Protein binding of temocillin is lower in plasma from patients in intensive care units compared to healthy subjects: in vitro and in vivo studies

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Background and Aims

- ✓ Only the unbound concentration of β-lactam antibiotics is considered as potentially active (capable of binding to the bacterial target) [1].
- ✓ Therapeutic drug monitoring, however, generally measures total plasma concentrations only [2], and extrapolates unbound concentrations from SmPc data that are most often obtained in healthy subjects.
- ✓ In the companion poster (P2220), we study the pharmacokinetics of temocillin (TMO) in healthy volunteers. We show that protein binding is high in this population, as also reported in the SmPc of the product [3].
- ✓ The present study aims at comparing the protein binding of temocillin in plasma from healthy subjects and critically-ill patients (hospitalized in ICU).

Materials & Methods

- ✓ **In-vitro:** Plasma samples from 8 healthy donors (HD) and 10 critically-ill patient donors (PD) were spiked with TMO (8 to 350 mg/L) and incubated for 30 minutes at 37°C.
- ✓ **In-vivo:** Plasma samples were collected over 12 h from 8 healthy volunteers (HV) having received 2 g of temocillin as IV infusion over 40 minutes.
- ✓ Plasma concentrations were determined by HPLC-MS/MS for total (sample preparation including protein precipitation with methanol) and unbound (sample preparation including ultrafiltration with Amicon filter Ultra-15 device; NMWL 30K; Merck Millipore Ltd) TMO [4].
- ✓ Proteins were measured by the Folin-Ciocalteu method.
- ✓ Binding characteristics were calculated by Michaelis–Menten kinetics using GraphPad 4 software.

References

1 Craig. Infect Dis Clin North Am 2003;17:479-501. PMID: 14711073
2. Huttner et al. J Antimicrob Chemother. 2015;70:3178-83. PMID: 26188037
3. Belgian SmPC, last revision 05/2017; Eumedica (data on file)
4. Ngougni Pokem et al. Clin Biochem. 2015;48:542-5. PMID: 25712752

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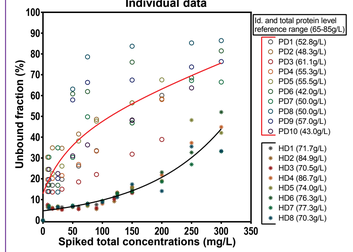
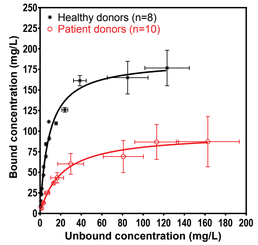
Results

In-vitro study: plasma samples from 8 healthy donors (HD) and 10 critically-ill patients donors (PD) spiked with temocillin (8 to 350 mg/L) and incubated for 30 minutes at 37°C.

A Unbound fraction *vs* total concentration of TMO in plasma samples from healthy donors (HD) *vs.* samples from critically-ill patients donors (PD). Individual data

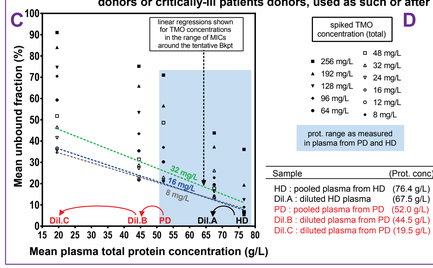


B Bound concentration *vs.* unbound concentration of TMO in plasma samples from healthy donors (HD) compared with critically-ill patients donors (PD). Pooled data



Mean unbound fraction *vs.* total protein concentration. Comparison between pooled plasma samples from healthy donors or critically-ill patients donors, used as such or after dilution in NaCl 0.9%. Pooled data

C



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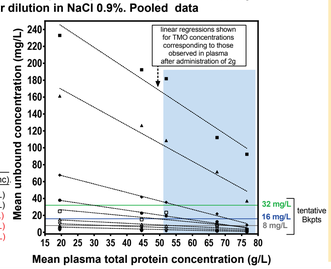


Figure A
- ✓ Unbound fraction increases with the total concentration with less variation between HD than between PD.
- ✓ In HD plasma, the unbound fraction is low (5-10%) for total concentrations < 100 mg/L, and increases up to 43% for higher total concentrations.
- ✓ In PD plasma, the unbound fraction is saturable and reaches 85% at the highest (supratherapeutic) concentrations tested.

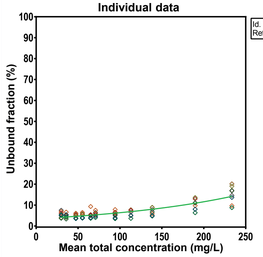
Figure B
- ✓ Protein binding of TMO is saturable in plasma from both HD or PD, but maximal binding capacity is lower in PD.

Figure C
- ✓ The unbound fraction increases when protein concentration decreases over the whole range of TMO concentrations tested.

Figure D
- ✓ The unbound concentration remains above MICs of 8, 16, 32 mg/L for total concentrations > 32, 64, 96 mg/L, respectively, if considering the range of protein concentrations observed in PD.

In-vivo study: plasma samples collected over 12 h from 8 HV having received 2 g of temocillin as IV infusion over 40 min.

E Unbound fraction *vs* total concentration of TMO Individual data



F Bound concentration *vs* unbound concentration of TMO. Pooled data

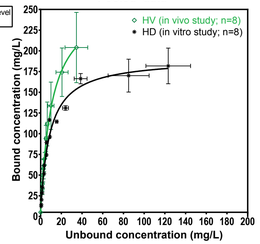


Figure E
- ✓ Unbound fraction increases with the total concentration.
- ✓ Unbound fraction is low (5-10%) for total concentrations < 150 mg/L, and increases up to 20% for higher total concentrations.

Figure F
- ✓ Protein binding of TMO is saturable in the plasma of HV.
- ✓ Binding of TMO is similar in vitro (spiked plasma) or in vivo (after drug administration) in healthy individuals.

Protein binding parameters of temocillin

Type of sample	Popu-lation studied	Plasma proteins ± SD (g/L)	Unbound fraction ± SD (%) at specified total conc.			Binding parameters (± SD)	
			50 mg/L	150 mg/L	230 mg/L	B_{max} (mg/L)	K_d (mg/L)
In vitro	HD	76.5±5.9	6.7±0.3	15.4±1.3	36.0±7.0	234.0±8.5	8.6±1.7
	PD	52±5.8	28.7±4.4*	67.9±12.4*	71.7±7.5*	107.9±2.3*	28.2±1.6*
In vivo	HV	70.4±4.5	7.3±2.0	5.1±1.0	15.0±3.8	261.7±8.6	10.7±0.7

B_{max}: maximal binding capacity;
K_d: dissociation constant (corresponds to the unbound TMO concentration for half of the maximal binding capacity)
Statistical analysis (one-way ANOVA): *, p< 0.05 as compared to HD data (analysis per column)

- ✓ Plasma proteins are higher in HD/HV than in PD.
- ✓ TMO unbound fraction increases with the total concentration over a clinically-achievable range of total concentrations: it is much higher in samples from PD than from HD/HV.
- ✓ TMO B_{max} is higher and K_d is lower in plasma from HD/HV than from PD, but binding parameters are similar in vitro and in vivo for samples from healthy people.

Discussion and Conclusions

- ✓ We confirm the high plasma protein binding of temocillin but show that it is lower and weaker in vitro (lower binding capacity and affinity) for samples from critically-ill patients than from healthy people.
- ✓ The reasons for reduced binding in patients' samples probably include lower protein concentration (reducing B_{max}) and displacement from binding sites by co-administered drugs (increasing K_d).
- ✓ As protein binding is similar in vitro and in vivo for healthy people, in vitro testing may be a useful tool to evaluate drug protein binding if using plasma samples from the appropriate population (to be verified with samples from critically-ill patients).

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