FR-PO139

Prophylactic and therapeutic tenapanor are vascular protective in a rat model of chronic kidney disease

Ann-Cathrine Jönsson-Rylander,[1] Margareta Behrendt,[1] Craig Plato,[2] Denise Schwabauer,[2] Peter J Greasley[1]

[1]AstraZeneca Gothenburg, Mölndal, Sweden; [2]Plato Biopharma Inc., Denver, CO, USA

Overview and conclusions

- Tenapanor is a minimally systemic, small-molecule inhibitor of the sodium/hydrogen exchanger NHE3. Tenapanor is in development for renal and constipation-related indications.
- We investigated the cardiovascular protective effects of prophylactic (Px) and therapeutic (Tx) tenapanor in a rat model of renal-insufficiency-induced, salt-sensitive arterial hypertension and chronic kidney disease (CKD).
- Compared with healthy controls (HCs), disease controls (DCs) had abnormal levels of urinary biomarkers and increased blood pressure, as well as impaired aortic vasoconstrictor and vasodilator function, and endothelial dysfunction at week 2 and week 6.
- Px tenapanor prevented and Tx tenapanor reversed existing renal-insufficiency- and NaCl-induced proteinuria and albuminuria, as well as reducing urinary sodium and phosphate excretion.
- At week 6, Px and Tx tenapanor treatment normalized or reduced arterial hypertension, vascular stiffness, vasoconstrictor function, and endothelium dependent and independent vasodilator function.
  - The benefit of Tx treatment was similar to that elicited by Px use, suggesting potential for reversal of existing disease.

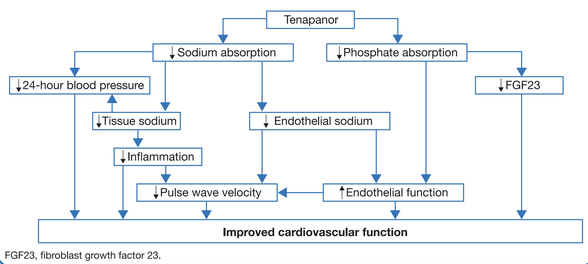
Background

- Tenapanor (AZD1722, RDX5791) is an inhibitor of NHE3 (also known as SLC9A3).[1]
  - NHE3 plays an important role in intestinal sodium/fluid homeostasis.[2]
- Preclinical and healthy volunteer studies have shown that tenapanor treatment reduces absorption of dietary sodium[1] and phosphate.[3] (See also Block *et al.*[4] oral presentation and other posters[5,6] at this meeting.)
- By reducing absorption of sodium and phosphate, it may be hypothesized that treatment with tenapanor has the potential to influence a number of pathways involved in increasing cardiovascular risk in patients with CKD (Figure 1).[7]
- The aim of this study was to investigate the cardiovascular protective effects of Px and Tx tenapanor treatment in a rat model of renal-insufficiency-induced, salt-sensitive arterial hypertension and CKD.

Methods

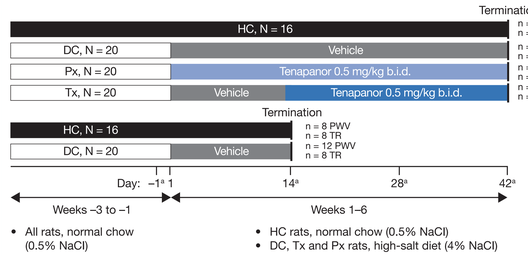
- 5/6 nephrectomized Sprague Dawley rats were fed 4% NaCl chow to induce salt-sensitive arterial hypertension (Figure 2).
- Oral tenapanor hydrochloride treatment (0.5 mg/kg twice daily) was initiated either at the start of NaCl intake (Px) or 2 weeks later (i.e. after disease establishment; Tx), and was administered for up to 6 weeks.
- DCs (vehicle-treated) and HCs (sham-operated, fed normal chow) were included to enable assessment of disease progression.
- Systemic hemodynamics and urinary biomarkers were assessed every 2 weeks, while *in vivo* arterial stiffness (pulse wave velocity [PWV][8]) and *ex vivo* vascular function (isometric tension recording [TR][9]) were evaluated at week 2 and week 6 (adapted from published methods).
  - The HC and DC animals undergoing PWV and TR evaluations at day 14 were functionally matched to HCs and DCs remaining in the study to serve as representative cohorts (Figure 2).

Figure 1. Study hypothesis: tenapanor affects a number of pathways associated with cardiovascular risk in patients with CKD.



FGF23, fibroblast growth factor 23.

Figure 2. Study design.



- All rats, normal chow (0.5% NaCl)
- HC rats, normal chow (0.5% NaCl)
- DC, Tx and Px rats, high-salt diet (4% NaCl)

With the exception of HC (naïve sham) rats, all animals were male Sprague Dawley rats who underwent uninephrectomy during week −3 and subtotal nephrectomy during week −2.
[a]Blood pressure was measured with a tail cuff on days −1, 14, 28 and 42 following 24 hours in a metabolic cage.
b.i.d., twice daily; DC, disease control; HC, healthy control; PWV, endpoint *in vivo* pulse wave velocity; Px, prophylactic tenapanor; TR, *ex vivo* aortic tension recording (endothelial and vascular smooth muscle cell function); Tx, therapeutic tenapanor.

Results

Endpoint analytes and systemic hemodynamics

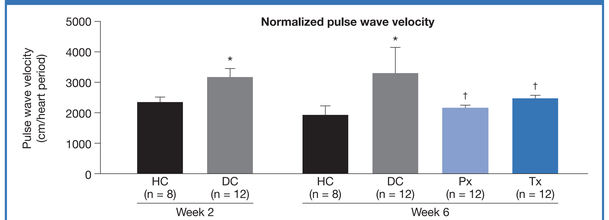
- At week 2, DCs had increased urinary protein and albumin, and increased systolic, diastolic and mean arterial blood pressure compared with HCs (all $p < 0.05$; Table 1).
- When compared with DCs at week 6, the Px and Tx tenapanor groups had reduced urinary albumin, protein, sodium and phosphate excretion ($p < 0.05$ vs DCs, Table 1).
- Systolic, diastolic and mean arterial blood pressure were increased in DCs at week 6 ($p < 0.05$ vs HCs), but were normalized with Px and Tx tenapanor treatment ($p < 0.05$ vs DCs, Table 1).

Table 1. Selected endpoint analytes and systemic hemodynamic data.

	Week 2		Week 6			
	HC (N = 16)	DC (N = 20)	HC (N = 16)	DC (N = 20)	Px (N = 20)	Tx (N = 20)
Urinary protein, mg/day	20.7 ± 1.4	48.3 ± 7.1*	21.2 ± 1.8	195 ± 33.5*	30.7 ± 2.8†	42.0 ± 6.3†
Urinary albumin, mg/day	0.23 ± 0.0	8.9 ± 2.0*	0.34 ± 0.0	76.7 ± 14.7*	2.05 ± 0.4†	7.35 ± 2.9†
Urinary sodium, mmol/day	2.71 ± 0.1	2.92 ± 0.1	3.10 ± 0.1	11.7 ± 0.6*	7.04 ± 0.5*†	6.22 ± 0.5*†
Urinary phosphate, mg/day	25.9 ± 1.3	23.0 ± 1.0*	28.8 ± 1.1	25.4 ± 0.9	6.28 ± 1.2*†	7.80 ± 1.1*†
Systolic BP, mmHg	128 ± 3.3	165 ± 6.5*	129 ± 3.7	211 ± 5.0*	136 ± 4.2†	143 ± 4.8†
Diastolic BP, mmHg	85.1 ± 2.6	114 ± 5.7*	84.4 ± 3.0	153 ± 5.6*	90.5 ± 3.1†	93.3 ± 3.6†
Mean arterial BP, mmHg	99.1 ± 2.8	132 ± 6.0*	98.8 ± 3.2	172 ± 5.4*	105 ± 3.5†	110 ± 4.0†
Heart rate, bpm	329 ± 7.4	350 ± 7.7	304 ± 8.2	372 ± 8.3*	334 ± 7.7†	325 ± 11.2†

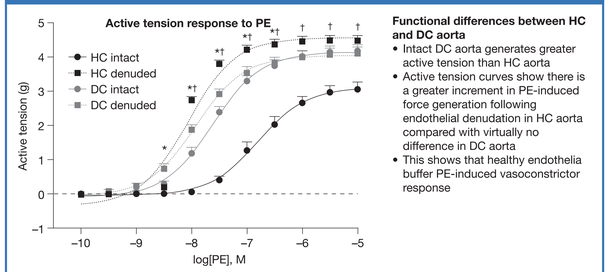
*$p < 0.05$ vs HCs within time period; †$p < 0.05$ vs DCs within time period.
Data are shown as mean ± standard error.
BP, blood pressure; bpm, beats per minute; DC, disease control; HC, healthy control; Px, prophylactic tenapanor; Tx, therapeutic tenapanor.

Figure 3. Px and Tx tenapanor reduced CKD-induced arterial stiffness *in vivo*.



*$p < 0.05$ vs HC within time period; †$p < 0.05$ vs DC within time period.
Pulse wave velocity data were normalized to cardiac cycle length ('heart period') and are shown as mean (+ standard error).
DC, disease control; HC, healthy control; Px, prophylactic tenapanor; Tx, therapeutic tenapanor.

Figure 4. Overview and context of *ex vivo* isometric tension recording experiments for evaluating vasoconstrictor and vasorelaxation properties of rat aorta.



*$p < 0.05$ for DC denuded vs HC denuded; †$p < 0.05$ for DC intact vs HC intact.
ACh, acetylcholine; $AR_{\alpha 1}$, adrenergic receptor α1; cGMP, cyclic guanosine monophosphate; DC, disease control; eNOS, endothelial nitric oxide synthase; HC, healthy controls; L-Arg, L-arginine; M, muscarinic receptor; NO, nitric oxide; PE, phenylephrine; ROS, reactive oxygen species; SNP, sodium nitroprusside.

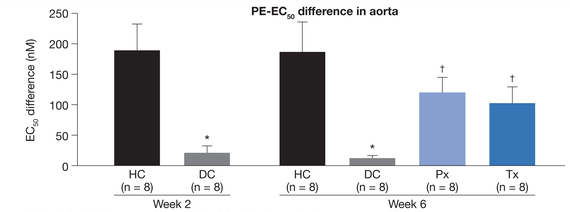
In vivo arterial stiffness

- Compared with HCs, *in vivo* arterial stiffness was elevated at weeks 2 and 6, reflected by increased normalized arterial PWV ($p < 0.05$ vs HCs; Figure 3).
  - At week 6, both Px and Tx tenapanor treatment reduced *in vivo* arterial stiffness ($p < 0.05$ vs DCs, Figure 3).

Ex vivo isometric tension recording

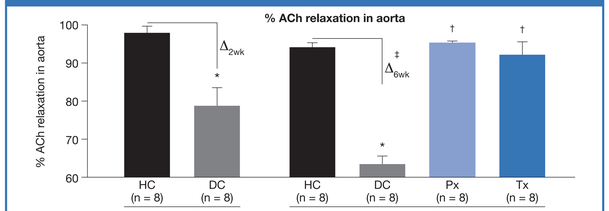
- DCs had impaired vascular function at weeks 2 and 6, as shown by the results of *ex vivo* isometric tension recording.
  - Functional differences between endothelial-intact and endothelial-denuded aorta were assessed to evaluate vasoconstriction and vasorelaxation properties of aorta as illustrated in Figure 4.
- Endothelial-mediated buffering of aortic vasoconstrictor response to phenylephrine (PE) was reduced in DCs at both weeks 2 and 6, as shown by much smaller PE half-maximal effective concentration (EC_{50}) differences (between endothelial-intact and endothelial-denuded aorta) compared with HCs ($p < 0.05$; Figure 5).
  - At week 6, both Px and Tx tenapanor treatment restored the endothelial-mediated buffering of PE-induced aortic contraction ($p < 0.05$ vs DCs; Figure 5), and reduced the PE-mediated vasoconstrictor force in intact vessel rings in a similar way to that shown in Figure 4.
- DCs had time-dependent impairment of endothelium-dependent vasodilator function, as shown by a progressive reduction in acetylcholine-induced vasorelaxation from weeks 2 to 6 ($p < 0.05$ vs HCs; Figure 6).
  - Renal-insufficiency-induced reductions in endothelium-dependent vasodilator response were completely prevented by Px tenapanor and reversed by Tx tenapanor treatment ($p < 0.05$ vs DCs).
- Endothelium-independent vasodilator function was time-dependently reduced in DCs from weeks 2 to 6, as shown by increases in sodium nitroprusside (SNP)-EC_{50} differences (between endothelial-intact and endothelial-denuded aorta) compared with HCs ($p < 0.05$; Figure 7).
  - At week 6, Px and Tx tenapanor treatment normalized and reversed, respectively, the the renal-insufficiency- and NaCl-induced impairment in endothelium-independent vasodilator function ($p < 0.05$ vs DCs).

Figure 5. Px and Tx tenapanor restore endothelial-mediated buffering of PE-induced aortic vasoconstriction.



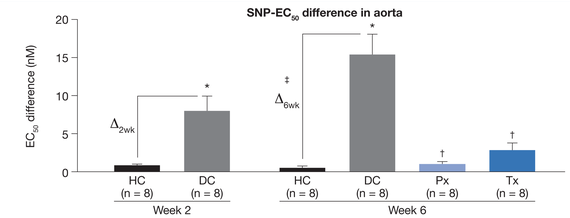
*$p < 0.05$ vs HC within time period; †$p < 0.05$ vs DC within time period.
Data are shown as the mean (+ standard error) PE-EC_{50} difference between endothelial-intact and endothelial-denuded aorta.
DC, disease control; EC_{50}, half-maximal effective concentration; HC, healthy control; PE, phenylephrine (a vasoconstriction agent); Px, prophylactic tenapanor; Tx, therapeutic tenapanor.

Figure 6. CKD time-dependently impaired endothelium-dependent vasorelaxation. Px tenapanor prevented and Tx tenapanor restored normal endothelium-dependent vasorelaxation.



*$p < 0.05$ vs HC within time period; †$p < 0.05$ vs DC within time period; ‡$p < 0.05$ vs Δ_{2wk}.
Data are shown as the mean % (+ standard error) relaxation of intact aorta in response to ACh.
ACh, acetylcholine (endothelial-dependent vasodilator); DC, disease control; HC, healthy control; Px, prophylactic tenapanor; Tx, therapeutic tenapanor.

Figure 7. CKD time-dependently impaired endothelium-independent vasodilator function. Px tenapanor prevented the impairment and Tx tenapanor restored normal endothelium-independent vasodilator function.



*$p < 0.05$ vs HC within time period; †$p < 0.05$ vs DC within time period; ‡$p < 0.05$ vs Δ_{2wk}.
Data are shown as the mean (+ standard error) SNP-EC_{50} difference between endothelial-intact and endothelial-denuded aorta.
DC, disease control; EC_{50}, half-maximal effective concentration; HC, healthy control; Px, prophylactic tenapanor; SNP, sodium nitroprusside (a nitric oxide-releasing compound that induces vasodilation); Tx, therapeutic tenapanor.

References

1. Spencer AG *et al. Sci Transl Med* 2014;6:227ra36.
2. Schultheis PJ *et al. Nat Genet* 1998;19:282–5.
3. Labonté ED *et al. J Am Soc Nephrol* 2015;26:1138–49.
4. Block G *et al.* Accepted abstract at the American Society of Nephrology Kidney Week, 3–8 November 2015, San Diego, CA, USA. (Abstract number 4247; oral presentation TH-OR114.)
5. Rosenbaum DP *et al.* Accepted abstract at the American Society of Nephrology Kidney Week, 3–8 November 2015, San Diego, CA, USA. (Abstract number 1938; poster SA-PO547.)
6. Stefansson B *et al.* Accepted abstract at the American Society of Nephrology Kidney Week, 3–8 November 2015, San Diego, CA, USA. (Abstract 4160; poster TH-PO655.)
7. Spencer A, Greasley P. *Curr Opin Nephrol Hypertens* 2015;24:410–16.
8. Fitch RM *et al. Cardiovasc Res* 2001;51:351–8.
9. Taddei S *et al. Hypertension* 1993;21:9–15.

Funding

This study was funded by AstraZeneca.

Disclosures

ACJR and MB are employees of AstraZeneca. CP is an employee of and has ownership interest in Plato BioPharma (Plato BioPharma has received research funding from AstraZeneca). DS is an employee of Plato BioPharma. PJG is an employee of and has ownership interest in AstraZeneca.

Acknowledgments

Medical writing support was provided by Steven Inglis (PhD) from Oxford PharmaGenesis, Oxford, UK, and was funded by Ardelyx, Fremont, CA, USA.

Supported by: ARDELYX AstraZeneca

Presented at the American Society of Nephrology Kidney Week 2015 (abstract 4071), 3–8 November 2015, San Diego, CA, USA