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Brain Penetration of Isavuconazole Following Single Dose Oral Administration to Wistar Rats

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ABSTRACT

Background: Area under the curve (AUC) has been reported as the pharmacokinetic/pharmacodynamic driver for treatment efficacy of triazole antifungal agents. Isavuconazole was evaluated *in vivo* in rats after p.o. administration (as isavuconazonium sulfate) to determine plasma and brain tissue concentrations.

Methods: A 25 mg/kg isavuconazole equivalent dose was administered orally (10 mL/kg of a NaCl 0.9% solution) as isavuconazonium sulfate to 24 non-fasting, non-infected Wistar rats. Blood (heparin) and brain (snap-frozen) samples were collected from three rats per time point (pre-dose, 0.25, 0.5, 1, 2, 3, 6, 8, and 24 h post-administration). Plasma and brain concentrations of isavuconazole were determined with a validated liquid chromatography–mass spectrometry/mass spectrometry method.

Results: Isavuconazole in brain reached peak concentrations approximately twice that in plasma (average individual brain/plasma ratio of 1.8 +/–16%) and declined in parallel to those in plasma. The $AUC_{0-\infty}$ was 54.3 µg.h/mL in brain compared with 30.7 µg.h/mL in plasma (**Table 1**). The isavuconazole concentrations in the brain are consistent with previous distribution studies where radiolabeled material were administered (quantitative whole-body autoradiography in rat[1]).

Conclusions: Brain penetration of isavuconazole was efficient in rats, as levels and overall exposure in brain were almost 2-fold higher than plasma.

INTRODUCTION

- Invasive fungal diseases (IFDs) present a significant therapeutic challenge particularly in immunocompromised patient populations.[2,3]
- CNS fungal infections pose an even greater challenge as brain penetration of an antifungal agent is a key determinant of its potential efficacy.[4]
- Isavuconazonium sulfate is the prodrug of the active triazole antifungal agent isavuconazole, which has been approved by the US Food and Drug Administration for the treatment of adults with invasive aspergillosis (IA) and invasive mucormycosis, and by the European Medicines Agency for the treatment of adults with IA, and for mucormycosis when treatment with amphotericin B is inappropriate, based on the results of Phase 3 clinical trials.[5,6]
- This study investigated the extent of isavuconazole penetration into the brain of male Wistar rats and compared those concentrations to that seen in plasma.
  - We also compared plasma and brain tissue concentrations of isavuconazole with those of voriconazole, an antifungal agent for which brain penetration has been established.[7]

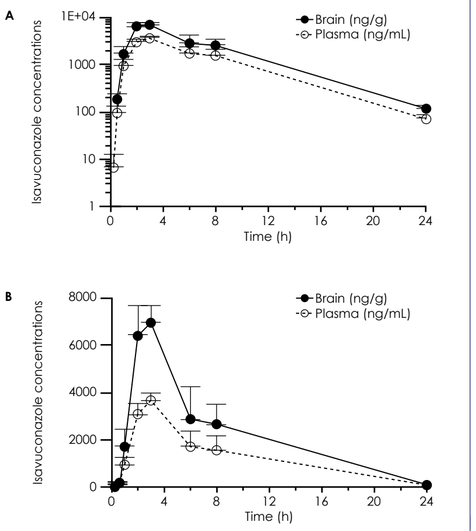
METHODS

- Two groups of 24 non-fasting, non-infected male Wistar rats (297–352 g; Harlan, The Netherlands) were used;
  - Animal procedures and handling complied with all applicable protocols and licenses; animals were individually housed in suspended, stainless steel cages during the acclimation and test periods, maintained at 20 to 26°C, and provided *ad libitum* access to food (except during dosing procedures) and water.
- Isavuconazonium sulfate (25 mg/kg isavuconazole equivalent in 0.9% NaCl – 10 mL/kg) or voriconazole (50 mg/kg – 10 mL/kg of Vfend® oral suspension diluted 1:8 with NaCl 0.9%) were administered as a single oral dose.
- Blood (1 mL) was collected via cardiac puncture into heparinized vials from 3 rats per time point (pre-dose, 0.25, 0.5, 1, 2, 3, 6, 8, and 24 h post-administration).
- Plasma proteins were precipitated by adding a 5-fold excess of acetonitrile (containing 0.5% trifluoroacetic acid) to the plasma samples.
- Samples were centrifuged, the supernatants collected then vortexed and 2 µL of supernatant was injected into the liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) system (Waters, Xevo TQ-S, Waters Corporation, Manchester, UK).
- A validated LC–MS/MS method was used to determine plasma concentrations of isavuconazole and voriconazole.
  - The lower limit of quantification for both analytes was 10 ng/mL in plasma and 50 ng/g in brain.
- Rat brains were collected immediately after terminal blood collection, weighed, frozen in liquid nitrogen and stored at −80°C until processing; brain isavuconazole concentrations were determined by LC–MS/MS:
  - Brains were diluted 1:5 with water before being homogenized for 60 sec at high velocity with an ultra-turrax tube disperser (IKA Werke GmbH & Co. KG, D-79219 Staufen, Germany).
  - Brains from untreated rats, spiked with known concentrations of isavuconazonium, isavuconazole, and voriconazole were used for calibration and quality control.

RESULTS

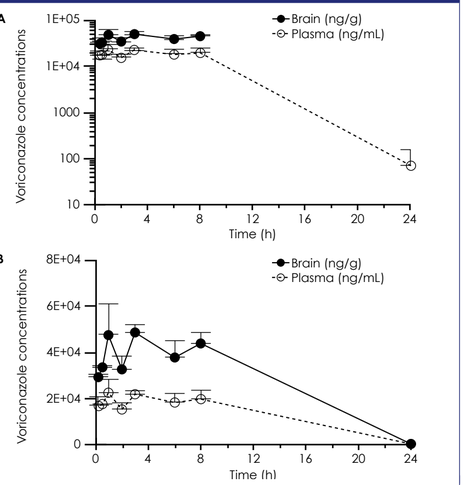
- The maximum plasma concentration of isavuconazole was reached within 3 h of a single oral dose of the prodrug and declined biphasically thereafter (**Figure 1A**).
- The concentrations of isavuconazole in brain paralleled those in plasma, with the maximum concentration in brain approximately double that observed in plasma (average individual brain/plasma concentration ratio, 1.8; coefficient of variation [CV], 16%) (**Figure 1B**).
- Plasma concentrations of voriconazole following a single oral dose were variable, with no clear maximum observed within 8 h post-dose (**Figure 2A**).

Figure 1. Mean isavuconazole concentrations (+SD) in Wistar rats following a single oral dose of 25 mg/kg eq. isavuconazole administered as isavuconazonium sulfate. (A) Lin/Log scale (B) Lin/Lin scale



- Similar to isavuconazole, brain concentrations of voriconazole paralleled those in plasma and the maximum concentration in brain was approximately double that observed in plasma (average individual brain/plasma concentration ratio, 2.2; CV, 13%) (**Figure 2B**).

Figure 2. Mean voriconazole concentrations (+SD) in Wistar rats following a single oral dose of 50 mg/kg. (A) Lin/Log scale (B) Lin/Lin scale



- Total exposure over the dosing period was almost double for brain tissue compared with plasma for both isavuconazole and voriconazole (**Table 1**).

Table 1. Derived pharmacokinetic parameters of isavuconazole and voriconazole in plasma and brain following a single oral administration in rats

Compound Matrix	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (µg/mL)	AUC_{last} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)
Isavuconazole					
Brain	3.7	3.0	6.95	53.7	54.3
Plasma	3.8	3.0	3.67	30.3	30.7
Voriconazole					
Brain	NR	3.0	48.07	321.5	NR
Plasma	2.1	1.0	21.97	304.5	304.7

$AUC_{0-\infty}$, area under the concentration–time curve from time 0 to the last measurable time point; $AUC_{0-\infty}$, area under the concentration–time curve from 0 to infinity; C_{max}, maximum concentration observed; NR, not reported; t_{max}, time of maximum concentration observed; $t_{1/2}$, terminal half-life.

CONCLUSIONS

- Isavuconazole demonstrated efficient brain penetration in rats.
  - Ratios of maximum isavuconazole concentrations in brain and plasma were similar to those for voriconazole, although absolute concentrations of voriconazole in both brain and plasma were less predictable.
- Brain isavuconazole concentrations in the current study were consistent with previous studies that assessed the distribution and fate of radiolabeled isavuconazonium sulfate in rat.[1]
  - The parallels between the concentration–time profiles in brain and plasma also support the lack of an effect of P-glycoprotein-mediated transport on isavuconazole.[8]
- Isavuconazole concentrations in the rat were similar to plasma levels reported in human following the recommended isavuconazole dose.[9]
  - Thus, these data may provide the basis for the observed efficacy of isavuconazole in the treatment of CNS fungal infections.[10-12]
- Taken together, these data indicate that isavuconazole may be an effective treatment option for CNS infections with susceptible fungal species.

References
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Disclosures
A-H. Schmitt-Hoffmann and J. Spickermann are employees of Basilea Pharmaceutica International Ltd.
M.J. Schneidkraut and R. Townsend are employees of Astellas Pharma Global Development, Inc. N. Azie was employed by Astellas Pharma Global Development, Inc. at the time of the study.

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