The selective mineralocorticoid receptor modulator AZD9977 reveals differences in mineralocorticoid effects of aldosterone and fludrocortisone

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Abstract

Introduction: AZD9977 is a novel mineralocorticoid receptor (MR) modulator, which in preclinical studies demonstrated organ protection without affecting aldosterone-regulated urinary electrolyte excretion. However, when tested in humans, using fludrocortisone as an MR agonist, AZD9977 exhibited similar effects on urinary Na+/K+ ratio as eplerenone. The aim of this study is to understand whether the contradictory results seen in rats and humans are due to the mineralocorticoid used.

Materials and methods: Rats were treated with single doses of AZD9977 or eplerenone in combination with either aldosterone or fludrocortisone. Urine was collected for five to six hours and total amounts excreted Na+ and K+ were assessed.

Results: AZD9977 dose-dependently increased urinary Na+/K+ ratio in rats when tested against fludrocortisone, but not when tested against aldosterone. Eplerenone dose-dependently increased urinary Na+/K+ ratio when tested against fludrocortisone as well as aldosterone.

Conclusions: The data suggest that the contrasting effects of AZD9977 on urinary electrolyte excretion observed in rats and humans are due to the use of the synthetic mineralocorticoid fludrocortisone. Future clinical studies are required to confirm the reduced electrolyte effects of AZD9977 and the subsequent lower predicted hyperkalemia risk.

Keywords
Aldosterone, AZD9977, fludrocortisone, mineralocorticoid receptor ligand, urinary electrolytes

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Introduction

Pathophysiological activation of the mineralocorticoid receptor (MR) promotes inflammation, oxidative stress and fibrosis and ultimately leads to target organ dysfunction and an increased risk of cardiovascular and renal disease.1,2 MR antagonists (MRAs) provide effective treatment for heart failure (HF),3,4 and several MRAs are currently in development for treatment of chronic kidney disease.5-7 A limitation with current MRAs is the mechanism-based risk of hyperkalemia, since MRAs interfere with MR-regulated renal sodium and potassium excretion.8

AZD9977 is a novel MR modulator that in preclinical studies exerted similar kidney protective effects as eplerenone, but in contrast to classical MRAs was devoid of acute effects on urinary Na+/K+ ratio, predicting a reduced hyperkalemia risk.9 Furthermore, AZD9977 attenuated the natriuretic effect of eplerenone, suggesting that AZD9977 binds to kidney epithelial MR, but displays a
differentiated mode of action compared with classical MR antagonists.9

AZD9977 was recently evaluated in humans in whom it was well tolerated and displayed pharmacokinetics (PK) compatible with further development.10 Effects on urine electrolytes were studied in healthy volunteers against the synthetic mineralocorticoid fludrocortisone. AZD9977 as well as eplerenone attenuated fludrocortisone-stimulated Na+ retention, suggesting that both compounds yielded similar MR occupancy. Hence the study did not prove the pharmacological differentiation of AZD9977 vs eplerenone observed previously in rats when tested against the endogenous mineralocorticoid aldosterone.9

The aim of the current study is to test whether the observed discrepancy between effects of AZD9977 on urinary electrolyte excretion in humans and lack thereof in preclinical models is due to the mineralocorticoid used. To assess this, antimineralocorticoid effects of AZD9977 and eplerenone were compared in rats using either aldosterone or fludrocortisone as mineralocorticoids.

Materials and methods

Experimental procedures to assess electrolytes effects in rats were approved by the local ethics review committee on animal experiments in the Gothenburg region (EA 141-2010). Fludrocortisone was purchased from Sigma-Aldrich, Germany, and aldosterone was purchased from Molekula Ltd, UK. Urinary Na+ and K+ concentrations were determined on a Radiometer ABL 700 blood gas analyzer (Radiometer, Copenhagen, Denmark).

Electrolyte effects in rats

Fludrocortisone experiment. Vehicle or 100 µg/kg fludrocortisone was administered orally four times per rat at two-hour intervals, which yielded plasma exposures of fludrocortisone equivalent to what was achieved in the study in humans described in Erlandsson et al.10 A rat PK study was carried out to set fludrocortisone doses: A total of 100 µg/kg fludrocortisone resulted in an average exposure of ~0.006 µM as in the clinical study.

Rats were placed in metabolic cages and 3, 10, 30 or 100 mg/kg AZD9977 or 1, 3, 10 or 30 mg/kg eplerenone was dosed two hours after the initial fludrocortisone administration by oral gavage (10 ml/kg). Urine was collected for six hours after AZD9977/eplerenone dosing and analyzed for Na+ and K+ content.

Aldosterone experiment. Rats were dosed via oral gavage with 2, 16, 54, 120 mg/kg AZD9977 or 1, 3, 10, 30, 100 mg/kg eplerenone (10 ml/kg) in a vehicle containing 2.7% NaCl. A total of 3 µg/kg aldosterone was administered subcutaneously within 15 minutes after dosing of either AZD9977 or eplerenone. Satellite animals were used to assess PK of aldosterone. The half-life of aldosterone was approximately 30 minutes and the average exposure during the first hour was ~0.003 µM. Aldosterone and fludrocortisone have similar in vitro potency as well as plasma protein binding and therefore exposures are directly comparable. Rats were placed in metabolic cages, and urine was collected for five hours after aldosterone administration and analyzed for Na+ and K+ content.

Satellite PK animals were used to assess drug exposure levels during the study duration with a total of 13 and 20 animals for AZD9977 and eplerenone, respectively. Maximum concentration (Cmax) was between 10 minutes and one hour both for AZD9977 and eplerenone. Taking PK properties and in vitro potencies on rat MR into account, a three-times higher dose of AZD9977 is required to achieve the same receptor occupancy as previously described.9

Comparison of clinical exposure response data with preclinical data

Clinical PK data previously presented by Erlandsson and colleagues10 were used to obtain the average exposure during the eight-hour study period after dosing to assess clinical vs preclinical urinary electrolyte effects at similar exposure levels. The clinical average plasma concentration was converted to unbound exposure levels using human plasma protein binding (18% unbound for AZD9977, 51% for eplerenone) and then divided by the in vitro potency to account for any in vitro potency difference between AZD9977 and eplerenone.9,10

Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology.11 Data are presented as group average ± SEM. Statistical analysis was by analysis of variance followed by Dunnett testing for multiple comparisons using Analyse-it for Microsoft Excel 4.51 (Analyse-it Software, Ltd).

Results

Fludrocortisone as mineralocorticoid

Fludrocortisone administration resulted in a reduced urinary Na+/K+ ratio (Figure 1), mainly as a result of reduced Na+ excretion (S1 Figure). Using fludrocortisone as the mineralocorticoid, AZD9977 as well as eplerenone caused dose-dependent increases in urinary Na+/K+ ratio (Figure 1) as a result of increased Na+ excretion (S1 Figure). At the highest dose, eplerenone but not AZD9977 treatment increased the urinary Na+/K+ ratio above baseline, but exposures at these doses were well above the
K⁺ excretion was slightly elevated after fludrocortisone administration, but not altered by either AZD9977 or eplerenone dosing (S1 Figure). This result is in sharp contrast to the observations in rats with elevated endogenous aldosterone, in which eplerenone, but not AZD9977, affected urinary Na⁺/K⁺ ratio. The increased urinary Na⁺/K⁺ ratio after eplerenone treatment was a result of increased Na⁺ excretion, since urinary K⁺ excretion was not measurably altered under these conditions (S2 Figure). Similar results were obtained when aldosterone was administered repeatedly and in absence of the NaCl load (S3 Figure). These results are in agreement with the observations in rats with elevated endogenous aldosterone, in which only eplerenone, but not AZD9977, affected the urinary Na⁺/K⁺ ratio.

**Discussion**

Despite clinical trial evidence demonstrating that MRAs reduce mortality and hospitalization in HF patients and a class I recommendation to use MRAs for treatment of HF with reduced ejection fraction, MRAs are among the most underutilized therapy for HF treatment. This is largely because of the mechanism-based risk for hyperkalemia, which is mainly mediated through the effects of MRAs on urinary electrolyte excretion.

Acute effect on urinary Na⁺/K⁺ ratio is an established translational biomarker for MR antagonism and is frequently used to characterize MRAs. Preclinically, acute effects of MRAs on urinary Na⁺/K⁺ are typically assessed in presence of elevated levels of the physiological mineralocorticoid aldosterone, while the synthetic,
orally available MR agonist fludrocortisone is used to activate MR in corresponding clinical trials. Studies comparing fludrocortisone with aldosterone and the pharmacological effects of MRAs in the presence of either fludrocortisone or aldosterone suggested that fludrocortisone apart from its oral bioavailability and prolonged half-life displays similar properties as aldosterone. The novel MR modulator AZD9977 is differentiated from classical MRAs since it does not alter acute urinary electrolyte excretion over a wide dose range in the presence of constantly elevated endogenous aldosterone levels in preclinical testing.9

Unexpectedly, when effects of AZD9977 on acute urinary electrolyte excretion were evaluated in a clinical trial in the presence of fludrocortisone, administration of AZD9977 increased the urinary Na⁺/K⁺ ratio to a similar extent as eplerenone.10 While the study did not prove that AZD9977 lacks effect on acute urinary electrolyte handling in humans, it did support that AZD9977 interacts with MR at expected therapeutic exposure levels.

To evaluate whether the unexpected effect of AZD9977 on urinary electrolytes seen in the clinical study is related to the use of fludrocortisone, we conducted preclinical studies with fludrocortisone in rats using a protocol that matched the clinical trial protocol. As shown here, AZD9977 dose-dependently increased urinary Na⁺/K⁺ ratio also in rats when tested against fludrocortisone. Modeling of preclinical and clinical data suggest that the observed acute effects of eplerenone and AZD9977 on urinary electrolytes in the presence of fludrocortisone quantitatively translate from rats to humans. In the preclinical study using fludrocortisone, at drug exposure levels corresponding to those assessed clinically,10 AZD9977 and eplerenone dosing resulted in an approximate two- to three-fold increased urinary Na⁺/K⁺ ratio compared with rats receiving fludrocortisone only (Figures 1 and 2), which was a similar increase as observed in humans. Thus, eplerenone and AZD9977 are equally efficacious in humans and rats with regard to inhibiting fludrocortisone-induced Na⁺ retention in the exposure range achieved in the clinical study (Figure 2). At exposures in rats exceeding the achieved clinical exposures, eplerenone, but not AZD9977, causes a further increase in urinary Na⁺/K⁺ ratio. This suggests that AZD9977 may act as a partial antagonist whereas eplerenone acts as a full antagonist upon fludrocortisone administration.

A different experimental protocol was used to study the effects of AZD9977 and eplerenone in the presence of fludrocortisone using administration of exogenous fludrocortisone as compared with previously described experiments using endogenous aldosterone elevations.9 We therefore extended the previous studies with aldosterone in rats and show here that AZD9977 lacks effects on acute electrolyte excretion also when tested against exogenously administered aldosterone. The data presented here suggest that the key difference regarding effects of AZD9977 on urinary electrolytes between the preclinical studies with aldosterone and clinical studies using fludrocortisone are related to the use of different MR agonists rather than species differences or differences in experimental setup. Despite the overall similar physiological effects of aldosterone and fludrocortisone, the use of AZD9977 and eplerenone highlight that fludrocortisone appears to affect urinary Na⁺ handling differently from aldosterone. To our knowledge, there are no publicly available data to date describing disparate molecular genomic or nongenomic effects of fludrocortisone compared with aldosterone. At the exposures achieved in this study, fludrocortisone can also activate glucocorticoid receptors, which may contribute to the observed differences in urinary electrolyte handling. Further studies are warranted to explore the molecular mechanism behind the differentiated effects of aldosterone and fludrocortisone.

![Figure 3](image-url)

Figure 3. (a) AZD9977 does not affect the urinary Na⁺/K⁺ ratio after administration of an NaCl bolus and a single dose of aldosterone whereas ((b) and gray bar in (a)) eplerenone dose-dependently elevates the urinary Na⁺/K⁺ ratio. Doses in mg/kg are indicated below each bar. Mean ± SEM, n = 7–8 per group, *p < 0.05 vs aldosterone alone. Data are assembled from two experiments.
The minimal urinary electrolyte effects of AZD9977 observed in rats in the presence of the physiological MR ligand aldosterone may therefore translate to a negligible urinary electrolyte effect for AZD9977 at therapeutic exposure in patients as well and support that AZD9977 may be superior to classical MRAs with regards to potassium retention. Further clinical trials will clarify whether AZD9977 treatment in patients has a reduced effect on plasma potassium compared with MRAs.

Conclusions
The presented data using the MR modulator AZD9977 suggest different mineralocorticoid effects of the synthetic mineralocorticoid fludrocortisone compared with the physiological mineralocorticoid aldosterone. The data suggest that fludrocortisone-based pharmacodynamic clinical studies may be appropriate for testing traditional MRAs as previously argued, but studies assessing MR modulators should be conducted in the presence of aldosterone. While fludrocortisone studies in healthy volunteers translate reasonably well to potassium retention in patients on MRAs, such translation for MR modulators may not be as straightforward.

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