

Abstract

Background: Significant reduction of serum uric acid (sUA) was discovered in a multiple dose study of RDEA806. Efforts have been devoted to confirm the clinical laboratory findings, to elucidate the likely active moiety and mechanism responsible for the sUA lowering effect, and to evaluate the potential drug-drug interaction (DDI) in rats based on potential interactions with renal transporters.

Method: To confirm the clinical laboratory measurement of sUA by colorimetry in the multiple dose study and to eliminate the possible interference from RDEA806 and its metabolites, uric acid levels in plasma were measured using high-performance liquid chromatography equipped with tandem mass spectrometry (LC-MS/MS). To determine the likelihood of RDEA806 or its metabolites decreasing uric acid production, the effect on xanthine oxidase and purine nucleoside phosphorylase activity was measured. In addition to this, hypoxanthine and xanthine levels in plasma from humans dosed with RDEA806 were measured by LC-MS/MS. To correlate urine levels of RDEA806 and its metabolites with the change in uric acid excretion into urine, uric acid, RDEA806, and its metabolites in urine were also analyzed from a single dose study in healthy volunteers. Evaluation of a DDI potential to kidney transporters OAT1 and OAT3 was conducted in rats by measuring renal excretion of tenofovir (PMPA) following co-dosing of RDEA806 or its metabolite RDEA594 with tenofovir disoproxil fumarate. The inhibitory potentials of RDEA806 and its metabolites on URAT1, a transporter responsible for uric acid reabsorption, were explored *in vitro* using URAT1-expressing oocytes.

Results: Results from LC-MS/MS analysis of the BID 400-mg RDEA806 dosing group showed a mean change of -42.6% of uric acid level in plasma, which is similar to serum analysis (-47.9%) using colorimetry, thus confirming the uric acid lowering effect and eliminating the possible interference from RDEA806 and its metabolites in serum analysis. Statistical analyses (t-test) comparing the RDEA806- and placebo-dosed groups showed no conclusive difference in hypoxanthine or xanthine levels between RDEA806 and placebo groups; therefore, the possibility of RDEA806 or its metabolites acting as a xanthine oxidase inhibitor is unlikely. This was confirmed by the lack of inhibition of xanthine oxidase and purine nucleoside phosphorylase enzymatic activity *in vitro*. Excretion of uric acid in urine showed a linear correlation ($r > 0.98$) with the excreted amount of RDEA594, a major metabolite of RDEA806, but not with RDEA806 or other metabolites (M1 and M3). There is no major difference in PMPA renal excretion following co-dosing of RDEA806 or RDEA594 with tenofovir disoproxil fumarate in rats, indicating that neither compound has an effect on the kidney OAT1 and OAT3 transporters. *In vitro* experiments revealed that two metabolites of RDEA806, RDEA594 and M3, inhibited the uptake of uric acid on URAT1-expressed oocytes.

Conclusions: These studies show that the likely mechanism of lowering sUA following multiple dosing of RDEA806 is due to the increased urinary excretion of uric acid. Among RDEA806 and its metabolites excreted in urine, RDEA594 was determined to be the active moiety due to its abundance in urine, the excellent correlation between its amount and the amount of uric acid excreted in urine, and inhibitory potential to URAT1-expressing oocytes.

Introduction

Significant reduction of serum uric acid (sUA) was discovered in clinical studies of RDEA806. Efforts have been devoted to confirm the clinical laboratory findings, to elucidate the likely active moiety and mechanism responsible for the sUA lowering effect, and to evaluate the potential drug-drug interaction (DDI) in rats based on potential interactions with renal transporters.

Methods

- Confirmation of colorimetric analysis of sUA in human by more specific high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis
- In vitro* analysis of RDEA806 or its primary metabolite (RDEA594) as xanthine oxidase or purine nucleoside phosphorylase inhibitors
- Quantification of hypoxanthine and xanthine levels in plasma by LC-MS/MS from humans dosed with RDEA806
- In vitro* evaluation of the inhibitory potential of RDEA806 and its metabolites on URAT1, a transporter responsible for uric acid re-absorption, using URAT1-expressing oocytes
- Correlation analysis of RDEA806 and RDEA594 with uric acid excretion in human urine following dosing of RDEA806
- Evaluation of DDI potential to kidney transporters hOAT1 and hOAT3 by measuring renal excretion of tenofovir (PMPA) following co-dosing of RDEA806 or its metabolite RDEA594 with tenofovir disoproxil fumarate

Results

Figure 1. Reduction in sUA Confirmed by Colorimetric and LC-MS/MS Analyses Following Multiple Doses of RDEA806

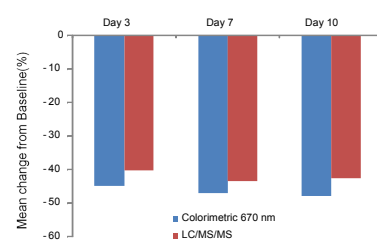


Figure 2. RDEA806 and its Metabolites are Unlikely to be Xanthine Oxidase Inhibitors in the *In Vitro* Assay

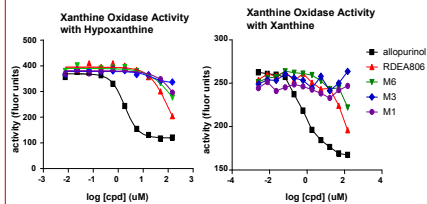


Figure 3. RDEA806 and its Metabolites are Unlikely to be Purine Nucleoside Phosphorylase Inhibitors in the *In Vitro* Assay

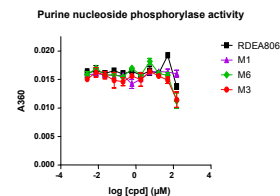


Figure 4. Higher Excretion of Urinary Uric Acid in 24h Following a Single Dosing of RDEA806 Indicating Uricosuric Effect

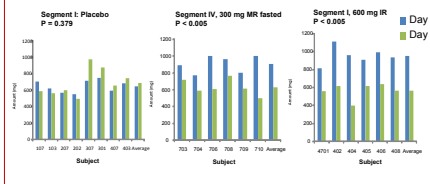
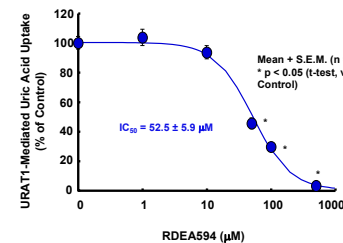


Figure 5. Inhibitory Effect of RDEA594 on Uric Acid Uptake by URAT1 Expressed in *Xenopus* Oocytes



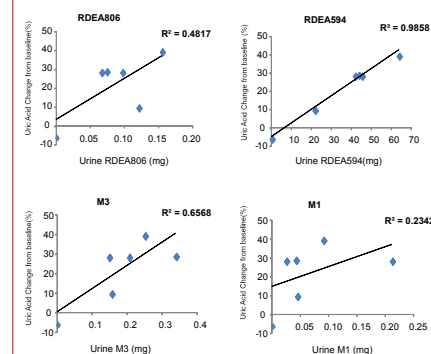
- Although RDEA594 is a modest inhibitor of URAT1, it reaches high concentrations at the site of action, the urine

Table 1. Concentration of RDEA806, RDEA594 and Other Metabolites in Urine Following Single Dose of 600 mg RDEA806 Immediate-Release Capsule

Time (hr)	Average Concentration (uM)			
	RDEA806	RDEA594	M3	M1
0	0.00	0.00	0.00	0.00
0 - 6	0.167	103	0.184	0.371
6 - 12	0.193	138	0.455	0.270
12 - 24	0.159	33.6	0.946	0.263
24 - 48	0.0171	6.16	0.0999	0.0385
48 - 72	0.00224	0.814	0.0164	0.00

- Only RDEA594 urine concentrations reach sufficient levels to inhibit URAT1 metabolite

Figure 6. Excellent Correlation Between RDEA594 and Uric Acid Changes in Urine Following Single Doses of RDEA806



Data from phase 1 clinical study in NHV dosed 300 - 600 mg as a single dose

Table 2. Minor Change of Tenofovir (PMPA) Urinary Excretion in Rats Following Co-administration of Tenofovir Disoproxil Fumarate with RDEA806 or RDEA594

Group	Mean ± %CV Urinary Excretion of Tenofovir (PMPA), %			
	Tenofovir Only	Tenofovir + RDEA806	Tenofovir + RDEA594	NA
1	46 ± 5	49 ± 5	NA	NA
2	51 ± 3	NA	56 ± 10	NA

- Tenofovir disoproxil fumarate was dosed
- Based on minimal changes in excretion, there doesn't seem to be an impact on OAT1 and OAT3 transporters

Conclusions

- Unexpected significant decrease in sUA observed with RDEA806 in Phase 1.
- Neither RDEA806 nor its metabolites are xanthine oxidase or PNP inhibitors.
- Statistically significant increase in uric acid excretion was seen in Phase 1 indicating a uricosuric effect.
- Excretion of uric acid correlated ($r^2 > 0.98$) with RDEA594 but not RDEA806, M1, or M3 in urine.
- RDEA806 or RDEA594 do not appear to significantly inhibit or induce OAT1 and OAT3.
- RDEA594, the active moiety of RDEA806, is an inhibitor of URAT1 transporter and reaches pharmacologically active concentrations in urine.