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In vitro selection of resistance to temocillin in *Enterobacter aerogenes*

Thi Thu Hoai Nguyen[1], Jean-Marie Pages[2], Youri Glupczynski[3], Françoise Van Bambeke[1], Paul M.Tulkens[1]

[1] Cellular and Molecular Pharmacology, Louvain Drug Research Institute, Université catholique de Louvain, Bruxelles, Belgium
[2] UMR-MD-1, Transporteurs membranaires, Chimiorésistance et Drug Design, Université de la Méditerrané, Marseille, France
[3] Laboratoire de microbiologie, Clinique universitaire UCL de Mont-Godinne, Yvoir, Belgium

Introduction

Temocillin (TMO; 6-methoxy-ticarcillin) is a directed-spectrum β-lactam active against Gram-negative bacteria (but not *P. aeruginosa*) even if expressing ESBL. TMO is indeed resilient to all classical and extended-spectrum TEM, SHV and CTX-M enzymes and to AmpC β-lactamase. For this reason, TMO is often proposed as an alternative to carbapenems in severe nosocomial infections when *Pseudomonas* can be excluded.[1]

The activity of β-lactams is described as time-dependent, Due to inter- and intra-patient variations in pharmacokinetic parameters resulting in often difficult-to-predict blood levels even with continuous infusion,[2] and in the absence of a fast, reliable method for assay of β-lactam serum levels, there is a risk of creating situations where TMO concentrations will fall below the MIC.

Our objective was to assess the risk of emergence of resistance to TMO if bacteria are exposed to sub-MIC concentrations. *E. aerogenes* was used here as a typical target organism.

Methods

Four clinical isolates susceptible to meropenem (MEM) but with different susceptibilities to cefepime (FEP) and TMO (see Table 1) were subjected to a multi-step selection approach with 15 sequential subcultures in medium containing half-MIC of TMO (with daily MIC read-out [microdilution] and readjustment of TMO concentration; see general protocol in ref. 3), followed by 12 daily sub-cultures on antibiotic-free agar (revertant).

Bacteria were examined for (i) susceptibility to TMO, FEP and MEM (MIC) (with and without the broad spectrum efflux transporter inhibitor Phe-Arg-β-Naphthylamide [PAβN][4] for TMO) and (ii) expression of Omp36 porin (the main porin in *E. aerogenes*)[5] using dotblot with specific antibody (validated with positive [ATCC13048] and negative [EA27] control strains by western blot and ELISA). Degradation of TMO and MEM in the presence of TMO-exposed bacteria was examined by incubating these strains with sub-inhibitory concentrations of TMO or MEM for up to 24 h followed by assay of filtered culture medium for residual antibiotic content (MIC and disk diffusion assay against susceptible *E. aerogenes* and *E. coli*; controls; same protocol with bacteria unexposed to temocillin [initial strains] and with sterile broths [to assess spontaneous degradation]).

Results

Figure1 shows that the MIC of all 4 strains increased markedly for TMO (6 to 10 $\log_2$ dilutions) when exposed to 0.5 x MIC for successive days, with reversion for 1 strain upon removal of TMO.

Figure 2 shows the detection of Omp36 porin by western- and dot-blot in one typical control positive and one typical control negative strains

Table 1 summarizes the observations made before exposure to TMO, after 13 days of exposure, and 10 days after TMO removal concerning (i) the MIC of TMO, FEP, and MEM, and (ii) the expression of Omp36 porin.

There was a concomitant increase in MIC of FEP (for 3 strains) and MEM (for 2 strains), with reversal for 2 out of 3 strains for FEP and for both strains for MEM.

No correlation between changes in MIC and Omp36 expression could be evidenced.

The MICs of bacteria exposed to TMO measured in the presence of PAβN were considerably reduced (4 to 32-fold) for 3 strains (2114/2, 2502/4, and 3511/1).

There was no evidence of loss of TMO and MEM activity in the culture medium during incubation with TMO-exposed bacteria *vs.* controls beyond a partial spontaneous loss of MEM in sterile broth (chemical instability; not seen with TMO which has been shown to be more chemically stable in aqueous solutions[2]).

The whole experiment was repeated at one month interval with essentially comparable results.

Figure 1: Development of resistance and reversion

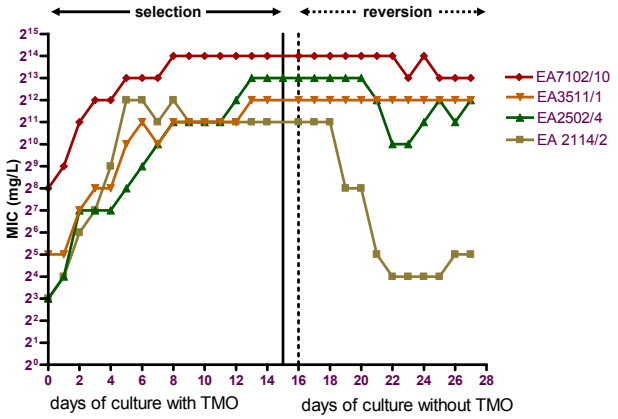

Figure 2: Blot using anti-Omp36 antibody

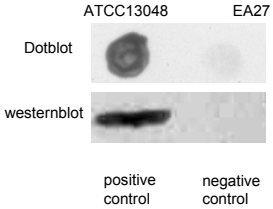

Table 1: Summary of antibiotic resistance profiles in strains subjected to temocillin (TMO) selection

strains	Initial				TMO-exposed				Revertant			
	MIC (mg/L) [a]			Omp36 express. [b]	MIC (mg/L)			Omp36 express.	MIC (mg/L)			Omp36 express.
	TMO	FEP	MEM		TMO	FEP	MEM		TMO	FEP	MEM	
2114/2 [c]	8	2	0.25	22.7	**2048**	**> 128**	**16**	-3.57	**32**	4	0.5	-4.52
2502/4 [c]	8	2	0.125	9.11	**8192**	4	0.25	38.6	**4096**	1	0.125	23.6
3511/1 [c]	**32**	2	0.125	9.51	**4096**	**32**	0.125	21.6	**4096**	**8**	0.5	28.6
7102/10 [d]	**256**	**32**	1	0.03	**16384**	**> 128**	4 [e]	10.9	**8192**	**64**	1	13.6

[a] figures in bold indicate values > the R breakpoint for *Enterobacteriaceae* (EUCAST for MEM [8 mg/L] and FEP [4 mg/L]; BSAC for TMO [8 mg/L for systemic infections)
[b] dotblot using anti-Omp36 antibody; signal quantified for grey value after subtraction of the signal of a porin-negative strain (ImageJ software); negative values indicate a signal lower than the background
[c] ESBL TEM 24 (+) ; [d] ESBL (-) and AmpC (+) [high level] ; [e] Intermediate (I) according to EUCAST

Conclusions

This study stresses the risks of triggering resistance to TMO as well as to other β-lactams by suboptimal exposure to TMO.

The underlying mechanism(s) of this fast emergence of resistance seems related neither to change in the expression of the Omp36 porin nor to TMO degradation, but could be related, in part, to the overexpression of efflux transporter(s)

The message for clinicians is that suboptimal treatments with temocillin should be avoided, which may justify the use of the maximal registered dosage as long as the susceptibility of the offending organism is not known (see ref. 2 for a calculation of temocilin target attainment rate as a function of the MIC).

References

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