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DRUG INTERACTIONS

Tenapanor administration and the activity of the H^+-coupled transporter PepT1 in healthy volunteers

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AIM

Tenapanor (RDX5791/AZD1722), an inhibitor of gastrointestinal Na^+/H^+ exchanger NHE3, is being evaluated for the treatment of patients with constipation-predominant irritable bowel syndrome and the treatment of hyperphosphataemia in patients with chronic kidney disease on dialysis. By reducing intestinal H^+ secretion, inhibition of NHE3 by tenapanor could indirectly affect H^+-coupled transporter activity, leading to drug–drug interactions. We investigated the effect of tenapanor on the activity of the H^+-coupled peptide transporter PepT1 via assessment of the pharmacokinetics of cefadroxil – a compound transported by PepT1 – in healthy volunteers.

METHODS

In this open-label, two-period crossover, phase 1 study (NCT02140281), 28 volunteers received in random order: a single dose of cefadroxil 500 mg for 1 day; and tenapanor 15 mg twice daily over 4 days followed by single doses of both cefadroxil 500 mg and tenapanor 15 mg on day 5. There was a 4-day washout between treatment periods.

RESULTS

Cefadroxil exposure was similar when administered alone or in combination with tenapanor {geometric least-squares mean ratios [(cefadroxil + tenapanor)/cefadroxil] (90% confidence interval): area under the concentration–time curve 93.3 (90.6–96.0)%; maximum concentration in plasma 95.9 (89.8–103)%}. Tenapanor treatment caused a softening of stool consistency and an increase in stool frequency, consistent with its expected pharmacodynamic effect. No safety concerns were identified and tenapanor was not detected in plasma.

CONCLUSIONS

These results suggest that tenapanor 15 mg twice daily does not have a clinically relevant impact on the activity of the H^+-coupled transporter PepT1 in humans. This may guide future research on drug–drug interactions involving NHE3 inhibitors.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Gastrointestinal NHE3 indirectly affects intestinal H^+-coupled transporters.
- Tenapanor, a locally-acting, minimally-absorbed NHE3 inhibitor, is being investigated for the treatment of patients with IBS-C and for the treatment of hyperphosphataemia in patients with chronic kidney disease on dialysis.
- Cefadroxil, a β-lactam antibiotic, is transported by the H^+-coupled transporter PepT1.

WHAT THIS STUDY ADDS

- Repeated oral dosing of tenapanor did not have a clinically relevant effect on the pharmacokinetics of the model PepT1-transported drug cefadroxil in healthy volunteers.
- This suggests that pharmacological inhibition of NHE3 by tenapanor is unlikely to have an impact on uptake of drugs mediated by the H^+-coupled transporter PepT1 in humans.

Tables of Links

TARGETS
Transporters [2]
NHE3
PepT1

LIGANDS
Cefadroxil
Tenapanor

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in http: //www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2].

Introduction

Sodium/hydrogen (Na^+/H^+) exchanger isoform 3 (NHE3) [3] plays an important role in sodium transport and fluid homeostasis in the gastrointestinal tract [4, 5]. Tenapanor (RDX5791, AZD1722) is a minimally-absorbed, highly-selective, small-molecule inhibitor of gastrointestinal NHE3 that reduces the uptake of dietary sodium and phosphate [3, 6]. Tenapanor is currently in development for the treatment of patients with constipation-predominant irritable bowel syndrome (IBS-C) (ClinicalTrials.gov identifiers: NCT01923428 [7], NCT02621892 [8], NCT02686138 [9]), and for the treatment of hyperphosphataemia in patients with chronic kidney disease (CKD) on dialysis (ClinicalTrials.gov identifiers: NCT02081534 [10], NCT02675998 [11]).

An important step in drug development is to identify any possible drug–drug interactions. As tenapanor has minimal systemic availability, it is unlikely to have effects outside of the gut. In the small intestine, a region of mildly acidic pH (6.1–6.8) adjacent to the luminal surface of the epithelium, known as the acid microclimate, is important for the absorption of molecules from the gut [12]. A range of H^+-coupled transporter proteins use the acid microclimate as a driving force for the uptake of molecules across the apical brush-border membrane [13, 14]. This includes transporters of dipeptides and tripeptides, amino acids, vitamins and organic cations [13]. NHE3 is present in the apical membrane of gastrointestinal epithelial cells and is expressed more highly in the small intestine than in the colon [15, 16]. NHE3 has a major role in the uptake of dietary sodium from the intestinal lumen, in an electroneutral manner, through proton exchange [4]. This H^+ secretion into the gut lumen plays an important role in generating and maintaining the acid microclimate [12, 17]. An NHE3 inhibitor could therefore reduce apical H^+ secretion, alter the pH of the microclimate and potentially lead to indirect inhibition of H^+-coupled transporter activity.

H^+-coupled peptide transporter 1 (PepT1) is highly expressed throughout the small intestine and is localized to the brush-border membrane. It is a high-capacity transporter, with relatively low affinity for a wide range of compounds [13, 18]. In addition to transporting dipeptides and tripeptides arising from protein digestion, PepT1 has also been shown to transport a range of hydrophilic oral drugs, including many β-lactam antibiotics [13, 14, 19]. Inhibiting NHE3 activity, by either removing extracellular Na^+ or using the NHE3-selective inhibitor S1611, reduces transepithelial peptide transport by PepT1 *in vitro*, suggesting that NHE3 contributes to PepT1 transport activity by maintaining the acid microclimate through Na^+/H^+ exchange [20, 21]. Therefore, by pharmacologically inhibiting NHE3, tenapanor has the potential to affect PepT1-mediated drug uptake.

Based on these theoretical considerations, this phase 1 study was conducted to assess whether tenapanor has a clinically relevant impact on the transport activity of the H^+-coupled transporter PepT1 in humans, by evaluating the pharmacokinetics of the model PepT1-transported drug cefadroxil [14, 20, 22] when administered alone or in combination with tenapanor.

Methods

The drug/molecular target nomenclature used in this article conforms to the Concise Guide to PHARMACOLOGY 2015/16 [2].

Study design

This was a phase 1, randomized, open-label, two-period crossover study (ClinicalTrials.gov identifier: NCT02140281) conducted by a contract research organization at a single study centre (Quintiles, Overland Park, KS, USA). The study was conducted in accordance with the Declaration of Helsinki, and International Conference on Harmonisation and Good Clinical Practice guidelines. The study protocol and an amendment were approved by the MidLands Independent Review Board (Overland Park, KS, USA). All study volunteers provided written informed consent before undergoing any study procedure.

Participants

Healthy men and women, aged 18–50 years, with a body mass index of 18–30 kg m^{-2} and weighing 50–100 kg, were eligible for enrolment in the study. Women of childbearing potential could not be pregnant and had to use effective contraception during the study period. Men were also required to use effective contraception. Volunteers were required to have suitable veins for cannulation or repeated venepuncture.

Key exclusion criteria were: a history or presence of gastrointestinal, hepatic or renal disease, or any other condition known to interfere with the absorption, distribution, metabolism or excretion of drugs; loose stools [Bristol Stool Form Scale (BSFS) score of 6 or 7 [23]] for 2 or more days in the week before study drug administration; use of medications or supplements known to affect stool consistency and/or gastrointestinal motility, including fibre supplements, antidiarrhoeal agents, prokinetic drugs, enemas, probiotic medications or supplements; or salt or electrolyte supplements containing sodium, potassium, chloride or bicarbonate formulations during the week before randomization.

Study treatments

Following screening, all volunteers underwent two treatments in a 1: 1 randomized sequence (Figure 1): (i) a single oral dose of cefadroxil 500 mg administered in the morning; and (ii) tenapanor 15 mg, administered orally twice daily for 4 days, followed by single doses of both tenapanor 15 mg and cefadroxil 500 mg (administered in the morning) on day 5 (this treatment combination hereafter referred to as cefadroxil + tenapanor). There was a washout period of at least 4 days between the two treatment periods.

For each treatment regimen, volunteers were admitted to the study centre on the morning of the day before study drug administration (day –1). Volunteers who received cefadroxil alone were discharged on the day of treatment (day 1), after samples had been collected for pharmacokinetic measurements. Those receiving cefadroxil + tenapanor were resident at the centre until the end of sample collection on day 5. Volunteers returned to the study centre for follow-up assessments 7–10 days after administration of the final treatment dose.

Volunteers underwent an overnight fast of at least 8 h before study drug administration. Cefadroxil and tenapanor were administered 5–10 min before meals (breakfast and/or dinner), and all volunteers could consume only the same standardized meals and drinks offered during the residential period.

Pharmacokinetic assessments

Blood samples were collected predose and 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 10 and 12 h postdose on day 1 of the cefadroxil treatment period and at the same time points on day 5 of the cefadroxil + tenapanor treatment period to assess the pharmacokinetics of cefadroxil. Blood samples collected predose and 1, 2 and 4 h postdose on day 5 were also used to assess plasma concentrations of tenapanor.

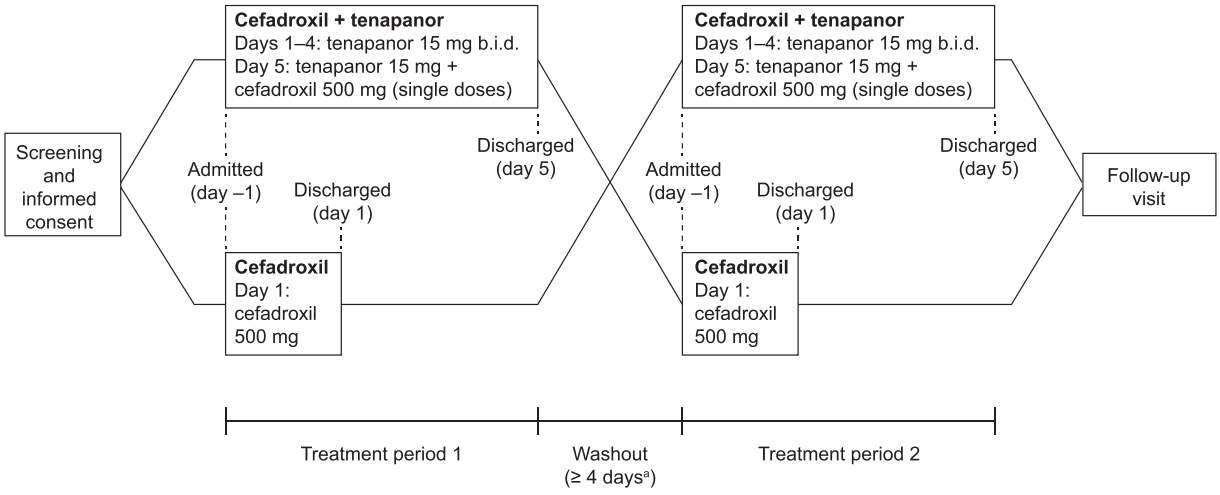

Figure 1

Study design. Participants were admitted to the study centre on the morning of the day before study drug administration (day –1) and discharged on the last day of treatment (day 1 or day 5) following sample collection for pharmacokinetic measurements. [a]At least 4 days between the last dose of the first treatment period and the first dose of the second treatment period. b.i.d., twice daily

Samples for determination of drug concentration in plasma were analysed by Covance Laboratories Inc. (Madison, WI, USA). Cefadroxil and its deuterated internal standard were isolated from plasma using protein precipitation and analysed using liquid chromatography followed by tandem mass spectrometric detection. Before being used to analyse cefadroxil levels in the study samples, the method was validated in the range 30.0–30 000 ng ml^{-1}. Tenapanor and its deuterated internal standard were extracted from samples using liquid–liquid extraction. After evaporation under nitrogen, the residue was reconstituted and analysed using liquid chromatography followed by tandem mass spectrometric detection. Before being used to analyse tenapanor concentrations in the samples collected, the method was validated in the range 0.500–100 ng ml^{-1}.

Pharmacokinetic parameters were derived using standard noncompartmental methods with Phoenix WinNonlin Professional version 6.3 (Pharsight Corp., Mountain View, CA, USA). All pharmacokinetic computations were performed using either this software or SAS® version 9.4 (SAS Institute Inc., Cary, NC, USA). The parameters determined were as follows: the maximum concentration in plasma (C_{max}); area under the concentration–time curve in plasma from time zero (predose) to time of last quantifiable concentration (AUC_{0-t}), calculated by linear up/log down trapezoidal summation; area under the concentration–time curve in plasma from time zero (predose) extrapolated to infinite time (AUC), calculated by linear up/log down trapezoidal summation and extrapolated to infinity by addition of the last quantifiable concentration (C_t) divided by the apparent elimination rate constant (λ_z; *i.e.* $AUC = AUC_{0-t} + C_t/\lambda_z$).

Pharmacodynamic assessments

Stool frequency and stool consistency (as measured by the BSFS [23]) were assessed daily on day –1 of the first treatment period and on days 1–5 of the cefadroxil + tenapanor treatment period. Assessments were over 24-h intervals following the morning dosing, except for day 5 of cefadroxil + tenapanor treatment (12-h interval).

Safety assessments

Safety assessments included vital signs (at screening, on entry to the study clinic on day –1, and at the end of each treatment period), physical examinations (at screening and at the end of the second treatment period), clinical laboratory evaluations (clinical chemistry, haematology and urinalysis: at screening, before dosing on day 1 of each treatment period, and at the end of the second treatment period), electrocardiograms (at screening and at the end of each treatment period) and adverse event (AE) monitoring (throughout the study: from screening until the follow-up visit).

Statistical analyses

Pharmacokinetic assessments were summarized using descriptive statistics for all plasma measurements of cefadroxil exposure. The pharmacokinetic analysis set consisted of all volunteers who received cefadroxil 500 mg and provided evaluable pharmacokinetic profiles during either treatment period.

Following natural log transformation, the observed C_{max}, AUC and AUC_{0-t} were analysed separately using a mixed effects analysis of variance model, with sequence, period and treatment as fixed effects, and volunteer nested within sequence as a random effect. The point estimate and 90% confidence interval (CI) for the difference between treatments was constructed and exponentially back-transformed to provide point and CI estimates for the ratio of interest ([cefadroxil + tenapanor]/cefadroxil).

Assuming no effect of tenapanor on the pharmacokinetics of cefadroxil and a standard deviation (SD) of 0.3 or less for the change in log-transformed pharmacokinetic variables, a sample size of 24 volunteers was expected to provide a 90% probability of the two-sided 90% CI for the ratio ([cefadroxil + tenapanor]/cefadroxil) being completely contained within 80–125%. The study therefore aimed to include 28 volunteers.

Summary statistics were determined for pharmacodynamic evaluations of stool frequency and stool consistency. The pharmacodynamic (i.e. stool) analysis and safety analysis sets included all volunteers who received at least one dose of tenapanor or cefadroxil and had at least one postdose measurement. All statistical analyses were performed using SAS version 9.4.

Results

Study participants

Twenty-eight volunteers (18 men) were enrolled in this study. All volunteers completed the study, receiving all treatments according to study protocol, and were included in pharmacokinetic and safety analyses. One participant was excluded from pharmacodynamic (stool) analysis, as only predose data were available. Mean ± SD age of the volunteers was 32 ± 10 years (range 19–49 years) and mean ± SD body mass index was 26.0 ± 2.8 kg m^{-2} (range 19.4–29.8 kg m^{-2}).

Pharmacokinetics

Cefadroxil plasma concentration–time curves were similar whether cefadroxil was administered alone or in combination with tenapanor (Figure 2). Pharmacokinetic parameters of cefadroxil were also similar when cefadroxil was given alone or in combination with tenapanor [geometric least-squares mean ratio (90% CI), (cefadroxil + tenapanor)/cefadroxil: AUC, 93.3 (90.6–96.0)%; AUC_{0-t}, 93.4 (90.7–96.1)%; C_{max}, 95.9 (89.8–102.5)%; Table 1]. Median time to maximum plasma concentration was 1.5 h and the geometric mean of the apparent terminal half-life was approximately 2 h for both cefadroxil and cefadroxil + tenapanor treatments. The plasma concentration of tenapanor was below the lower limit of quantitation (0.5 ng ml^{-1}) in all samples collected and analysed during the study.

Stool frequency and consistency

Stool frequency increased with tenapanor treatment, from a predose mean ± SD of 1.4 ± 0.5 bowel movements per day, measured on the day before study drug administration (day –1), to a mean ± SD of 1.8 ± 0.8 bowel movements per

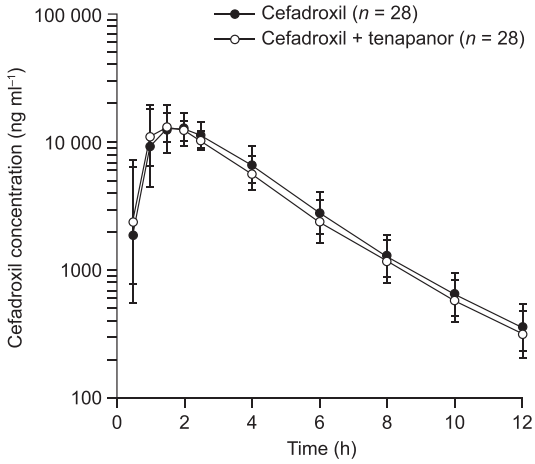

Figure 2

Cefadroxil plasma concentration *vs.* time following cefadroxil administration alone and in combination with tenapanor. Data shown as geometric mean (± standard deviation). Cefadroxil: a single dose of cefadroxil 500 mg administered on the morning of day 1. Cefadroxil + tenapanor: tenapanor 15 mg twice daily administered from day 1 to day 4, followed by single doses of both tenapanor 15 mg and cefadroxil 500 mg, administered concurrently on the morning of day 5

day for the entire 5-day tenapanor dosing period. Mean ± SD BSFS scores increased from 3.7 ± 1.0 on day –1 to 5.4 ± 1.2 for the overall tenapanor dosing period.

Safety and tolerability

Tenapanor was generally well tolerated and no safety concerns were identified. No serious AEs or discontinuations due to AEs occurred during the study. Overall, 11 volunteers reported AEs, all of which were mild in intensity and resolved. Almost all AEs were gastrointestinal in nature. Ten volunteers reported AEs during administration of tenapanor 15 mg twice daily (days 1–4), while one volunteer reported an AE following cefadroxil + tenapanor administration (day 5). The most common AEs (reported by at least two volunteers) were abdominal distension (n = 4), abdominal pain (n = 4), abnormal gastrointestinal sounds (n = 3), diarrhoea (n = 2) and flatulence (n = 2). No trends or clinically relevant changes in clinical laboratory results, vital signs, electrocardiograms or physical examinations were observed during the study.

Discussion

This phase 1 study aimed to investigate whether gastrointestinal NHE3 inhibition by tenapanor has a clinically relevant impact on the transport activity of the H^+-coupled transporter PepT1 in humans. NHE3 inhibition has been suggested to reduce H^+ secretion into the gut, potentially affecting the acid microclimate and thereby reducing H^+-coupled transporter activity [20, 21]. PepT1 is involved in the uptake of several drugs [14, 18, 19], and apical dipeptide transport and uptake assays across a range of extracellular pH values suggest that the optimal transport activity of PepT1 occurs at pH 6.5, which is within the *physiological* pH range of the acid microclimate at the mucosal surface of the intestine (pH 6.1–6.8). To test whether NHE3 inhibition by tenapanor affects PepT1 transport activity, the pharmacokinetics of cefadroxil (a compound transported by PepT1) were compared when cefadroxil was administered alone and in combination with tenapanor in 28 volunteers. Our results suggest that repeated dosing with tenapanor 15 mg twice daily has no clinically relevant effect on PepT1 activity.

Our study was performed in line with regulatory guidance for transporter-based *in vivo* drug–drug interaction studies [24, 25]. The tenapanor dose of 15 mg twice daily is at the lower end of the range tested so far for the treatment of patients with IBS-C or the treatment of hyperphosphataemia in patients with CKD on dialysis [7, 10]. Additional data may be needed to confirm whether the lack of effect on cefadroxil absorption observed in our study is also seen at higher doses of tenapanor. Tenapanor was administered for 4 days to

Table 1

Pharmacokinetic parameters of cefadroxil when administered alone or in combination with tenapanor

	Cefadroxil (n = 28)	Cefadroxil + tenapanor (n = 28)	Geometric least-squares mean ratio,[a] % (90% CI)
AUC (ng h ml^{-1})	53 800 (16.8)	50 200 (16.6)	93.3 (90.6–96.0)
AUC_{0-t} (ng h ml^{-1})	52 700 (16.7)	49 200 (16.4)	93.4 (90.7–96.1)
C_{max} (ng ml^{-1})	14 800 (23.2)	14 200 (21.6)	95.9 (89.8–102.5)
t_{max} (h)[b]	1.5 (1.0–4.0)	1.5 (1.0–4.0)	
$t_{½}$ (h)	2.0 (11.1)	2.1 (11.8)	

Unless otherwise stated, data are shown as geometric mean (GCV, %). AUC, area under the plasma concentration–time curve from time zero to infinity; AUC_{0-t}, area under the plasma concentration–time curve from time zero to the last quantifiable concentration; CI, confidence interval; C_{max}, maximum observed plasma concentration; GCV, geometric coefficient of variation; t_{max}, time to C_{max}; $t_{½}$, apparent terminal half-life.
[a](cefadroxil + tenapanor)/cefadroxil.
[b]Data are shown as median (range).

ensure that the pharmacodynamic effects reached a steady state before administration of a therapeutically relevant dose of cefadroxil. *In vitro* studies have convincingly shown that cefadroxil has moderate affinity for, and is transported by, PepT1 [19]. Furthermore, studies in knockout mice indicate that PepT1 plays a key role in the rate and extent of absorption of cefadroxil following oral administration [22]. Cefadroxil is therefore a recommended agent for studies examining the pharmaceutical relevance of H^+-coupled peptide transporters [19, 26].

Pharmacological activity of tenapanor was evident owing to changes in stool consistency and frequency, consistent with NHE3 inhibition and previous findings in healthy volunteers [27]. In mouse models, deletion of the NHE3 gene results in severe impairment of sodium–fluid volume homeostasis with subsequent chronic diarrhoea [4], and a decrease in calcium absorption [28]. However, our study showed that pharmacological inhibition of NHE3 in humans resulted in a phenotype nowhere near as extreme as that observed in NHE3 knockout mouse models. The ratios of plasma cefadroxil AUC, AUC_{0-t} and C_{max} for cefadroxil alone and cefadroxil in combination with tenapanor were within the bioequivalence range (80–125%), indicating that there were no clinically significant differences in cefadroxil uptake between the two treatments.

Other clinical studies that have investigated the effect of changes in the acid microclimate on PepT1 transport activity have shown inconsistent results [29, 30]. Amiloride, an inhibitor of Na^+/H^+ exchange, was found to decrease the gastrointestinal uptake of the β-lactam antibiotic amoxicillin in healthy volunteers [29]. By contrast, in another study, jejunal perfusion with amiloride and amoxicillin in healthy volunteers did not affect amoxicillin absorption in the gut [30]. Our study showed no evidence of an indirect effect of tenapanor on PepT1 transport activity. This lack of effect suggests that inhibition of gastrointestinal NHE3 by tenapanor may not be sufficient to alter the pH of the acid microclimate to an extent that could significantly affect PepT1 function. Although the reported pH of the acid microclimate varies across studies and in different regions of the intestine, values for the proximal jejunum typically range from pH 6.1 to 6.8 [31–35]. *In vitro* studies have shown that, although PepT1 activity is reduced as the pH increases, PepT1 is still active at pH 7.0 [20]. It therefore seems unlikely that any increase in the pH of the acid microclimate as a consequence of tenapanor inhibition of NHE3 would be sufficient to cause complete loss of PepT1 activity. Furthermore, most H^+-coupled transporters are spread over a large area of the intestine and are high-capacity and low-affinity transporters, making it difficult to inhibit the transport of any one particular compound effectively and hence difficult to create a relevant drug–drug interaction. PepT1 is considerably more abundant in the small intestine than in the colon [36]; however, as this expression pattern is similar to that of NHE3 [15, 16], it seems unlikely that differences in the relative regional expression of gastrointestinal PepT1 and NHE3 account for the lack of drug–drug interaction seen here. Throughout our phase 3 programme for tenapanor we are investigating any data trends that may reflect drug–drug interactions; none have been identified to date.

In conclusion, our study found that repeated dosing of tenapanor 15 mg twice daily had no clinically relevant effect on cefadroxil exposure in humans. This suggests that pharmacological inhibition of NHE3 by tenapanor is unlikely to have any clinically relevant impact on the activity of the H^+-coupled transporter PepT1 in the gut. This may guide future research on drug–drug interactions during the development of NHE3 inhibitors.

Competing Interests

S.J. and C.H. are employees of, and have ownership interest in, AstraZeneca. J.P., B.S. and M.K. are employees of AstraZeneca. D.P.R. is an employee of, and has ownership interest in, Ardelyx Inc. E.L. is an employee of Quintiles. This study was funded by AstraZeneca.

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Contributors

S.J., J.P. and C.H. designed the research. E.L. was the principal investigator of the study. M.K. was responsible for the statistical analyses. All authors contributed to the interpretation of the results and the writing of the manuscript.

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