Supplementary Material

Manuscript title: Pharmacokinetics of Caspofungin in Critically Ill Patients in Relation to Liver Dysfunction: Differential Impact of Plasma Albumin and Bilirubin Levels

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Supplementary tables

Supplementary Table S1

PK parameters on days 2, 5 and 10 for the patients completing all days of the study; median (range) (n=21)

	PK day 2	**PK day 5**	**PK day 10**
C_{max} (mg/L)	5.4 (3.4-7.7)	5.0 (3.6-9.0)	5.3 (3.6-9.4)
C_{min} (mg/L)	1.1 (0.6-2.3)	1.2 (0.74-2.7)	1.1 (0.49-2.6)
$AUC_{0–24}$ (mg*h/L)	62.9 (40.0-108)	57.4 (44.0-113)	58.4 (37.2-105)
CL (L/h)	0.81 (0.46-1.52)	0.93 (0.44-1.46)	0.90 (0.47-1.88)
V1 (L)	11.0 (7.6-18.7)	12.3 (8.21-18.2)	11.2 (7.7-19.4)
V2 (L)	6.4 (2.6-13)	5.3 (2.3-7.1)	5.5 (3.3-6.6)
k10 (/h)	0.077 (0.048-0.11)	0.079 (0.053-0.10)	0.077 (0.061-0.12)

Abbreviations: C-max, observed concentration 1 hour post-infusion;; C-min, observed concentration 24 hours post-infusion; $AUC_{0–24}$, area under the concentration-time curve from 0 to 24 h; CL, clearance; V1, central volume of distribution; V2, peripheral volume of distribution; k10, the elimination rate constant from V1 at equilibrium between V1 and V2

Supplementary Table S2

Correlation coefficients between PK parameters and ALT, ALP and GFR on day 5

Median (range)	**ALT (**n=37**)** 0.86 (0.15-8.0)	**ALP** (n=37) 1.8 (0.9-8.0)	**GFR** (n=22) 89 (25-117)
AUC_{0-24} (mg*h/L)	- 0.01;p=0.98	- 0.31;p=0.07	0.13;p=0.57
CL (L/h)	- 0.03;p=0.86	0.30;p=0.08	- 0.13;p=0.56
V1 (L)	- 0.07;p=0.67	0.19;p=0.26	- 0.31;p=0.07
k10 (/h)	0.14;p=0.44	0.14;p=0.43	0.40;p= 0.07

ALT, alanine aminotransferase; ALP, alkaline phosphatase; GFR, glomerular filtration rate as estimated by the Modification of Diet in Renal Disease Study equation (patients with continuous renal replacement therapy excluded); AUC_{0-24}, area under the concentration-time curve from 0 to 24 hours; CL, clearance; V1, central volume of distribution; k10, the elimination rate constant from V1 at equilibrium between V1 and V2.

Supplementary Table S3A

PK parameters on day 5 in patients with and without CRRT; median (range)

	CRRT (n= 15)	**non-CRRT** (n=22)	***p* value**
C_{max} (mg/L)	5.5 (4.0-7.5)	6.3 (3.6-9.0)	0.32
C_{min} (mg/L)	1.3 (0.5-2.7)	1.2 (0.7-2.5)	0.59
AUC_{0-24} (mg*h/L)	59.7 (44.4-113)	57.6 (44.0-97.1)	0.87
CL(L/h)	0.87 (0.44-1.58)	0.89 (0.51-1.38)	0.82
V1 (L)	11.9 (7.5-20.0)	11.9 (7.8-16.1)	0.70
V2 (L)	5.6 (1.5-7.1)	5.1 (3.9-7.1)	0.11
k10 (/h)	0.074 (0.053-0.12)	0.082 (0.057-0.10)	0.10

CRRT, continuous renal replacement therapy mainly performed as continuous venovenous haemodialysis; C_{max}, maximal concentration; C_{min}, minimal concentration; AUC_{0-24}, area under the concentration-time curve from 0 to 24 hours; CL, clearance; V1, central volume of distribution; V2, peripheral volume of distribution; k10, the elimination rate constant from V1 at equilibrium between V1 and V2.

Supplementary Table S3B

PK parameters on day 5 in patients with and without ECMO; median (range)

	ECMO (n=9)	**non-ECMO** (n=28)	***p* value**
C_{max} (mg/L)	6.8 (4.6-8.4)	5.4 (3.6-9.0)	0.11
C_{min} (mg/L)	1.2 (0.5-2.1)	1.2 (0.7-2.7)	0.98
AUC_{0-24} (mg*h/L)	63.3 (51.1-93.0)	57.8 (44.0-113)	0.48
CL (L/h)	0.92 (0.72-1.22)	0.88 (0.44-1.58)	0.79
V1 (L)	12,1 (7.5-13.6)	11.9 (7.8-20.1)	0.89
V2 (L)	5.4 (1.5-5.8)	5.3 (2.4-7.1)	0.86
k10 (/h)	0.080 (0.063-0.12)	0.077 (0.053-0.11)	0.41

ECMO, extracorporeal membrane oxygenation; C_{max}, maximal concentration; C_{min}, minimal concentration; AUC_{0-24}, area under the concentration-time curve from 0 to 24 hours; CL, clearance; V1, central volume of distribution; V2, peripheral volume of distribution; k10, the elimination rate constant from V1 at equilibrium between V1 and V2.

Supplementary Table S4

Comparison of PK data from day 2 in the present study with results from three studies in ICU patients and one in healthy volunteers

	The present study	**Sinnollareddy et al (1)**	**Muilwijk et al (2)**	**van der Elst et al (3)**	**Stone et al (4)**
Patients	ICU patients	ICU patients	ICU patients	ICU patients	Healthy men
Time of sampling	Day 2	1st week	Day 3	Day 3	Day 1
Dose (mg)	70/50 or 70/70	No data	70/50 or 70/70	Varying doses[1]	70
	Median (IQR)	Mean±SD	Median (IQR)	Median (range)	GM (90% CI)
Cmax (mg/L)	5.1 (1.1-8.8)	3.9+2,1	7.5 (6.0-8.2)	7.4 (4.7-14.7)	12.0 (10.9, 13.3)
Cmin (mg/L)	1.1 (0.5-2.3)	1.5+0.9	2.2 (1.4-2.5)	1.7 (1.1-3.9)	1.4 (1.2. 1.7)
AUC_{0-24} (mg*h/L)	61.1 (32.8-108)	52.0+27.6	88.7 (72.2-97.5)	78.1 (61.4-129.4)	118 (104, 135)
Clearance (L/h)	0.84 (0.46-1.78)	NA	0.57 (0.54-0.77)	0.66 (0.37-1.26)	0.59 (0.58, 0.68)
V1 (L)	11.2 (6.68-26.4)	NA	7.7 (6.1-9.0)	9.1 (5,5-13.2)	NA
V2 (L)	6.9 (2.6-12.9)	NA	NA	8.6 (5.2-59.0)	NA
k10 (/h)	0.072 (0.048-0.11)	NA	NA	NA	NA

[1]Doses ranged from 35 mg to 100 mg; most patients received 70/50 mg

ICU, intensive care unit; IQR, interquartile range; SD, standard deviation; GM, geometric mean; CI, confidence interval; NA, not analyzed; C_{max}, maximal concentration; C_{min}, minimal concentration; AUC_{0-24}, area under the concentration-time curve from 0-24 hours; CL, clearance; V1, central volume of distribution; V2, peripheral volume of distribution; k10, the elimination rate constant from V1 at equilibrium between V1 and V2.

Supplementary Information 1

Measurement of caspofungin in plasma by LC-MS/M

A fully validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was used. Caspofungin was obtained from Merck & CO (Kenilworth, NJ) and the internal standard (IS; rel-(R-S)-Voriconazole-d3) was purchased from Toronto Research Chemicals, (Toronto, Canada). In brief, plasma was diluted with mobile phase A (0.1% formic acid), followed by protein precipitation by acetonitrile with 0.1% formic acid (mobile phase B) including IS. The final extract was injected onto LC-MS/MS, with an initial separation on a C18-column (Acquity BEH, Waters, Milford, MA) using a gradient run. The analyte was detected by operating in positive electrospray ionisation (ESI) mode utilizing selected reaction monitoring, with transitions 547→137 m/z for caspofungin and 353→284 m/z for the IS. The calibration curve was linear over 0.035 – 23.1 µg/mL. Between-run precision of quality control samples was below 5.0% (CV%) and between-run accuracy between -5.7% to 11.4%, without any observed analytical interferences.

Supplementary Information 2

Pharmacokinetic analysis

Based on caspofungin dosing history, observed caspofungin concentrations and a previously published population pharmacokinetic (PK) model (3) individual PK parameters were derived. The original PK model was developed based on data from 21 adult intensive care unit patients with rich PK sampling (11 samples day 3 and 7, and trough sampling at other study days). Caspofungin PK was described by a two-compartment model with the elimination rate constant (k10) and the structural parameters clearance (CL), volume of the central compartment (V1), volume of the peripheral compartment (V2), and inter-compartmental clearance (Q). The model included inter-individual variability (IIV) on CL, V1 and V2, with correlation between IIV on CL and V1, and a proportional residual error (14.8 %). The model was implemented in the non-linear mixed-effects modelling software NONMEM (5) and applied to the data to obtain empirical Bayes estimates (EBEs) using Bayesian estimation (MAXEVAL=0 option). Potential time-dependent differences in PK was investigated by comparing EBEs obtained when using all data with the EBEs derived when each day of sampling was treated separately (day 2, 5, and 10).

REFERENCES

S1. Sinnollareddy MG, Roberts JA, Lipman J, Akova M, Bassetti M, De Waele JJ, Kaukonen KM, Koulenti D, Martin C, Montravers P, Rello J, Rhodes A, Starr T, Wallis SC, Dimopoulos G. 2015. Pharmacokinetic variability and exposures of fluconazole, anidulafungin, and caspofungin in intensive care unit patients: Data from multinational Defining Antibiotic Levels in Intensive care unit (DALI) patients Study. Crit Care 19:33.

S2. Muilwijk EW, Schouten JA, van Leeuwen HJ, van Zanten AR, de Lange DW, Colbers A, Verweij PE, Burger DM, Pickkers P, Bruggemann RJ. 2014. Pharmacokinetics of caspofungin in ICU patients. J Antimicrob Chemother 69:3294-9.

S3. Martial LC, Bruggemann RJ, Schouten JA, van Leeuwen HJ, van Zanten AR, de Lange DW, Muilwijk EW, Verweij PE, Burger DM, Aarnoutse RE, Pickkers P, Dorlo TP. 2016. Dose Reduction of Caspofungin in Intensive Care Unit Patients with Child Pugh B Will Result in Suboptimal Exposure. Clin Pharmacokinet 55:723-33.

S4. Stone JA, Xu X, Winchell GA, Deutsch PJ, Pearson PG, Migoya EM, Mistry GC, Xi L, Miller A, Sandhu P, Singh R, deLuna F, Dilzer SC, Lasseter KC. 2004. Disposition of caspofungin: role of distribution in determining pharmacokinetics in plasma. Antimicrob Agents Chemother 48:815-23.

S5. Beal SL SL, Boeckmann AJ, and Bauer RJ (eds) NONMEM 7.3.0 Users Guides. (1989–2013).