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Role of MexAB-OprM in intrinsic resistance of *Pseudomonas aeruginosa* to temocillin compared to ticarcillin.

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Abstract

Objectives: Temocillin (6-methoxy-ticarcillin; TEM) is a narrow spectrum beta-lactam resistant to most narrow and extended-spectrum beta-lactamases and AmpC enzymes (JAC 2009, 63, 243–5). It knows a revival for the treatment of multiresistant ESBL-producing Gram (-). TEM, however, has no useful activity against Pa with MICs = or > 128 mg/L. Our aim was to examine whether efflux could explain this apparent intrinsic resistance.
Methods: 10 clin. and lab. strains and ATCC PAO1 (as reference). The expression of genes encoding efflux pumps was determined by RT-PCR for mexA and mexX or by PCR for mexC and mexE. The genomic information was combined with antibiogram data to define the efflux status of each strain. MICs were measured by microdilution in cation-adjusted Mueller-Hinton broth (pH 7.4, 24 h) with Phe-Arg-beta-naphthylamide (PAβN, 50 mg/L) used as broad-spectrum efflux inhibitor.
Results: The Table shows that MICs were high against PAO1 but considerably reduced in the presence of PABN (from >1024 to 64 mg/L). Clinical strains overexpressing MexAB showed high MIC and were poorly susceptible to PABN. All strains laboratory deficient for MexAB-OprM efflux pump showed drastically reduced MICs (2 - 4 mg/L). Deletions of MexXY or MexEF caused only a partial reduction of MIC (128 mg/L), which was further reduced by addition PAβN.
Conclusions: The data strongly suggest that the intrinsic resistance of Pa to temocillin in clinical isolates and in the reference strain PAO1 is primarily due to efflux through MexAB transporter, with potential additional role played by the other efflux pumps. In view of the interest of TEM in an era of increasing resistance related to beta-lactamases, our results trigger further current efforts in designing clinically useful inhibitors of the MexAB transporter.

Background and aim

Temocillin, a 6-α-methoxy derivative of ticarcillin, is resistant to most β-lactamases, including ESBLs, and is, therefore, considered a useful alternative to carbapenems for treatment of infections caused by multi-resistant ESBL-producing Gram-negative bacteria when *P. aeruginosa* can be excluded (1). Temocillin, indeed, has no useful activity against the latter organism, with MICs against most clinical isolates being ≥ 128 mg/L.

Intrinsic, high-level resistance of *P. aeruginosa* to β-lactams is usually ascribed to insufficient permeability of the outer membrane and/or which is usually ascribed to poor permeation or reduced PBP binding. However, expression of multidrug efflux systems in Gram-negative bacteria may, today, represent an increasingly important mechanism (2). To date, 3 multidrug efflux systems have been described to export β-lactams, namely (from lesser to most effective) MexXY-OprM, MexCD-OprJ, and MexAB-OprM. We wondered whether the apparent intrinsic resistance of *P. aeruginosa* to temocillin was not primarily the result of such efflux.

Results

Table 1. MICs of temocillin and ticarcillin against *P. aeruginosa* strains with known expression of the efflux Mex components in Mueller-Hinton broth (MHB) and in MHB supplemented with the broad spectrum efflux transporter inhibitor Phe-Arg-β-naphthylamide (PAβN; 50 µg/mL)

Strains	Origin or Ref.	Description	Expression of Efflux system					MIC (mg/L)	
			AB [a]	XY [a]	OprM [a]	CD [b]	EF [b]	Temocillin (+PAβN)	Ticarcillin (+PAβN)
Reference strain									
PAO1	ATCC		1	1	1	-	-	256 (64)	32 (16)
Clinical isolates									
12	d		**3.97**	9.04	ND	+	+	**512** (128)	64 (64)
11	d		**3.56**	5.68	ND	-	-	**>512** (64)	32 (32)
156	d		0.33	0.95	ND	-	+	512 (64)	256 (32)
68	d		0.87	44.94	ND	-	-	512 (64)	32 (16)
333A	d		**2.17**	2.29	ND	-	-	**>1024** (1024)	128 (128)
34	d		**6.86**	1.26	ND	-	-	**>1024** (512)	256 (128)
168B	d		1.15	0.89	ND	-	-	256 (32)	16 (16)
Engineered strains									
FB1	3	PAO1Δ(*mexB::FRT*)	ND	ND	ND	ND	ND	**2**	0.5
PAO1 *mexAB*	4	PAO1Δ(*mexAB::FRT*)	**0[e]**	1.08	ND	-	+	**4** (2)	2 (2)
PAO200	4	PAO1Δ(*mexAB-oprM*)	**0[e]**	1.26	ND	-	-	**4** (0.5)	2 (0.5)
CB536	5	PAO1Δ(*mexCD-oprJ*)	1.09	1.65	ND	-	+	128 (16)	8 (1)
CB603	5	PAO1Δ(*mexEF-oprN*)	1.21	1.06	0.51	-	-	128 (32)	16 (16)
CB602	5	PAO1Δ(*mexXY-oprM*)	1.10	0.06	0.55	-	+	64 (16)	16 (16)
PAO1Δ(oprM)		PAO1 Δ(*oprM*)	ND	ND	ND	ND	ND	**2**	0.5
4098	6	Clinical strain	1.26	1.62	0.33	-	-	256 (128)	32 (32)
4098E	6	4098 overproducing OprM	**5.41**	1.31	**3.19**	-	-	**1024** (512)	64 (32)
4098ET	6	4098E Δ(*oprM*)	2.18	0.04	**0.02**	-	-	**2** [f]	2 [f]

[a] Real-time PCR (threshold ratio compared to PAO1; values of ≥ 2 and 5 are considered to denote highly significant overexpression of *mexAB* and *mexXY*, respectively. [b] RT-PCR (qualitative detection [+ / -]). [c] Phe-Arg-β-naphthylamide (broad spectrum efflux inhibitor) used at 50 mg/L. [d] isolated from Intensive Care patients with a clinical diagnostic of health care-associated pneumonia. [e] complete absence of detection. [f] No growth, PAβN MIC = 25 mg/L.

As compared to PAO1, strains overexpressing MexAB-OprM show an increaed MIC of temocillin (blue) while those deleted for the expression of at least one of the proteins constituting the pump show low MICs (green)

→ **Intrinsic resistance of *P. aeruginosa* to temocillin is primarily due to efflux through MexAB-OprM**

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Materials & Methods

Bacterial strains and susceptibility testing. *P. aeruginosa* strain ATCC PAO1 was used as reference. Typical clinical isolates (isolated from ICU patients with clinically-suspected hospital-acquired pneumonia) and laboratory strains with specific disruption(s) of the gene(s) encoding 4 main efflux transporters known to efflux antibiotics in *P. aeruginosa* have been used for MICs determinations (see Table 1 for genotype). MICs were measured by microdilution in cation adjusted Mueller-Hinton broth (MHB; pH 7.4, 24 h) and without or with PaβN (50 mg/L) used as an broad spectrum efflux inhibitor.
Strains characterization. The expression of *mexA*, *mexX and oprM* (constitutively expressed in reference and clinical strains) was measured by quantitative Real-Time PCR (based on previous studies, we considered and increase in the detection thresholds of ≥ 2 [*mexA and oprM*] and ≥ 5 [*mexX*] over the value observed in the PAO1 strain as being highly significant), and that of *mexC* and *mexE* by semi-quantitative RT-PCR.
Pharmacologic agents. Temocillin was provided by Eumedica (Brussels, Belgium). Ticarcillin (disodium salt) and PaβN (Phe-Arg-β-napthylamide) were purchased from Sigma-Aldrich (St. Louis, MO).

Conclusions

- Intrinsic resistance of *P. aeruginosa* to temocillin is primarily due to efflux through MexAB-OprM transporter with potential additional role played by the other efflux pumps.
- Ticarcilin is also a substrate of the transporters but to a much lesser extent.
- Impairment of the MexAB-OprM brings the MIC of temocillin to values that are lower than its current susceptibility breakpoint for *Enterobacteriaceae* (16 mg/L according to both BSAC and the Belgian labelling) as well as the PK/PD breakpoint (8 mg/L) proposed for a daily administration of 4 g (7). This may further trigger current efforts in designing clinically useful efflux inhibitors of the MexAB-OprM transporter.

References

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