

BRIEF REPORT

Influence of Rifampin-Mediated Organic Anion-Transporting Polypeptide 1B1/1B3 Inhibition on the Pharmacokinetics of Clazosentan

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Clazosentan is a selective endothelin A receptor antagonist in development for the prevention and treatment of vasospasm postsubarachnoid hemorrhage. It is a substrate of organic anion-transporting polypeptide 1B1/1B3 based on preclinical data. This randomized, double-blind, two-period, cross-over study investigated the pharmacokinetics, safety, and tolerability of an intravenous infusion of clazosentan (15 mg/hour for 3 hours) after the intravenous administration of placebo or rifampin (600 mg/100 mL in 30 minutes). A total of 14 healthy male participants were enrolled resulting in 13 completers. Clazosentan exposure was three to four times higher after organic anion-transporting polypeptide 1B1/1B3 inhibition, as reflected by the geometric mean ratio (90% confidence interval) of area under the plasma concentration-time curve from zero to infinity: 3.88 (3.24–4.65). Clearance and volume of distribution decreased to a similar extent. Elimination half-life was not affected. A similar pattern but a higher incidence and frequency of adverse events were observed when clazosentan was given with rifampin than with placebo.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Clazosentan is a selective endothelin A receptor antagonist formulated for parenteral use that is in clinical development for the treatment of aneurysmal subarachnoid hemorrhage. Clazosentan is mainly excreted unchanged in feces, and its exposure is increased in hepatically impaired patients.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study investigated the effect of inhibition of the uptake transporters organic anion-transporting polypeptide 1B1/1B3 on the pharmacokinetics of clazosentan.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Following rifampin-mediated inhibition of the uptake transporters organic anion-transporting polypeptide 1B1/1B3, exposure to clazosentan increased threefold to fourfold. Clearance and volume of distribution decreased to a similar extent, whereas the elimination half-life was not affected.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The pharmacokinetics and safety data gathered in this study suggest that organic anion-transporting polypeptide 1B1/1B3 plays an important role in the disposition of clazosentan.

Endothelin-1 (ET-1) is one of the most potent vasoconstrictors known.¹ ET-1 concentration is increased in a number of different diseases such as aneurysmal subarachnoid hemorrhage.² As a selective endothelin A receptor antagonist, clazosentan inhibits ET-1-mediated vasoconstriction and has demonstrated efficacy by reducing the frequency and severity of cerebral vasospasm following severe aneurysmal subarachnoid hemorrhage.^{3–5} Cerebral vasospasm is considered one of the major causes of morbidity and mortality in these patients.⁶ Clazosentan (at a dose of 15 mg/hour) is in development for the prevention and treatment of vasospasm associated with aneurysmal subarachnoid hemorrhage (NCT03585270).

The pharmacokinetics (PK) of clazosentan are characterized by dose-proportional exposure during the investigated dose range 3–60 mg/hour.⁷ The volume of distribution at steady state (V_{ss}) and clearance (CL) ranged between 23.0–32.4 L and 35.5–43.9 L/hour, respectively. The terminal elimination half-life ($t_{1/2}$) was approximately 2 hours. Clazosentan is mainly excreted as unchanged drug via bile,⁸ and the PK of clazosentan are affected by hepatic, but not renal, impairment.^{9,10} Tolerability was limited by dose and duration of infusion.⁷ The maximum tested doses were 60 mg/hour for 6 hours and 30 mg/hour for 12 hours. Dose-limiting adverse events (AEs) in healthy participants were headache, nausea, and vomiting.

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In vitro studies have shown that clazosentan is a substrate of the organic anion-transporting polypeptide (OATP) 1B1/1B3 (data on file). As per US Food and Drug Administration¹¹ and European Medicines Agency (EMA)¹² recommendations, interactions between clazosentan and an OATP1B1/1B3 inhibitor need to be investigated in humans. Several studies have revealed that single-dose administration of rifampin leads to the inhibition of OATP1B1/1B3,¹³ and rifampin is considered a reference inhibitor of OATP1B1/1B3 as per US Food and Drug Administration guidance.¹¹ This study investigated the impact of rifampin-mediated OATP1B1/1B3 inhibition on the PK of clazosentan.

MATERIAL AND METHODS

Participants

Healthy male participants aged between 18 and 65 years with a body mass index between 18.0 and 30 kg/m² were enrolled in this study. The screening visit included medical and drug use history recording, physical examination, assessment of body weight and height, and clinical laboratory, vital sign, and standard electrocardiogram data.

Written informed consent was obtained from each participant prior to any study procedure. The protocol was approved by the ethics committee (Medisch Ethische Toetsings Commissie, Assen, The Netherlands). This study was performed according to good clinical practice and in accordance with the principles of the Declaration of Helsinki.

Study design

This single-center, randomized, double-blind, placebo-controlled study had a two-period cross-over design.

The sample size was based on a precision estimate. A total of 14 participants were enrolled to one of the two treatment sequences A-B or B-A (1:1 ratio). Treatment A consisted of rifampin placebo (saline, 100 mL) immediately followed by clazosentan (15 mg/hour), and treatment B consisted of rifampin (600 mg/100 mL) immediately followed by clazosentan (15 mg/hour). All drugs were administered intravenously (i.v.). Drugs were administered in sequential order: rifampin or its placebo from 0 to 30 minutes followed by clazosentan from 30 minutes to 3 hours 30 minutes. Infusion material was masked, and the same rate of infusion was applied for saline and rifampin to ensure the blinding.

A light breakfast was administered approximately 30 minutes prior to the start of the rifampin/placebo infusion.

PK assessments

Blood samples of about 3.4 mL were collected in ethylene di-amine tetra acetic acid tubes at predose and every 30 minutes from the start to the end of clazosentan infusion, 2, 5, 10, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 7, and 21 hours after the stop of clazosentan infusion. After centrifugation, plasma was transferred into a polypropylene tube and stored at -70°C ($\pm 5^{\circ}\text{C}$) pending analysis.

Plasma concentrations of clazosentan were determined using a validated liquid chromatography coupled to tandem mass spectrometry assay⁹ with a lower limit of quantification of 4 ng/mL, and the method was linear in the concentration

range 4–4,000 ng/mL. An analysis of quality-control samples of all runs showed that interbatch coefficient of variation (precision) was $< 3.9\%$, whereas the average intrabatch accuracy was in the range 1.1–5.8%.

Noncompartmental PK analyses were performed using Professional WinNonlin 8.0 software (Pharsight Corp., Mountain View, CA). C_{max} (maximum plasma concentration) was directly obtained from the plasma concentration-time profiles, area under the plasma concentration-time curve (AUC) from zero to time *t* of the last measured concentration above the limit of quantification (AUC_{0-t}) and AUC from 0–3 hours after the start of clazosentan infusion (AUC₀₋₃) were calculated using the trapezoidal method.¹⁴ AUC from 0 to infinity (AUC_{0-∞}) was calculated by combining AUC_{0-t} and area under the plasma concentration-time curve extra. Area under the plasma concentration-time curve extra represents an extrapolated value obtained by C_t/λ_z , where C_t is the last plasma concentration measured above the lower limit of quantification, and λ_z represents the elimination rate constant determined by log-linear regression analysis. Terminal elimination half-life was calculated as $\ln 2/\lambda_z$. CL and V_{ss} were calculated as follows: $\text{CL} = \text{Dose}/\text{AUC}_{0-\infty}$ and $V_{ss} = \text{CL} ((\text{AUMC}/\text{AUC}) - (\text{infusion time}/2))$, where AUMC was the area under the first moment curve.

Safety assessments

Safety and tolerability were evaluated based on AE, vital sign (supine blood pressure), 12-lead electrocardiogram, clinical laboratory, and physical examination data at screening, predose, and up to 24 hours postdose in each period.

RESULTS

Demographics

The mean (standard deviation (SD)) age and body mass index were 52.9 (13.6) years and 25.2 (2.8) kg/m², respectively. All participants were white except for one Asian participant.

A total of 13 participants completed the study per protocol, and one participant discontinued the study for personal reasons after completion of the first period (rifampin + clazosentan) and was not included in the PK analysis.

PK

The PK parameters are presented in **Table 1**, and the plasma concentration-time profiles of clazosentan are depicted in **Figure 1**.

Exposure to clazosentan was 3–4 times higher when administered after rifampin, as reflected by the ratio (90% confidence interval) of the geometric mean of C_{max} (3.13 (2.53–3.88)), AUC₀₋₃ (3.37 (3.07–3.70)), AUC_{0-t} (3.89 (3.24–4.66)), and AUC_{0-∞} (3.88 (3.24–4.65)). Rifampin administration led to a marked decrease in CL and V_{ss} of clazosentan of 3.9-fold and 2.4-fold, respectively. The ratio (90% confidence interval) of the geometric mean of $t_{1/2}$ of clazosentan (1.09 (0.96–1.24)) was not affected by rifampin (**Table 1**).

A participant with outlier plasma concentrations 30 minutes and 1 hour after the stop of clazosentan infusion led to a second peak of the mean plasma concentration-time curve.

Safety and tolerability

Overall, 13 participants (92.9%) reported 44 treatment-emergent AEs. The pattern and time of the onset of AEs was comparable between treatments. The incidence of AEs was higher following treatment B (12 participants, 29 AEs) when compared with treatment A (7 participants, 15 AEs). The most common AE was headache (10 of 14

Table 1 Summary of pharmacokinetic variables of clazosentan when infused at a dose of 15 mg/hour for 3 hours after a short (30 minutes) intravenous infusion of placebo (treatment A) or rifampin (treatment B) and geometric mean ratios for the comparison of treatments (n = 13)

Parameters	Treatment A	Treatment B	Treatment B/A
C_{max}	519 (400–672)	1,613 (1,445–1,802)	3.13 (2.53–3.88)
AUC_{0-3}	1,077 (963–1,204)	3,628 (3,263–4,033)	3.37 (3.07–3.70)
AUC_{0-t}	1,381 (1,094–1,744)	5,326 (4,710–6,022)	3.89 (3.24–4.66)
$AUC_{0-\infty}$	1,395 (1,106–1,759)	5,376 (4,755–6,078)	3.88 (3.24–4.65)
$t_{1/2}$	1.36 (1.16–1.61)	1.51 (1.40–1.64)	1.09 (0.96–1.24)
CL	32.3 (25.6–40.7)	8.37 (7.40–94.6)	0.26 (0.21–0.31)
V_{ss}	23.5 (20.1–27.5)	9.90 (8.68–11.3)	0.42 (0.36–0.49)

Data are expressed as geometric mean (95% confidence interval) for treatments A and B and as ratio of geometric mean (90% confidence interval) for treatment B/A.

AUC_{0-3} , area under the plasma concentration-time curve from time zero to 3 hours (ng hour/mL); $AUC_{0-\infty}$, area under the plasma concentration-time curve from zero to infinity (ng hour/mL); AUC_{0-t} , area under the plasma concentration-time curve from time zero to time t of the last measured concentration above the limit of quantification (ng hour/mL); CL, clearance (L/hour); C_{max} , maximum observed plasma concentration (ng/mL); $t_{1/2}$, terminal half-life (h); V_{ss} , volume of distribution at steady state (L).

participants, 15 AEs) with a higher incidence in treatment B (8 participants, 9 AEs) when compared with treatment A (5 participants, 5 AEs). The incidence and frequency of AEs commonly reported after clazosentan infusion, i.e., nausea and vomiting, were also higher following treatment B when compared with treatment A.

The severity of AEs was also unbalanced between treatments: five participants (9 AEs) reported moderate AEs of headache following treatment B compared with a single participant (1 AE) following treatment A. All of the other AEs were of mild intensity. The duration of AEs of headache and nausea was longer following treatment B compared with treatment A. All AEs resolved without sequelae.

No clinically relevant changes in clinical laboratory, vital signs, or body weight were determined. Incidence, nature, and frequency of electrocardiogram abnormalities reported in the study were balanced between treatments.

DISCUSSION

In this study, clazosentan exposure was threefold to fourfold higher upon pretreatment with the OATP1B1/1B3 inhibitor rifampin when compared with placebo. CL and V_{ss} were 3.9 and 2.4 times lower, respectively, after the administration of rifampin, and terminal elimination half-life was not affected.

Clazosentan was administered i.v. at 15 mg/hour and is a therapeutically relevant dose because it was investigated in several efficacy trials.⁴⁻⁶ Although multiple dosing with rifampin leads to the induction of the cytochrome P450 3A4 enzyme, single-dose administration of

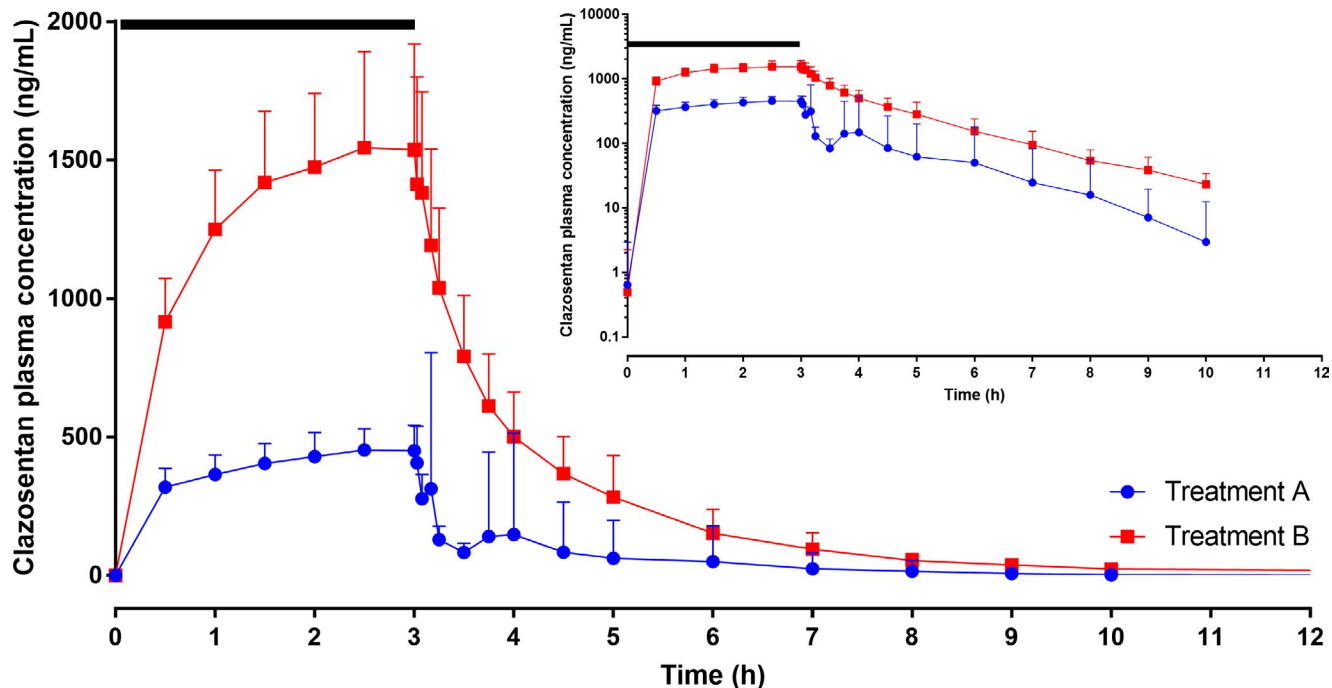


Figure 1 Arithmetic mean (\pm standard deviation) plasma concentration-time profile (with linear scale, semilogarithmic scale shown as inset) of clazosentan after infusion of 15 mg/hour clazosentan for 3 hours following a short intravenous infusion of placebo (treatment A) or rifampin (treatment B); $n = 13$.

600 mg i.v. rifampin leads to an exposure that triggers relevant inhibition of the OATP1B1/1B3 transporters.^{11,15} Because clazosentan is not a substrate of the cytochrome P450 3A4 enzyme,⁸ the effect of rifampin on the PK of clazosentan was only triggered by its inhibitory effect on OATP1B1/1B3. Considering that steady-state conditions were reached approximately 2–3 hours after the start of clazosentan infusion and clazosentan does not accumulate,⁷ the effects observed in the present study can be extrapolated to longer infusion of clazosentan. The exposure to oral rifampin has been shown to be affected by food intake.¹⁶ Because a breakfast was given to the participants prior dosing to improve the tolerability of clazosentan, administration of rifampin as an i.v. infusion was preferred. In addition, although plasma concentrations of rifampin were not measured, the short i.v. infusion of rifampin ensured the compliance of administration. The administration of placebo allowed to apply a double-blind approach to investigate the safety and tolerability in an unbiased manner. Genotyping for OATP1B1 was not performed in this study. The low variability observed in the PK data (coefficient of variation < 30% for each PK parameter) and comparable extent of increase in exposure in all participants when clazosentan was administered after rifampin suggest that the results were not affected by interindividual differences in the OATP1B1 genotype.

Considering the excretion via the bile as an unchanged drug,⁸ the fast distribution phase, and the small volume of distribution, it is likely that the excretion of clazosentan requires active transport. Because no major metabolites have been found in the human distribution, metabolism, and excretion study,⁸ the contribution of metabolizing enzymes is likely limited, and therefore the rate-determining process in the disposition of clazosentan, i.e., its hepatic clearance, is likely hepatic uptake mediated by transporters. The inhibition of hepatic uptake by OATP1B1/1B3 led to a decrease in CL and therefore an increase in exposure. Several other endothelin A receptor antagonists, for example, bosentan¹⁷ and tezosentan,¹⁸ are also substrates of OATP transporters. In this context, when given on top of cyclosporine, an inhibitor of several transporters including OATP1B1/1B3, the exposure to tezosentan was fourfold higher, and CL and V_{ss} were 4.0 and 2.9 times lower, respectively.¹⁸ The changes in the PK parameters of clazosentan observed after the infusion of rifampin were of similar extent. The inhibition of biliary excretion by cyclosporine led to an increase in the renal excretion of tezosentan.¹⁸ A similar conclusion is, however, not possible here because of the lack of urine sampling. As observed with other drugs, e.g., statins, inhibition of the uptake transporters OATP1B1/1B3 may prevent distribution into tissues, leading to a decrease in V_{ss} .¹⁹ The increase in exposure to clazosentan and the decrease in CL when administered after rifampin administration suggest that OATP1B1/1B3 is mainly involved in the disposition of clazosentan.

Clazosentan administered after rifampin or its placebo was safe and tolerated, although the incidence and frequency of AEs commonly reported after infusion of clazosentan, i.e., headache, nausea, and vomiting, were higher after rifampin than placebo. As previously reported,

the incidence, frequency, and severity of the AEs of headache, nausea, and vomiting increase with dose and/or duration of clazosentan infusion.⁷ The maximum tested dose of 60 mg/hour for 3 hours led to a similar safety profile as that observed here when clazosentan was administered on top of rifampin. The safety and tolerability data reported in this article also support the fact that the increase of approximately fourfold in exposure when clazosentan was administered on top of rifampin, corresponding to a dose of 60 mg/hour, led to the higher incidence, frequency, and severity of AEs.

Because rifampin is the reference drug for OATP1B1/1B3 inhibition, interactions with other inhibitors would likely trigger similar or lower increases in the exposure to clazosentan. Potential dose adjustment or prohibited coadministration of clazosentan with medications inhibiting OATP1B1/1B3 (e.g., cyclosporine) will be defined based on emerging clinical data from the ongoing pivotal study.

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Conflict of Interest. P.E.J., M.U., and J.D. are employees of Idorsia Pharmaceuticals, Ltd., the sponsor of the study, and possess stock options/shares. C.V.P. and P.D. had support from QPS for the submitted work and were employed by QPS in the previous 3 years.

Author Contributions. All authors wrote the manuscript. P.E.J., J.D., and M.U. designed the research. P.D. and C.V.P. performed the research. P.E.J. analyzed the data.

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