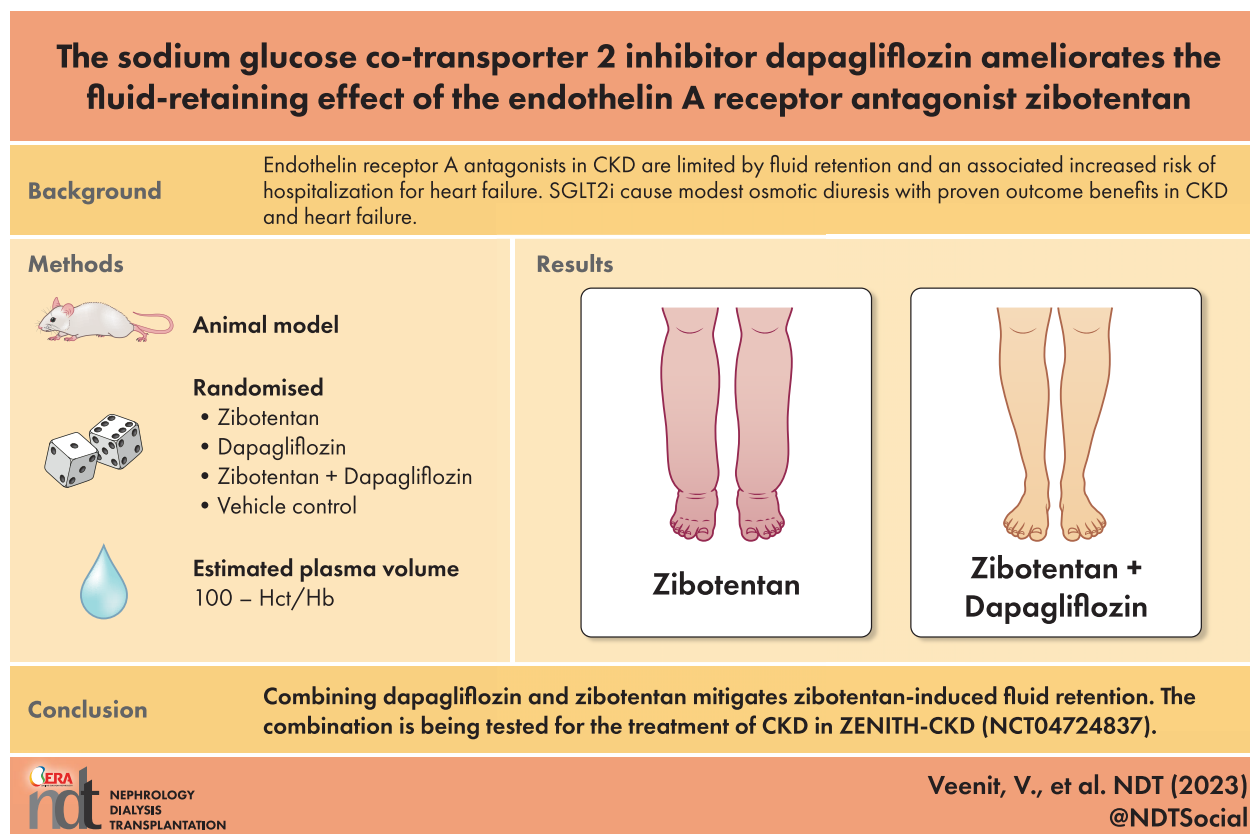# The sodium glucose co-transporter 2 inhibitor dapagliflozin ameliorates the fluid-retaining effect of the endothelin A receptor antagonist zibotentan

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GRAPHICAL ABSTRACT

The sodium glucose co-transporter 2 inhibitor dapagliflozin ameliorates the fluid-retaining effect of the endothelin A receptor antagonist zibotentan

Background
Endothelin receptor A antagonists in CKD are limited by fluid retention and an associated increased risk of hospitalization for heart failure. SGLT2i cause modest osmotic diuresis with proven outcome benefits in CKD and heart failure.

Methods

Animal model

Randomised
• Zibotentan
• Dapagliflozin
• Zibotentan + Dapagliflozin
• Vehicle control

Estimated plasma volume
100 − Hct/Hb

Results

Zibotentan

Zibotentan + Dapagliflozin

Conclusion
Combining dapagliflozin and zibotentan mitigates zibotentan-induced fluid retention. The combination is being tested for the treatment of CKD in ZENITH-CKD (NCT04724837).

NEPHROLOGY DIALYSIS TRANSPLANTATION

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What is already known about this subject?

- The development of endothelin receptor A antagonists (ET$_A$RA) in chronic kidney disease (CKD) has been limited by fluid retention and an associated increased risk for hospitalization for heart failure. Sodium–glucose co-transporter 2 inhibitors (SGLT2i) cause modest osmotic diuresis with proven outcome benefits in CKD and heart failure.

What this study adds?

- Combining an SGLT2i with an ET$_A$RA may provide both superior efficacy and reduced risk of fluid retention. The present data indicate that the SGLT2i dapagliflozin mitigates the fluid-retaining effects of the ET$_A$R antagonist zibotentan in a rodent model.

What impact this may have on practice or policy?

- Dapagliflozin and zibotentan are currently under clinical investigation as a combination treatment for CKD (ZENITH-CKD; NCT04724837). The present preclinical data support further clinical investigation of combination treatment with zibotentan and dapagliflozin.

ABSTRACT

Background. Endothelin A receptor antagonists (ET$_A$RA) slow chronic kidney disease (CKD) progression but their use is limited due to fluid retention and associated clinical risks. Sodium–glucose co-transporter 2 inhibitors (SGLT2i) cause osmotic diuresis and improve clinical outcomes in CKD and heart failure. We hypothesized that co-administration of the SGLT2i dapagliflozin with the ET$_A$RA zibotentan would mitigate the fluid retention risk using hematocrit (Hct) and bodyweight as proxies for fluid retention.

Methods. Experiments were performed in 4% salt fed WKY rats. First, we determined the effect of zibotentan (30, 100 or 300 mg/kg/day) on Hct and bodyweight. Second, we assessed the effect of zibotentan (30 or 100 mg/kg/day) alone or in combination with dapagliflozin (3 mg/kg/day) on Hct and bodyweight.

Results. Hct at Day 7 was lower in zibotentan versus vehicle groups [zibotentan 30 mg/kg/day, 43% (standard error 1); 100 mg/kg/day, 42% (1); and 300 mg/kg/day, 42% (1); vs vehicle, 46% (1); $P < .05$], while bodyweight was numerically higher in all zibotentan groups compared with vehicle. Combining zibotentan with dapagliflozin for 7 days prevented the change in Hct [zibotentan 100 mg/kg/day and dapagliflozin, 45% (1); vs vehicle 46% (1); $P = .44$] and prevented the zibotentan-driven increase in bodyweight (zibotentan 100 mg/kg/day + dapagliflozin 3 mg/kg/day = –3.65 g baseline corrected bodyweight change; $P = .15$).

Conclusions. Combining ET$_A$RA with SGLT2i prevents ET$_A$RA-induced fluid retention, supporting clinical studies to assess the efficacy and safety of combining zibotentan and dapagliflozin in individuals with CKD.

Keywords: bodyweight, dapagliflozin, fluid retention, hematocrit, zibotentan

INTRODUCTION

In 2017, 698 million people were diagnosed with chronic kidney disease and, in 1.2 million people, chronic kidney disease (CKD) was attributed as the primary cause of death [1]. Endothelin 1 (ET-1), first described in the late 1980s [2], has been shown to modulate renal physiology and pathophysiology. ET-1 regulates kidney vascular tone and electrolyte and fluid homeostasis under normal physiological conditions [3] and is implicated in proinflammatory and fibrotic pathophysiological processes which are hallmarks of kidney disease [4–6]. ET-1 levels increase in CKD and are associated with increasing urinary albumin-to-creatinine ratio (UACR) levels and decreasing levels of glomerular filtration rate [3, 7, 8]. Accumulating evidence suggests that the pathological effects of ET-1, including proteinuria, vasoconstriction and inflammation, are driven primarily via its action on the endothelin A receptor (ET$_A$R) [4, 5, 9].

Preclinical studies have demonstrated beneficial effects of ET$_A$ receptor antagonists (ET$_A$RA) in diabetic models of kidney disease by ameliorating established glomerular damage, inflammation and UACR [10–13]. Clinical studies have demonstrated that ET$_A$RA reduce albuminuria, slow kidney function loss over time and reduce the risk of kidney outcomes. However, clinical use of ET$_A$RA administration is limited due to sodium and fluid retention, leading to bodyweight increase, edema and potentially congestive heart failure, particularly in patients with reduced glomerular filtration rate in whom volume homeostasis is often impaired [14].

Sodium–glucose co-transporter 2 inhibitors (SGLT2i) block sodium and glucose reabsorption in the renal proximal tubule leading to glycosuria and osmotic diuresis [15–18]. These effects lead to reductions in HbA1c, total body water, weight and blood pressure. In large clinical trials, SGLT2i, including dapagliflozin, reduced the risk of heart failure and kidney disease progression [18, 19]. The modest osmotic diuretic effects of SGLT2i make them an attractive option to combine with ET$_A$RAs in order to mitigate the sodium and fluid retention induced by these agents. An exploratory *post hoc* analysis of a large clinical trial (Study Of Diabetic Nephropathy With Atrasentan (SONAR)) demonstrated that patients who initiated both the ET$_A$RA atrasentan and an SGLT2i experienced a greater reduction in albuminuria and a reduction in bodyweight whilst body weight increased in participants who initiated ET$_A$RA alone [20].

The aim of the current study was to address the hypothesis that combined initiation of SGLT2i with dapagliflozin and a

selective $ET_A RA$ zibotentan facilitates optimal fluid homeostasis. Consequently, we designed preclinical *in vivo* studies in Wistar rats fed a 4% salt diet. In the first experiment, increasing doses of zibotentan (30, 100 and 300 mg/kg) were administered once daily and the effects on hematocrit (Hct) and bodyweight were assessed as surrogate measures of fluid retention. In a subsequent study, zibotentan (30 and 100 mg/kg) was administered with and without dapagliflozin (3.0 mg/kg) to determine whether co-administration of dapagliflozin altered zibotentan-mediated effects on Hct and body weight.

MATERIALS AND METHODS

Animals

Experimental procedures were approved by the Local Ethics Review Committee on Animal Experiments (Göteborg region). Male, 8- to 9-week-old Wistar rats (Charles River, Germany) were housed in pairs in plastic cages, Makrolon type 3, in a temperature (20–22°C) and humidity (40%–60%) controlled facility with a 12 h light–dark cycle (lights on 06:00 am), and had free access to food (4% salt in chow diet #D20042901, Research Diets, USA) and tap water. The animal bedding consisted of aspen chips, gnaw blocks, shredded paper and a home for enrichment.

Experimental procedures

The study was divided into two experiments. In Experiment 1, the effect of zibotentan on Hct was determined when zibotentan was administered peroral (p.o.) daily for 14 days. Prior to administration of study drugs, the animals were randomized based on body weight into the following groups: (i) vehicle; (ii) Zibo 30 mg/kg; (iii) Zibo 100 mg/kg; and (iv) Zibo 300 mg/kg. Animals were weighed daily prior to dosing throughout the study. Blood samples (20 μL) for bioanalysis were taken at 4 and 24 h after the first dose via the tail vein from conscious animals and 24 h after the last dose via retroorbital route from anesthetized animals. Blood samples were collected via tail vein (100 μL) for Hct measurements on Days 7 and 14, 1–2 h after dosing (between 9:30 am and 12:00 pm). Twenty-four-hour urine was collected for the measurement of urinary glucose, urine volume and electrolytes between doses 9 and 10. Animals were terminated 24 h after the last dose.

In Experiment 2, the effect of zibotentan and dapagliflozin (alone or in combination) on Hct and body weight was determined. Seventy-two animals were randomized based on body weight and Hct prior to administration of study drugs into the following groups: (i) vehicle; (ii) zibotentan (Zibo) 30 mg/kg; (iii) Zibo 100 mg/kg; (iv) dapagliflozin (Dapa) 3 mg/kg; (v) Zibo 30 mg/kg + Dapa 3 mg/kg; and (vi) Zibo 100 mg/kg + Dapa 3 mg/kg. For practical reasons, the study was conducted in two consecutive slots with 36 animals (6/group) in each slot. Animals were weighed daily prior to dosing and throughout the study. Compounds were administered p.o. daily for 7 days. Blood samples for bioanalysis were collected at 4 and 24 h after the first dose and 24 h after the final dose for Hct analysis on Day 7 as described for Experiment 1. Twenty-four-hour urine was collected between the third and

fourth dose. Twenty-four-hour food and water intake were measured between Day 2 and 3 and between Day 6 and 7. With two animals per cage, food and water intake per animal in a cage was assessed by dividing by two.

Experimenters were blinded to the treatment allocation of animals during termination, blood sampling and sample analyses.

On the day of termination, animals were anesthetized using 5% isoflurane, and blood was collected via the retroorbital route (in heparin-coated microvette, Sarsted) and centrifuged at 3500 r.p.m. at 4°C for 10 min. Plasma was collected and stored at –80°C degrees prior to analysis. Animals were then euthanized by removing the heart.

Compounds and dose selection

In Experiment 1, zibotentan (AZ11922817) was dosed orally at 30 mg/kg, 100 mg/kg or 300 mg/kg. Drugs were dissolved in saline [30% (w/w) triethylene glycol (TEG) 2% (w/w) EtOH 0.5% (w/w) hydroxypropyl methylcellulose (HPMC) 10 000 c.p.s. 0.1% (w/w) Tween 80 67.4% purified water]. The volume of injection was 10 mL/kg.

In Experiment 2, zibotentan was dosed orally at 30 and 100 mg/kg with or without 3 mg/kg dapagliflozin (AZ13219875). Drugs were dissolved in saline [30% (w/w) TEG 2% (w/w) EtOH 0.5% (w/w) HPMC 10 000 c.p.s. 0.1% (w/w) Tween 80 67.4% purified water]. The volume of injection was 10 mL/kg.

Dapagliflozin dose selection was performed according to human dose-equivalent estimation of 3 mg/kg in rat scaling to a 10 mg dose in human. Zibotentan dose selection was estimated from prior work with the $ET_A RA$ sitaxentan where 30, 100 and 300 mg/kg reduced Hct in rats [21]. The corresponding doses of zibotentan, taking pharmacokinetics, plasma protein binding and ET_A affinity differences into account were 30, 100 and 300 mg/kg with a free exposure in rat scaling respectively to a 250, 750 and 2500 mg dose in human.

Plasma and urine biochemistry

Plasma and urine albumin, creatinine, glucose, urea, potassium and sodium levels were analyzed using an ABX Pentra 400 instrument (Horiba Medical, Irvine, CA, USA) according to the manufacturer's protocol. Osmolality was calculated according to the formula: $(2 \times Na + K + urea + glucose)/creatinine$.

Hematocrit and hemoglobin analysis

For Hct and hemoglobin (Hb), 100 μL of blood from tail vein was collected and measured using CG8 cartridges and analyzed with an iSTAT instrument (Abbott Point of Care Inc, Abbot Park, IL, USA). Estimated plasma volume was calculated according to the formula: $100 – Hct/Hb$.

Analysis of blood creatinine by LC-MS

Ten μl of water was briefly mixed with 10 μL of blood or calibration standards in a 96-well plate. Calibration samples contained creatinine in 90% acetonitrile at concentrations

ranging from 0.35 to 70 µM. One hundred microliters of internal standard solution (1 µM D3-creatinine in 90% acetonitrile and 10% water) was added to all samples and vortexed for 5 min. Separation was achieved in HILIC mode on a Waters BEH-Amide 5 cm × 2.1 mm column using 95% acetonitrile and 5% buffer (5 mM ammonium formate + 0.0625% formic acid) as A-phase and buffer (10 mM ammonium formate + 0.125% formic acid) as B-phase. Mass spectrometric detection was performed on a Waters Xevo TQ triple quadropol instrument.

Statistical analysis

The data for each feature measured was checked for a normal distribution and where appropriate a log transformation was applied to data with a non-normal distribution using R. Since the data were grouped by one factor zibotentan doses or combined therapy, a one-way ANOVA was used to analyze traits measured at a single time point (GraphPad version 8 or R statistics package emmeans) and a linear mixed effects model (nlme package) in R was used to analyze multiple measures data for bodyweight and provided estimated marginal means (EMM) for the difference in grams corrected for baseline bodyweight between an experimental group and vehicle. The differences between groups were tested using either a Dunnett test (emmeans package in R or GraphPad v8) when comparing treated groups to the vehicle group or a Tukey comparison (emmeans package in R or Graphpad v8) for specific pairwise comparisons of treated groups. Analysis of estimated plasma volume and calculated osmolality were performed using Brown–Forsythe and Welch ANOVA for multiple comparisons (GraphPad v9). For specific pairwise comparisons of treated groups. All model assumptions were checked using the model diagnostic plots in R statistics. Results are expressed as the mean ± standard error of the mean or mean ± standard deviation where described. Significance was set at $P < .05$.
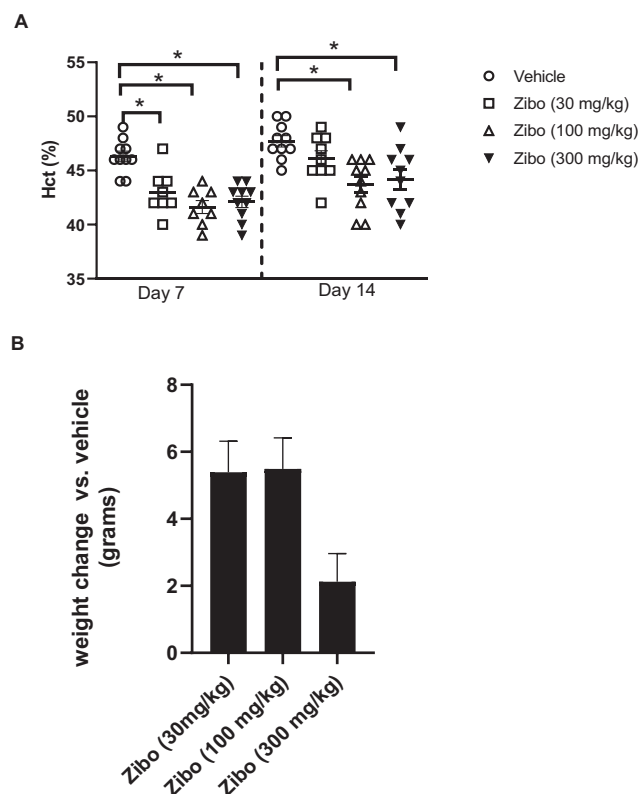
RESULTS

Zibotentan causes fluid retention

On Day 7 of dosing, zibotentan (30, 100 and 300 mg/kg/day) significantly reduced Hct compared with vehicle treatment (Fig. 1). These effects were sustained at Day 14 in the two highest zibotentan doses (100 and 300 mg/kg/day) (Fig. 1). Body weight was numerically and consistently higher throughout the study in all three zibotentan groups (Fig. 2), with least square mean differences compared with vehicle of 5.4 g [standard error (SE) 2.6], 5.5 (2.6) and 2.2 (2.7) g for the zibotentan 30, 100 and 300 mg/kg/day dose groups, respectively. None of these differences reached statistical significance (all P-values >.13).

Dapagliflozin mitigates zibotentan-induced fluid retention

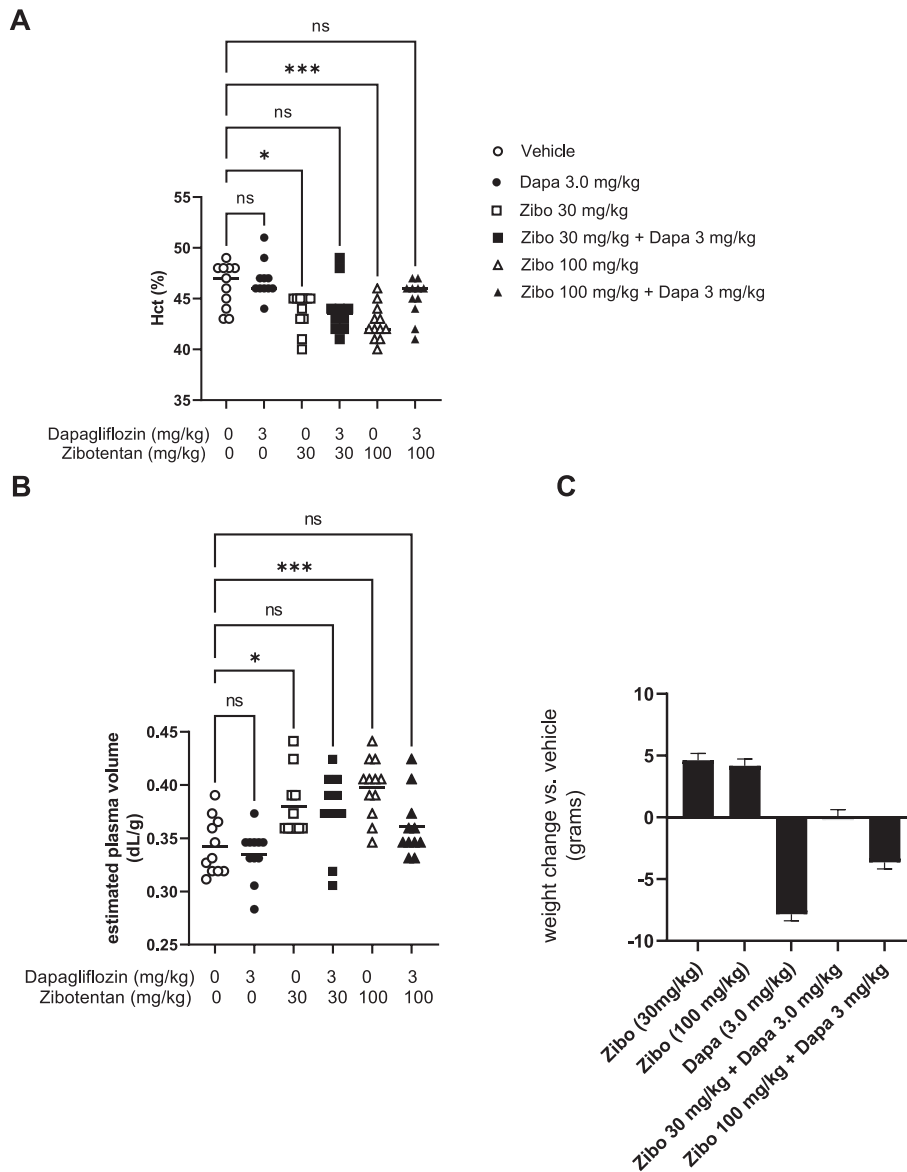Co-administration of dapagliflozin (3 mg/kg/day) and zibotentan (30 or 100 mg/kg/day) was investigated in a

second experiment. At Day 7, both zibotentan doses caused a significant reduction in Hct compared with the vehicle group (Fig. 2A). The decrease in Hct observed with zibotentan monotreatment was ameliorated in the zibotentan–dapagliflozin combination groups. Plasma Hb levels were reduced by zibotentan (30 and 100 mg/kg) and this effect was mitigated by Day 7 of co-administration with dapagliflozin (Table 1). Consistently, estimated plasma volume was significantly increased by zibotentan (30 and 100 mg/kg) and mitigated by co-administration with dapagliflozin (Fig. 2B).

Twenty-four-hour urine volume and urea excretion were unchanged in zibotentan (30 and 100 mg/kg) mono-treatment groups compared with vehicle (Supplementary data, Table S1) between doses 3 and 4. Both 24-h urine volume and urea excretion significantly increased in all dapagliflozin-treated groups with or without co-administration of zibotentan. Urinary potassium excretion was unchanged across all groups. Urinary sodium excretion was not significantly different between groups but tended to be reduced by both zibotentan doses and increased by dapagliflozin with or without co-administration of zibotentan (Supplementary data, Table S1).

Both zibotentan 30 mg/kg and 100 mg/kg increased body weight from baseline compared with vehicle by 4.6 g (SE 1.8; $P = .05$) and 4.2 g (SE 1.8; $P = .09$) (Fig. 2C). Body weight significantly decreased in the dapagliflozin 3 mg/kg group compared with vehicle by 7.8 (SE 1.8) g ($P < .001$).

Figure 2: Co-administration of zibotentan with dapagliflozin reverses zibotentan induced fluid retention. (**A**) Effect of zibotentan (30 and 100 mg/kg/day) with or without 3 mg/kg/day dapagliflozin and vehicle on Hct on Day 7 of dosing. (**B**) Effect of zibotentan (30 and 100 mg/kg/day) with or without 3 mg/kg/day dapagliflozin and vehicle on estimated plasma volumes on Day 7 of dosing. (**C**) Body weight presented as the difference in grams corrected for baseline body weight between groups. $N = 8$–12/group, *$P < .05$, *$P < .01$, *$P < .001$ vs vehicle. Data presented as mean $\pm$ SEM.

Adding dapagliflozin 3 mg/kg to zibotentan 30 mg/kg or 100 mg/kg prevented the body weight increase observed in the zibotentan mono-treatment groups (Fig. 2C) with differences in body weight compared with vehicle of 0.1 (SE 1.8) and –3.7 (SE 1.8) g.

Zibotentan does not alter dapagliflozin-driven glucosuria, food intake or water intake

Dapagliflozin significantly increased 24-h urine glucose excretion vs vehicle treatment. There was no effect of zibotentan on urinary glucose (Fig. 3A). Furthermore, co-administration of dapagliflozin with zibotentan did not change dapagliflozin-mediated glycosuria. Dapagliflozin increased 24-h food intake and water intake compared with vehicle and

zibotentan mono-treated groups (Fig. 3B and C). Dapagliflozin and zibotentan significantly increased water intake and food intake compared with vehicle, and to a similar level observed in animals administered dapagliflozin alone.

DISCUSSION

ET_ARAs are in development for the treatment of CKD, however concern exists about the development of these agents in these patients due to the risk of fluid retention. Strategies that mitigate fluid retention may enable the use of ET_ARAs in the future. Because SGLT2i exert modest diuretic effects, this study tested the hypothesis that use of the Et_ARA zibotentan with the SGLT2i dapagliflozin mitigates ET_ARA-induced fluid retention. We demonstrated
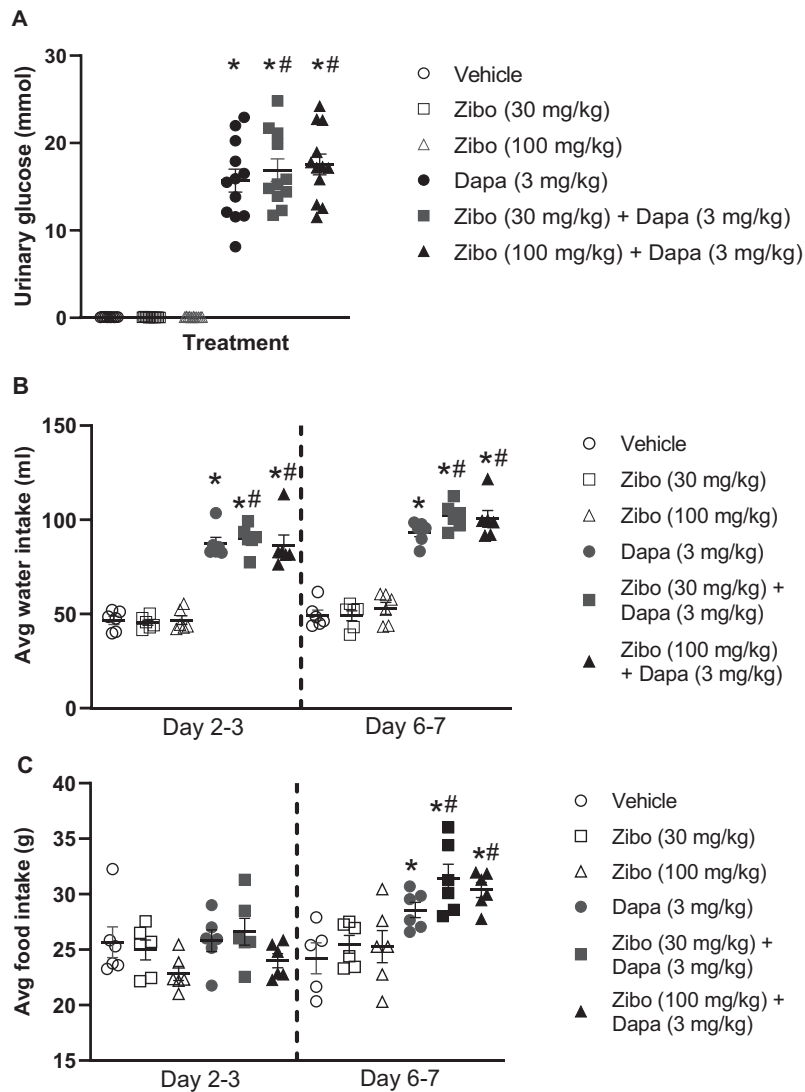
Figure 3: Effect of zibotentan, dapagliflozin and the combination on 24-h urinary glucose excretion, food intake and water intake. (**A**) Effect of 30 mg/kg/day and 100 mg/kg/day zibotentan with or without 3 mg/kg/day dapagliflozin or vehicle on 24-h urinary glucose excretion. (**B** and **C**) Twenty-hour water intake and 24-h food intake respectively. For (B) and (C), each point represents mean food intake and water intake per animal with two animals/cage. $N = 10–12$/group, $^*P < .05$ vs vehicle, $^#P < .05$ vs zibotentan mono-treatment groups. Data presented as mean $\pm$ SEM.

that zibotentan increases fluid retention in a dose-dependent fashion. Combining zibotentan with dapagliflozin, particularly zibotentan 30 mg/kg + dapagliflozin 3.0 mg/kg in the present study, completely prevented the change in Hct and body weight driven by zibotentan mono-treatment mediated fluid retention, suggesting that combination treatment may be an effective strategy to reduce fluid retention in the clinic.

This study measured changes in Hct and body weight as common and well established surrogates for fluid retention [22]. ET_AR antagonism has been shown to reduce Hct and increase body weight acutely, an effect that is reversible upon treatment cessation and consistent with a hemodilution-driven effect as opposed to a loss of red blood cell mass or an increase in fat mass, respectively [21]. In patients with type 2 diabetes and CKD, changes in Hct and body weight during 6 weeks of treatment with the ET_ARA atrasentan were associated with a higher risk of edema and heart failure

hospitalization supporting their use as early proxies of fluid retention during ET_ARA treatment. Although the lowest tested zibotentan dose (30 mg/kg) resulted in a decrease in Hct after 7 days in our study, the effect dissipated after 14 days. The lack of a sustained effect could be due to limitations in the sensitivity of Hct to detect subtle changes in fluid retention [23], or due to limitations of the employed experimental 4% salt induction model given compensatory physiological mechanisms of the kidney to achieve fluid homeostasis in the longer term [24]. By driving fluid retention through salt-loading there was a robust window to measure decreases in Hct and Hb pharmacologically, but salt-loading also reduced sensitivity to further increases in Hct or Hb levels. Thus, a clinically observed SGLT2i mechanism was not detectable in the salt-fed rats [25].

Body weight gain, another surrogate measure of fluid retention [26], increased during zibotentan treatment, although

Table 1: Effect of zibotentan, dapagliflozin and the combination on baseline corrected Hb.

Parameters	Vehicle	Zibo (30 mg/kg)	Zibo (100 mg/kg)	Dapa (3 mg/kg)	Zibo (30 mg/kg) + Dapa (3 mg/kg)	Zibo (100 mg/kg) + Dapa (3 mg/kg)
Basal Hb (g/L)	159.4 ± 2.44	160.2 ± 1.62	158.5 ± 2.00	157.9 ± 2.34	159.3 ± 2.17	158.7 ± 1.04
Day 3 Hb (g/L)	159.1 ± 2.20	144.4 ± 2.32*	147.5 ± 1.96*	164.4 ± 1.42	151.3 ± 1.62*	152.4 ± 1.02
Day 7 Hb (g/L)	157.5 ± 1.91	148.6 ± 1.86*	144.8 ± 1.69*	159.1 ± 1.88	149.7 ± 2.80	152.9 ± 1.99

Effect of 30 mg/kg and 100 mg/kg zibotentan with or without 3 mg/kg dapagliflozin, 3 mg/kg dapagliflozin alone and vehicle on Hb. $N = 8$–12/group, $*P < .05$ vs vehicle. Data analysis used one-way ANOVA, and Tukey's test for multiple comparisons. Data presented as mean ± SEM.

not as clearly as Hct in the present study. The highest dose of zibotentan, 300 mg/kg, resulted in a lower overall weight gain compared with other zibotentan doses which suggests a maximal pharmacological response was already achieved in the lower doses. Furthermore, since the estimated human-dose equivalent of rat zibotentan, 300 mg/kg, is 2500 mg or approximately 12 000% higher than the highest dose of zibotentan under clinical investigation, we conclude that further investigation of zibotentan 300 mg/kg is unlikely to be of therapeutic importance. Dapagliflozin acutely reduces body weight which is thought to reflect its diuretic effect, while during longer term treatment increased caloric loss may contribute to the body weight–lowering effects. That both the reduction in Hct and increase in body weight were abrogated when zibotentan was combined with dapagliflozin suggests that the observed effects are likely real and plausible.

Plasma volume, as calculated from Hct and Hb, confirmed the effects of zibotentan and dapagliflozin on fluid retention. Urinary sodium excretion trended in the expected directions; the failure to achieve statistical significance was likely due, at least in part, to high variability, small sample size and intrinsic challenges in detecting relatively small differences in urinary sodium excretion between groups of rodents consuming a high salt diet.

SGLT2i and ET$_A$RAs have been shown to slow the progression of CKD and reduce the risk of kidney failure in well-conducted high-quality placebo-controlled randomized clinical outcome trials [14, 27]. These two drug classes are believed to confer nephroprotection via common and different mechanistic pathways. SGLT2i cause a reduction in glomerular hyperfiltration through the restoration of tubulo-glomerular feedback. This acute reduction in glomerular hyperfiltration is associated with stabilization of kidney function during prolonged treatment [28]. In addition, SGLT2i improve tubular oxygenation and reduce tubular hypoxic stress, and exert metabolic effects, including activation of hypoxia inducible factor-1 and sirtuin-1 mediated pathways, leading to improved mitochondrial and cellular function [29]. ET$_A$RAs reduce glomerular hyperfiltration, albeit moderately, but they also improve endothelin-1 mediated injury to the podocytes, endothelial glycocalyx, mesangium (extracellular matrix accumulation and proliferation) and tubulointerstitial (fibrosis) through direct actions on these cells and compartments [4, 30, 31]. Our finding that zibotentan co-administration with dapagliflozin attenuated fluid retention without any modification of dapagliflozin's primary mechanism on increasing glucosuria supports this hypothesis.

Two other experimental studies investigated the effects of dapagliflozin and atrasentan as mono-therapies and in combination. In contrast to the *post hoc* analysis from the SONAR trial, in the first experimental study neither dapagliflozin nor atrasentan, alone or in combination, reduced UACR or improved kidney function in db/db mice [32]. In another experimental study db/db mice were treated with empagliflozin, atrasentan or ramipril in combination of two or all three drugs. Triple therapy showed a modestly larger kidney protective effect than dual therapy but acute effects on sodium and fluid homeostasis were not assessed [33]. Acute effects on markers of fluid retention and kidney protection were, however, analyzed in a small *post hoc* analysis of the SONAR trial. The results of this analysis showed that initiation of combined treatment with SGLT2i and the ET$_A$RA atrasentan is associated with less fluid retention, as measured by changes in bodyweight and N-terminal pro B-type natriuretic peptide (NT-proBNP), compared with initiation of atrasentan alone. In addition, the combination resulted in a larger reduction in UACR and blood pressure compared with mono-therapy, suggesting that the combination of both drug classes may exert beneficial synergistic effects that augment UACR reduction while offsetting fluid retention. This beneficial effect has not been described to date with traditional diuretics and would require a head-to-head study to be fully addressed. The ongoing ZENITH study (Zibotentan and Dapagliflozin for the Treatment of CKD (ZENITH-CKD); NCT04724837) is a randomized, dose-finding, controlled clinical trial to provide more definitive evidence about the efficacy and safety of combined zibotentan–dapagliflozin treatment in patients with CKD.

The present study has limitations. Firstly, since our experimental study was designed to assess pharmacological aspects of combined treatment with zibotentan and dapagliflozin we did not assess whether combination treatment conferred additional kidney protection; this requires additional studies in experimental models of kidney disease. Secondly, the lack of NT-proBNP is another limitation of our study particularly since clinical studies have demonstrated that changes in NT-proBNP during ET$_A$RA treatment predict heart failure [34]. Thirdly, this study used significantly higher human-equivalent doses of zibotentan than under investigation in human zibotentan–dapagliflozin combination CKD trial (ZENITH-CKD; NCT04724837).

In conclusion, zibotentan treatment causes dose-dependent fluid retention. Co-administration with the SGLT2i dapagliflozin minimizes this effect. If proven in a prospective clinical trial, this would make the combination of both agents an attractive therapeutic option for clinical practice since both drug classes individually have already been shown to markedly reduce the risk of kidney failure.

AUTHORS' CONTRIBUTIONS

P.B.L.H., P.J.G., C.A., V.V. and R.I.M. designed the experiments. H.J.L.H. and D.E.K. provided feedback on study plan and data produced. V.V. directed the rodent studies during the in-life phase and coordinated sample analysis. C.A. performed analysis confirming compound exposure. N.v.Z. performed the statistical analysis. V.V. and R.I.M. drafted the manuscript and all authors contributed to subsequent manuscript reviews. S.S. contributing to study/concept design in first line.

DATA AVAILABILITY STATEMENT

Data contained in this manuscript are submitted to US Patent US20220023295A1.

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