day −2 or −5. Each subject received a diet standardized for sodium content while in the CPU. Subjects received once- or twice-daily doses of tenapanor with ~240 ml of non-carbonated water on days 1 to 7. Safety assessments were performed at regular intervals and included clinical and vital signs, laboratory evaluations, electrocardiograms, and adverse event monitoring. Assessment of the pharmacological effects of tenapanor included changes in urinary and stool sodium, timing of bowel movements, and stool frequency and consistency (based on the Bristol Stool Form Scale). Stool sodium was determined by inductively coupled plasma optical emission spectrometry (RTI International), and plasma levels of tenapanor were determined by LC-MS/MS (MicroConstants Inc.).

Studies in 5/6th NPX rats

Two independent studies of tenapanor in 5/6th NPX rats were conducted at Plato BioPharma Inc. Rats (8 to 9 weeks old, 225 to 250 g; Charles River Laboratories) were subjected to uninephrectomy 1 week before subtotal nephrectomy and week-long recovery. After randomization by body weight, serum creatinine (>0.5), and blood urea nitrogen (>35), animals were fed a diet containing 4% NaCl or control chow (0.49% NaCl). Body weight, mortality, and morbidity were evaluated daily. Periodically, 24-hour urine and serum samples were collected. SBP and DBP were measured via indirect tail cuff pressure-volume plethysmography on days 1, 14, and 28. At day 28, cardiac and renal tissues were collected for determination of LVH and remnant kidney

weight normalized to tibia length. Albuminuria and clinical chemistry panels were obtained using standard clinical analyses.

In the first study, rats were dosed with vehicle (water) or tenapanor (0.3, 1, or 3 mg/kg per day; $n = 12$ per dose group) incepting concomitantly with high-salt feeding. Dosing occurred daily just before the feeding period. After 2 weeks on study, 24 rats initially enrolled in the vehicle group were split into serum clinical chemistry and SBP/DBP matched groups ($n = 12$), one of which began treatment with tenapanor (3.0 mg/kg per day). The remaining group remained on vehicle for the remainder of the study. Body fluid compartment volumes were measured serially via bioimpedance spectroscopy at days 0, 14, and 28 (*66*).

In the second study, four groups ($n = 12$) of NPX rats were dosed with vehicle (water), tenapanor (1 mg/kg), enalapril (10 mg/kg), or a combination of tenapanor (1 mg/kg) and enalapril (10 mg/kg) while consuming 4% NaCl. Non-NPX, vehicle-treated, naïve controls ($n = 10$) consumed chow with 0.49% NaCl. After day 28 SBP and DBP measurements, functional physiological evaluations were performed: (i) PWV was determined from direct arterial pressure measurements; (ii) cardiac performance parameters (including E/A) were determined in rats by noninvasive ultrasound under isoflurane anesthesia. The mid-transverse section of remnant kidneys was processed for histological evaluation. Urinary KIM-1 concentration (days 7 and 28) was determined by enzyme-linked immunosorbent assay. Remnant kidney was stained separately with hematoxylin and eosin and picrosirius red. Endpoint collection of clinical chemistry samples after anesthetization and cardiac functional measurements rendered them unsuitable for analysis. Drug levels in plasma samples collected on days 0, 7, 14, 21, and 28 were analyzed by LC-MS/MS.

Statistical analyses

Data were normally distributed, and statistical significance (α level 0.05) was determined in Prism 6 software (GraphPad Software Inc.) with two-way ANOVA tests with Bonferroni's post hoc correction except as noted. Data are expressed as means ± SEM.

SUPPLEMENTARY MATERIALS

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Fig. S1. Summary of mass balance study of ^{14}C-tenapanor.

Fig. S2. Effect of tenapanor on fecal and urinary sodium in rats on a very low sodium diet, and dose-dependent effect of tenapanor on stool form in rats.

Fig. S3. Measurements of serum bicarbonate and chloride, urine pH and urinary calcium, and stool form in humans after 7-day repeated, twice-daily dosing of placebo and 15 to 60 mg of tenapanor.

Fig. S4. Measurements of serum bicarbonate and chloride, urine pH, and urinary calcium in humans after 7-day repeated, once-daily dosing of placebo and 3 to 100 mg of tenapanor.

Fig. S5. Effect of tenapanor, or tenapanor and enalapril, on serum sodium, potassium, chloride, uric acid, glucose, and bicarbonate in NPX rats.

Table S1. Potency of tenapanor activity against NHE1, NHE2, NHE3, NaPillb, TGR5, ASBT, and Pit-1.

Table S2. PK parameters of tenapanor in rats, dogs, and humans.

Table S3. MDCK and PAMPA permeability data of tenapanor.

Table S4. Radiolabeled ADME and QWBA study design.

Table S5. QWBA data for ^{14}C-tenapanor in Long-Evans rats.

Table S6. QWBA data for ^{14}C-tenapanor in Sprague-Dawley rats.

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Supplementary Materials for

Intestinal Inhibition of the Na^+/H^+ Exchanger 3 Prevents Cardiorenal Damage in Rats and Inhibits Na^+ Uptake in Humans

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DOI: 10.1126/scitranslmed.3007790

The PDF file includes:

Figure S1

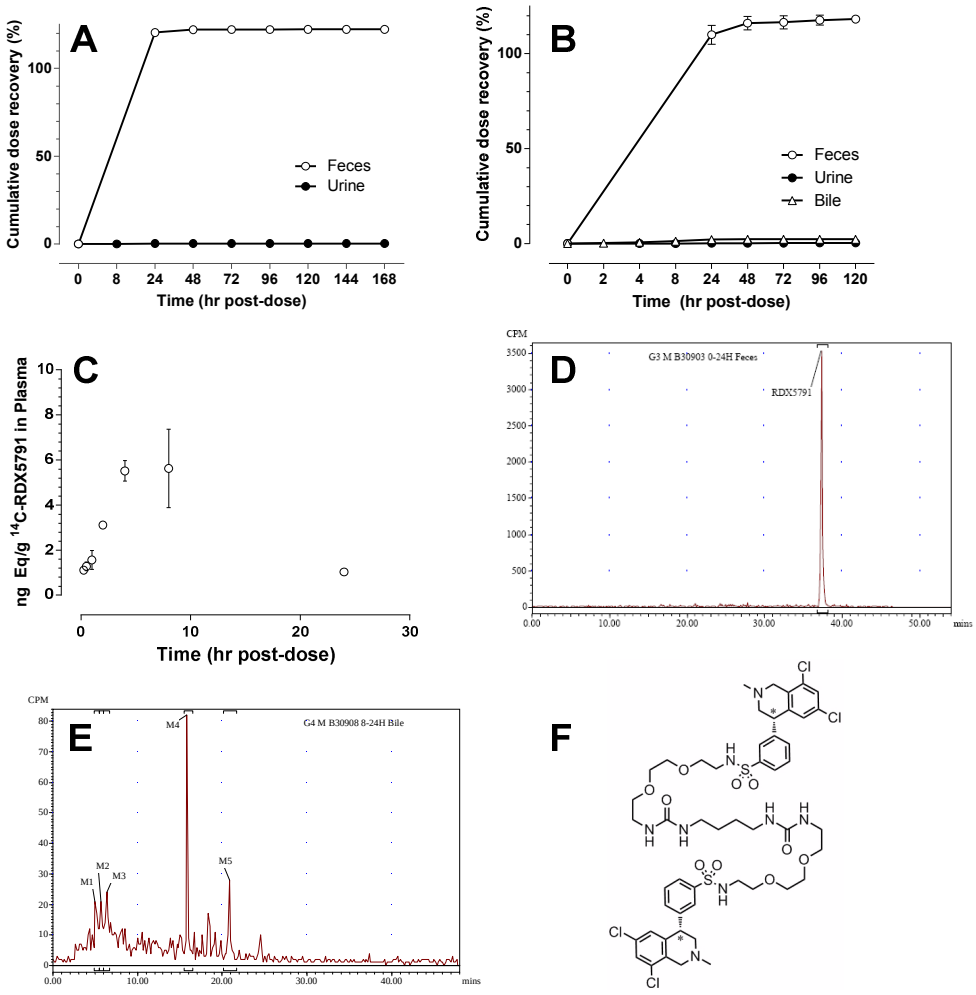

Figure S1. Summary of mass balance study of ^{14}C-tenapanor. Radiolabeled mass balance studies of ^{14}C- tenapanor. Determination of mass balance after dosing (A) normal and (B) bile duct cannulated (BDC) rats with ^{14}C- tenapanor shows the drug is excreted largely via feces in the first 24 hr after dosing. (C) Negligible ^{14}C- tenapanor -derived radioactivity was detected in plasma . (D) Detection of radioactivity in fecal extracts showed feces contained almost exclusively parent ^{14}C- tenapanor, while (E) bile contained more polar species and no detectable ^{14}C- tenapanor, which elutes at ~37.5 min in the method used in D and E. (F) The structure of ^{14}C- tenapanor. * = the labeled carbon in a non-metabolizable chiral position. Data in A-C are expressed as mean ± SEM.

Figure S2

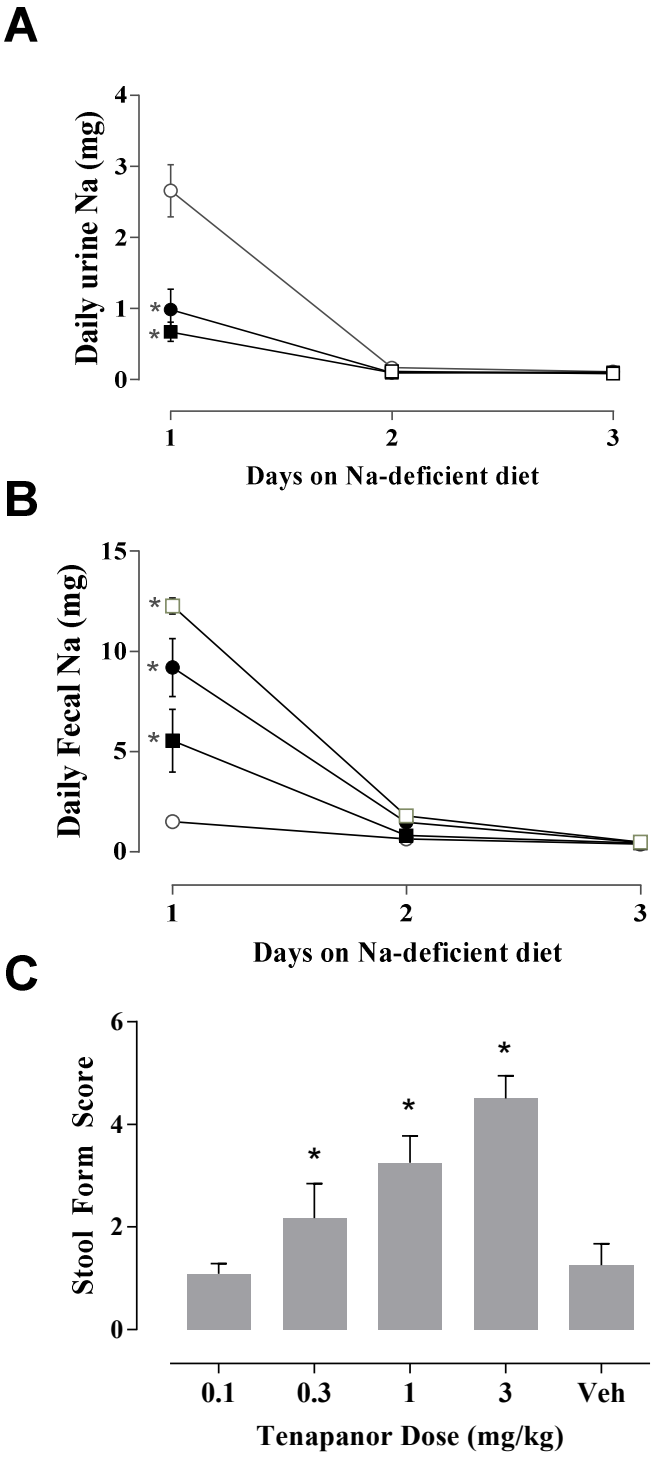
A
Daily urine Na (mg)
4
3
2
1
0
*
*
1
2
3
Days on Na-deficient diet
B
Daily Fecal Na (mg)
15
10
5
0
*
*
*
1
2
3
Days on Na-deficient diet
C
Stool Form Score
6
4
2
0
*
*
*
0.1
0.3
1
3
Veh
Tenapanor Dose (mg/kg)

Figure S2: Effect of tenapanor on fecal and urinary sodium in rats on a very low sodium diet, and dose-dependent effect of tenapanor on stool form in rats. Sprague-Dawley rats were fed a sodium deficient diet (0.01% Na^+) and dosed daily by oral gavage with vehicle (water, ○), or tenapanor at 0.3 mg/kg (■) or 1 mg/kg (●), or 3 mg/kg (□) on each of three consecutive days. Urine (A) and fecal (B) sodium were determined after 24 hour collections. (C) The effect of increasing doses of tenapanor on stool form score in rats consuming standard chow. Data are expressed as Mean ± SEM . * = $p < 0.05$ versus vehicle, 2-way ANOVA, Bonferroni's *posthoc* test.

Figure S3

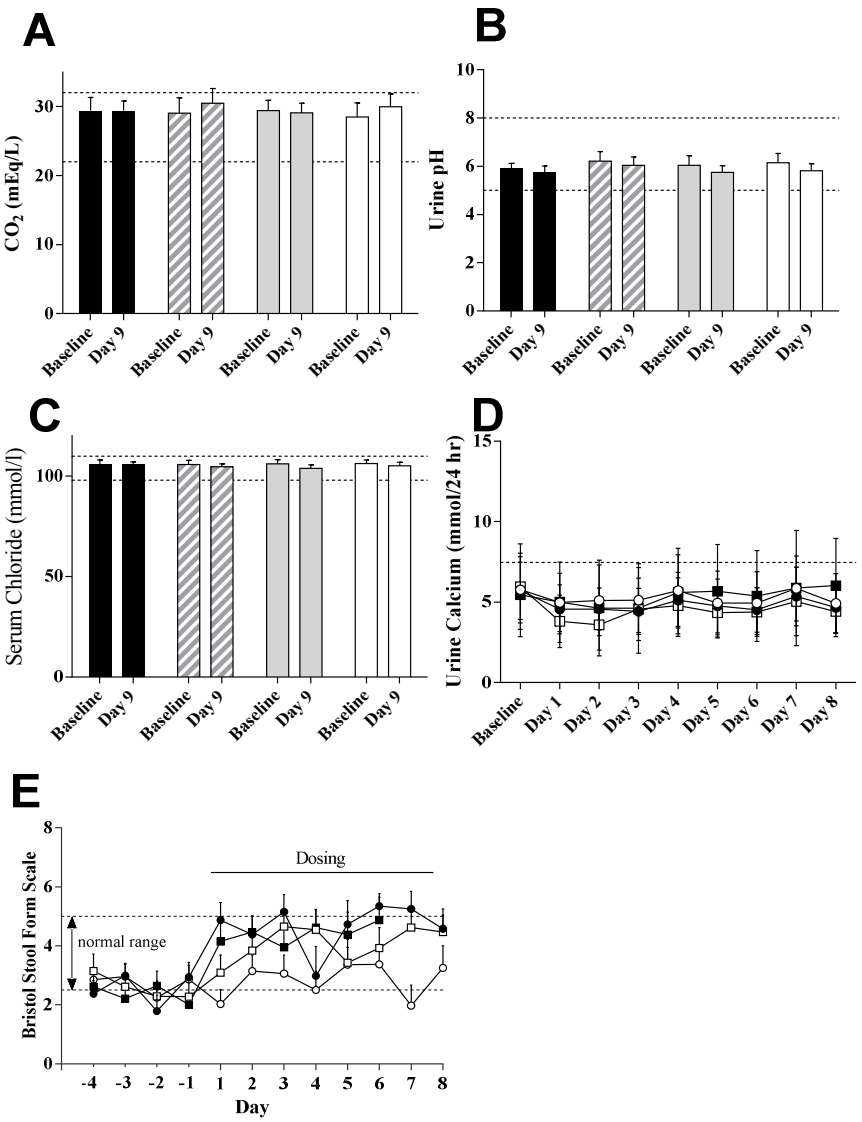
A
CO_2 (mEq/L)
0
10
20
30
Baseline
Day 9
B
Urine pH
0
2
4
6
8
10
C
Serum Chloride (mmol/l)
0
50
100
D
Urine Calcium (mmol/24 hr)
0
5
10
15
Baseline
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
E
Bristol Stool Form Scale
normal range
Dosing
-4
-3
-2
-1
1
2
3
4
5
6
7
8
Day

Figure S3: Measurements of serum bicarbonate and chloride, urine pH and urinary calcium, and stool form in humans after 7-day repeated, twice-daily dosing of placebo and 15 to 60 mg of tenapanor. Serum bicarbonate (A), urine pH (B), serum chloride (C), urinary calcium (D), or stool form (E). In A-C, white = placebo; black = 15 mg bid; hatched = 30 mg bid; grey = 60 mg bid. In D-E, ○ = placebo; ● 15 mg bid; □ = 30 mg bid; ■ = 60 mg bid. Dotted lines indicate normal ranges; in D, the lower bound of the normal range is zero. Data are expressed as mean ± SEM.

Figure S4

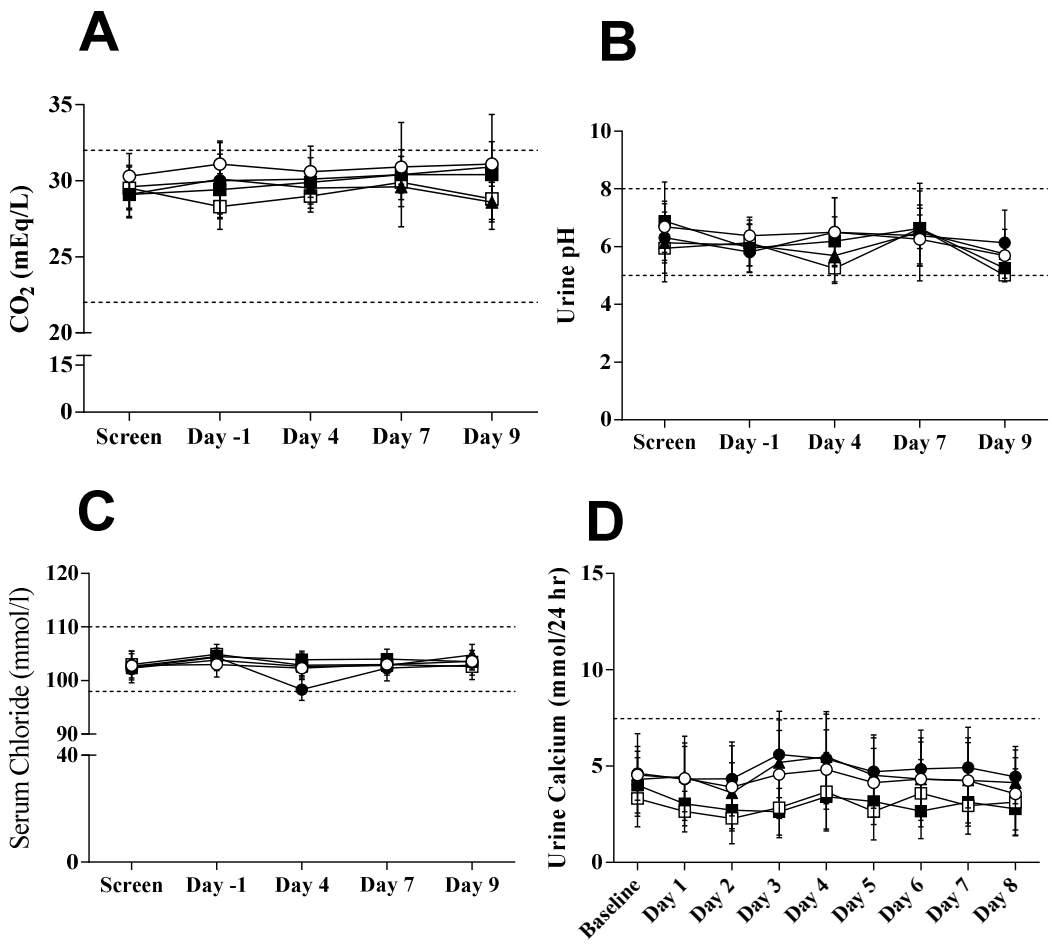

Figure S4: Measurements of serum bicarbonate and chloride, urine pH, and urinary calcium in humans after 7-day repeated, once-daily dosing of placebo and 3 to 100 mg of tenapanor. Tenapanor was dosed as a capsule at 3 mg qd, 10 mg qd, 30 mg qd, 100 mg qd, or placebo. There were no changes in serum bicarbonate (A), urine pH (B), serum chloride (C), or urinary calcium (D). ○ = placebo; ● 3 mg qd,; □ = 10 mg qd; ■ = 30 mg qd; ▲ = 100 mg qd. Dotted lines indicate normal ranges; in D, the lower bound of the normal range is zero. Data are expressed as mean ± SEM.

Figure S5

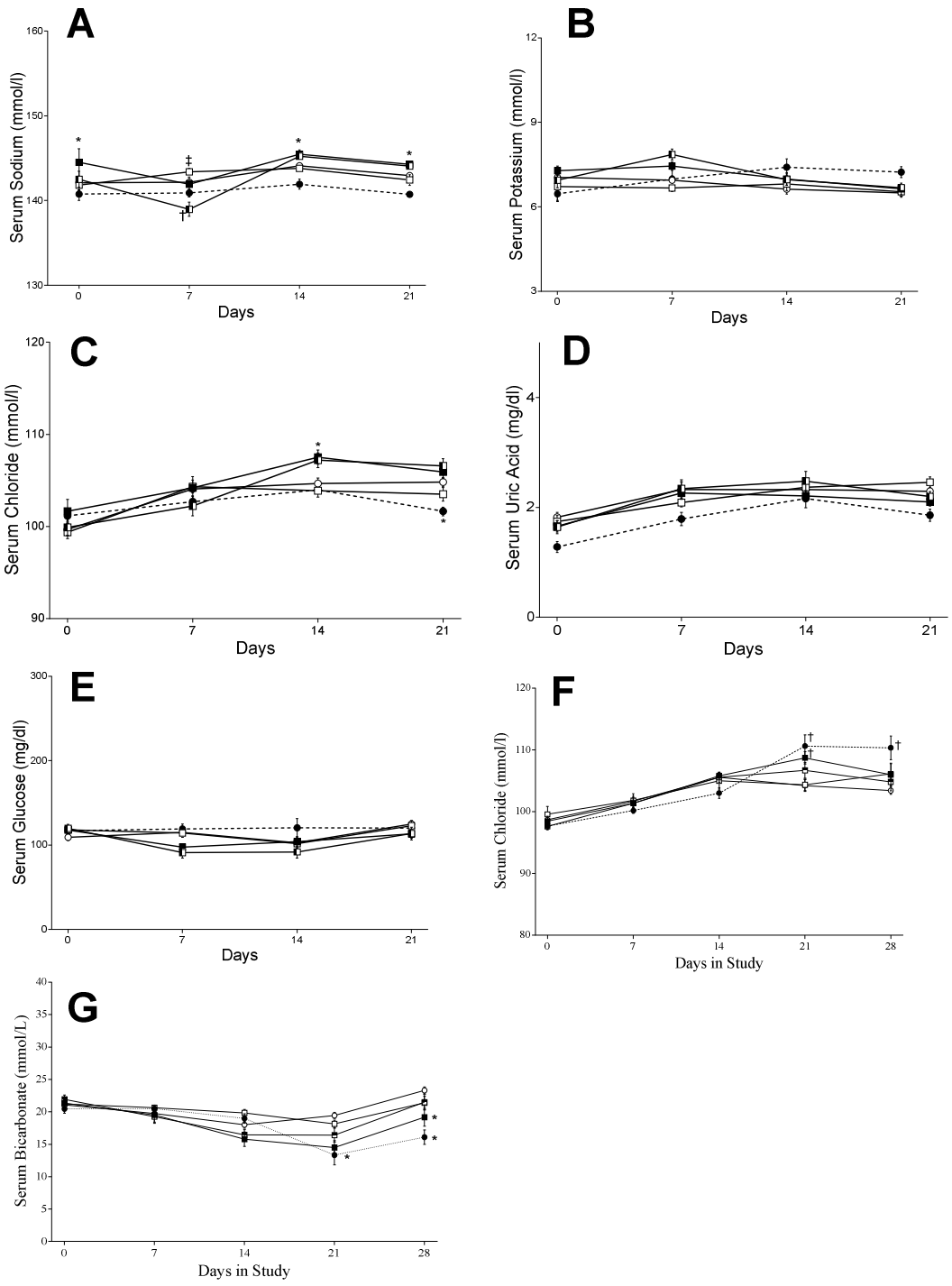
A
Serum Sodium (mmol/l)
Days
B
Serum Potassium (mmol/l)
Days
C
Serum Chloride (mmol/l)
Days
D
Serum Uric Acid (mg/dl)
Days
E
Serum Glucose (mg/dl)
Days
F
Serum Chloride (mmol/l)
Days in Study
G
Serum Bicarbonate (mmol/L)
Days in Study

Figure S5: Effect of tenapanor, or tenapanor and enalapril, on serum sodium, potassium, chloride, uric acid, glucose, and bicarbonate in NPX rats. Significance by 1-way ANOVA: * = $p < 0.05$ vs. naïve controls; † = $p < 0.05$ vs. NPX vehicle controls; ‡ = $p < 0.05$ vs. NPX + enalapril. A-E: Open circle = vehicle; open square = 10 mg/kg enalapril; closed square = 1.0 mg/kg tenapanor; split square = 10 mg/kg enalapril + 1 mg/kg tenapanor; closed circle = naïve controls. F-G: Open circle = vehicle; open square = 0.3 mg/kg tenapanor; split square = 1.0 mg/kg tenapanor; closed square = 3.0 mg/kg tenapanor prophylactic setting; closed circle and dotted line = 3.0 mg/kg tenapanor treatment setting. Data are expressed as mean ± SEM.

Table S1 Potency of tenapanor activity against NHE1, NHE2, NHE3, NaPiIIb, TGR5, ASBT, and Pit-1.

Target	IC50	Result
Human NHE1	>10 μM	No detectable inhibition of human NHE1
Human NHE2	>10 μM	No detectable inhibition of human NHE2
Human NHE3	5 ± 4 nM (n>50)	~5 nM inhibitor of human NHE3
Rat NHE3	10 ± 7 nM (n>50)	~10 nM inhibitor of rat NHE3
Human NaPi2b	>10 μM	No detectable inhibition of human NaPi2b
Human TGR5	>10 μM	No detectable agonism of human TGR5
Human ASBT	>10 μM	No detectable inhibition of human ASBT
Human PiT1	>10 μM	No detectable inhibition of human PiT1

Table S2 PK parameters of tenapanor in rats, dogs, and humans.

Dose (mg/kg)	Species[D]	Diet	Co-Dosed	Route	C_{max} (ng/mL)	AUC (ng•h/mL)	% Unbound[E]
30	Rat (n=3)	0.49% Na^+	-	PO gavage	<3	BQL	<0.001 **rat plasma**
10	Rat (n=5)	0.49% Na^+	-	PO gavage	BQL	BQL	
1	Rat (n=5)	0.49% Na^+	-	PO gavage	BQL	BQL	
1	5/6th NPX Rat (n=3)	4.0% NaCl	-	PO gavage	<1	BQL	
1	5/6th NPX Rat (n=4)	4.0% NaCl	Enalapril	PO gavage	<1	BQL	
10	Dog (n=4)	0.49% Na^+	-	PO gavage	BQL	BQL	<0.001 **dog plasma**
1	Dog (n=4)	0.49% Na^+	-	PO gavage	<1	BQL	
3 mg qd[A,B]	Human (n=8)	1200 mg Na^+	-	PO capsule	BQL	BQL	<0.001 **human plasma**
10 mg qd[A,B]	Human (n=8)	1200 mg Na^+	-	PO capsule	BQL	BQL	
30 mg qd[A,B]	Human (n=8)	1200 mg Na^+	-	PO capsule	BQL	BQL	
100 mg qd[A,B]	Human (n=8)	1200 mg Na^+	-	PO capsule	BQL	BQL	
150 mg qd[A,C]	Human (n=6)	1500 mg Na^+	-	PO capsule	BQL	BQL	
450 mg qd[A,C]	Human (n=6)	1500 mg Na^+	-	PO capsule	<1	BQL	
900 mg qd[A,C]	Human (n=6)	1500 mg Na^+	-	PO capsule	<1	BQL	

A = Human doses are expressed in total milligrams of the bis-hydrochloride salt of tenapanor.

B = Doses were administered on each of seven consecutive days.

C = Doses were administered on a single day.

D = All human subjects were healthy volunteers

E = % Unbound is the percentage of unbound plasma tenapanor determined by in vitro equilibrium dialysis experiments using the rapid equilibrium dialysis (RED) device (Pierce)

BQL = below the quantitative limit of 0.5 ng/mL

Note: Typically tenapanor concentrations were below the limit of quantification (0.5 ng/mL). C_{max} and AUC values are reported as <*x* to reflect that rare samples in some individuals had low concentrations of tenapanor within the quantitative range.

Table S3 MDCK and PAMPA permeability data of tenapanor.

Compound	MDCK Monolayer Permeability							
	A(pH 7.4) → **B**(pH 7.4)		**B**(pH 7.4) → **A**(pH 7.4)		**A**(pH 6.5) → **B**(pH 7.4)		**B**(pH 7.4) → **A**(pH 6.5)	
	P_{app}	**Recovery (%)**	**P_{app}**	**Recovery (%)**	**P_{app}**	**Recovery (%)**	**P_{app}**	**Recovery (%)**
Atenolol	0.5 ± 0.1	102 ± 2%	1 ± 0.1	107 ± 4	0.5 ± 0.04	102 ± 10	2 ± 0.5	107 ± 9
Propranolol	28 ± 1	95 ± 6%	18 ± 3	95 ± 6	7 ± 0.1	99 ± 1	26 ± 0.4	104 ± 1
Labetalol	6 ± 0.3	96 ± 0.6%	14 ± 2	104 ± 0.5	1 ± 0.1	99 ± 2	14 ± 0.1	104 ± 0.3
Colchicine	0.3 ± 0.1	101 ± 3.7%	3 ± 0.2	104 ± 2	0.3 ± 0.04	100 ± 1	3 ± 0.2	104 ± 2
Tenapanor[A]	<0.01 ± <0.01	86 ± 10	<0.01 ± <0.01	64 ± 5†	<0.01 ± <0.01	83 ± 1	0.02 ± 0.03	59 ± 2†
Tenapanor[B]	0.04 ± 0.003	102 ± 0.3	0.7 ± 0.1	100 ± 0.4	0.04 ± 0.01	98 ± 2	0.6 ± 0.001	102 ± 0.3

†The low recovery observed when tenapanor was added on the basolateral side of the monolayer improved to ~100% when additives like bovine serum albumin (2%) or Tween-80 (0.1%) were employed. The general behavior of tenapanor in the presence of additives was not significantly different (i.e., low P_{app}), but permeability artifacts for control compounds confounded interpretation of data gathered in the presence of additives.

[A] = < are values that were below the limit of quantitation

[B] = Tween-80 (0.1%) was added to transport buffer to reduce the potential interaction of tenapanor with the assay plate wells and Transwell inserts.

P_{app} = Apparent permeability rate in 10^{-6} x (cm/s)

Table S4 Radiolabeled ADME and QWBA study design.

Group	Rat Strain	Dose	Samples Analyzed	Collection Times
1	Sprague-Dawley	90-110 μCi/kg	Blood	0.25-72 h
2	Sprague-Dawley & Long Evans	90-110 μCi/kg	Blood, Carcasses for QWBA	0.5-504 h
3	Sprague Dawley	23-33 μCi/kg	Urine, Feces	0-168 h
4	Sprague Dawley	23-33 μCi/kg	Urine, Feces, Bile	0-120 h

Table S5 QWBA Data for ^{14}C-tenapanor in Long-Evans rats.

Tissue	ng Eq ^{14}C-Tenapanor/g in Long Evans Rats								
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h	72 h	168 h
Adrenal gland(s)	ND	ND	ND[a]	15.0[b]	BLQ	ND	ND	ND	ND
Arterial wall	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood	ND	ND	ND	BLQ	BLQ	ND	ND	ND	ND
Bone	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebellum	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	ND	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cecum	ND	ND	13.6	26.3	41.7	BLQ	38.9	ND	ND
Contents, cecum	ND	ND	279	13900	4480	218	668	ND	ND
Contents, esophageal	4220	550	1330	38.9	31.8	ND	ND	ND	ND
Contents, large intestine	ND	NR	ND	13800	17400	1190	642	BLQ	ND
Contents, small intestine	12700	20000	31100	1310	2500	37.5	63.1	BLQ	ND
Contents, stomach	41700	53100	23800	7310	6420	ND	45.9	ND	ND
Diaphragm	ND	ND	ND	BLQ	ND	ND	ND	ND	ND
Epididymis	ND	ND	ND	ND	ND	ND	ND	ND	ND
Esophagus	BLQ	BLQ	36.2	BLQ	BLQ	ND	ND	ND	ND
Exorbital lacrimal gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eye lens	ND	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	ND	ND	19.6	47.2	63	34.5	34.8	34.3	ND
Eye(s)	ND	ND	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	ND
Fat (abdominal)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fat (brown)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Harderian gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Intra-orbital lacrimal gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Kidney cortex	ND	12.4	20.4	35.5	39.4	ND	ND	ND	ND
Kidney medulla	ND	BLQ	BLQ	14.2	11.2	ND	ND	ND	ND
Kidney(s)	ND	10.8	17.3	31.6	31	ND	ND	ND	ND
Large intestine	ND	ND	ND	BLQ	10.6	BLQ	15.2	ND	ND
Liver	31.2	22.7	40.5	77.8	100	21.2	14.1	BLQ	ND
Lung(s)	ND	ND	BLQ	BLQ	11.6	ND	ND	ND	ND
Lymph node(s)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Meninges	ND	ND	ND	ND	ND	ND	ND	ND	ND
Muscle	ND	ND	ND	ND	ND	ND	ND	ND	ND
Myocardium	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nasal turbinates	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pancreas	BLQ	BLQ	BLQ	BLQ	BLQ	ND	ND	ND	ND
Pituitary gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Preputial gland	ND	ND	ND	ND	BLQ	ND	ND	ND	ND
Prostate gland	ND	ND	ND	ND	ND	ND	ND	ND	ND
Salivary gland(s)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Seminal vesicle(s)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skin (non-pigmented)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Skin (pigmented)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Small intestine	233	ND[a]	61.5	93.4	29.9	48.3	12.8	ND	ND
Spinal cord	ND	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	ND	ND	BLQ	10.4	ND	ND	ND	ND	ND
Stomach	75.4	32.2	35.4	38.1	BLQ	ND	ND	ND	ND
Testis(es)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Thymus	ND	ND	ND	ND	ND	ND	ND	ND	ND
Thyroid	ND	ND	ND	ND	ND	ND	ND	ND	ND
Urinary bladder	ND	NR	BLQ	ND	ND	ND	ND	ND	ND
Urine	ND	NR	25.4	44.5	ND	ND	ND	ND	ND

Footnotes Table S5

h = Hours

BLQ = Below limit of quantitation (<10.2 ng Eq ^{14}C- tenapanor /g)

ND = Not detectable (sample shape not discernible from background or surrounding tissue).

NR = Not represented (tissue not present in section)

a = Tissue appeared to be fat soaked.

b = Tissue ND due to flare of gastrointestinal contents.

c = ≥1 sample above the upper limit of quantitation (>107000 ng Eq ^{14}C- tenapanor /g).

Table S6 QWBA Data for ^{14}C-tenapanor in Sprague-Dawley rats.

Tissue	ng Eq ^{14}C-Tenapanor/g in Sprague Dawley Rats							
	0.5 h	1 h	2 h	4 h	8 h	24 h	48 h	72 h
Adrenal gland(s)	ND	ND	BLQ	BLQ	BLQ[a]	ND	ND	ND
Arterial wall	ND	ND	ND	ND	ND	ND	ND	ND
Bile	ND	ND	ND	ND	ND	ND	ND	ND
Blood	ND	ND	BLQ	ND	ND	ND	ND	ND
Bone	ND	ND	ND	ND	ND	ND	ND	ND
Bone marrow	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebellum	ND	ND	ND	ND	ND	ND	ND	ND
Brain cerebrum	ND	ND	ND	ND	ND	ND	ND	ND
Brain medulla	ND	ND	ND	ND	ND	ND	ND	ND
Brain olfactory lobe	ND	ND	ND	ND	ND	ND	ND	ND
Bulbo-urethral gland	ND	ND	ND	ND	ND	ND	ND	ND
Cecum	ND	ND	72.2	48	ND[b]	65.6	BLQ	ND
Contents, cecum	ND	ND	706	6420	9370	1510	26.9	ND
Contents, esophageal	2930	304	141	7350	33.9	17.3	ND	ND
Contents, large intestine	ND	ND	ND	240	25600	3590	51.6	BLQ
Contents, small intestine	24700	22700	21900	32100	1960	769	BLQ	ND
Contents, stomach	49800	43900	62000	98100[c]	401	624	ND	ND
Diaphragm	ND	ND	BLQ	BLQ	BLQ	ND	ND	ND
Epididymis	ND	ND	ND	ND	ND	ND	ND	ND
Esophagus	20.1	17.6	BLQ	ND	BLQ	ND	ND	ND
Exorbital lacrimal gland	ND	ND	ND	ND	ND	ND	ND	ND
Eye lens	ND	ND	ND	ND	ND	ND	ND	ND
Eye uveal tract	ND	ND	ND	ND	ND	ND	ND	ND
Eye(s)	ND	ND	ND	ND	ND	ND	ND	ND
Fat (abdominal)	ND	ND	ND	ND	ND	ND	ND	ND
Fat (brown)	ND	ND	ND	ND	ND	ND	ND	ND
Harderian gland	ND	ND	ND	ND	BLQ	ND	ND	ND
Intra-orbital lacrimal gland	ND	ND	ND	ND	ND	ND	ND	ND
Kidney cortex	ND	BLQ	20.2	34.1	24.7	ND	ND	ND
Kidney medulla	ND	BLQ	19.1	17.9	11	ND	ND	ND
Kidney(s)	ND	BLQ	19.5	29.3	21.2	BLQ	ND	ND
Large intestine	ND	ND	ND	BLQ	ND[b]	12.8	BLQ	ND
Liver	13.3	18.7	36.3	55.1	59.1	31	BLQ	BLQ
Lung(s)	ND	BLQ	BLQ	BLQ	11	ND	ND	ND
Lymph node(s)	ND	ND	ND	ND	ND	ND	ND	ND
Meninges	ND	ND	ND	ND	ND	ND	ND	ND
Muscle	ND	ND	ND	ND	ND	ND	ND	ND
Myocardium	ND	ND	ND	ND	ND	ND	ND	ND
Nasal turbinates	ND	ND	ND	ND	ND	ND	ND	ND
Pancreas	ND	BLQ	BLQ	BLQ	ND	ND	ND	ND
Pituitary gland	ND	ND	ND	ND	ND	ND	ND	ND
Preputial gland	ND	ND	ND	ND	ND	ND	ND	ND
Prostate gland	ND	ND	ND	ND	ND	ND	ND	ND
Salivary gland(s)	ND	ND	ND	ND	ND	ND	ND	ND
Seminal vescicle(s)	ND	ND	ND	ND	ND	ND	ND	ND
Skin (non-pigmented)	ND	ND	ND	ND	ND	ND	ND	ND
Small intestine	18	17.7	20.7	21.8	122	19.4	BLQ	ND
Spinal cord	ND	ND	ND	ND	ND	ND	ND	ND
Spleen	ND	ND	BLQ	10.4	ND	ND	ND	ND
Stomach	53.8	BLQ	25.2	13.6	BLQ	BLQ	ND	ND
Testis(es)	ND	ND	ND	ND	ND	ND	ND	ND
Thymus	ND	ND	ND	ND	ND	ND	ND	ND
Thyroid	ND	ND	ND	ND	ND	ND	ND	ND
Urinary bladder	ND	ND	83	BLQ	BLQ	65.8	ND	ND
Urine	BLQ	ND	28.6	55.4	41.4	22.1	ND	ND

Footnotes for Table S6

h = Hours

BLQ = Below limit of quantitation (<10.2 ng Eq ^{14}C- tenapanor /g)

ND = Not detectable (sample shape not discernible from background or surrounding tissue).

a = Tissue appeared to be fat soaked.

b = Tissue ND due to flare of gastrointestinal contents.

c = ≥1 sample above the upper limit of quantitation (>107000 ng Eq ^{14}C- tenapanor)