Contrasting effects of ertapenem (ETP) against intracellular *L. monocytogenes* (L.m.) and *S. aureus* (S.a.) in a model of THP-1 macrophages

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Background : ETP is a penem with an unusually long half-time, yielding efficacy against extracellular infections with a once-daily schedule. We have studied its activity in two types of intracellular infections (L.m., [cytosol]; S.a., [phagolysosomes]).

Methods : MIC and MBC were measured in TSB. Intracellular activity in THP-1 macrophages was determined as previously described by Carryn et al. for L.m. (AAC, 2002, 46:2095-2103), and by Seral et al. for S.a. (AAC, 2003, 47:2283-2292) with minor adaptations. Meropenem (MEM) was used for comparison. .

Results:

	L.m.				S.a.			
	MIC [a]	MBC	Activity [b] Broth	Activity [b] THP-1	MIC [a]	MBC	Activity [b] Broth	Activity [b] THP-1
ETP (155 mg/L) [c]	0.48	>64	-0.57 ± 0.05	+1.16 ± 0.08	0.11	0.25	-4.89 ± 0.16	-0.98 ± 0.21
MEM (50 mg/L) [c]	0.05	>64	-0.38 ± 0.05	-1.90 ± 0.05	0.15	0.25	-4.85 ± 0.16	-1.03 ± 0.23

[a] arithmetic dilutions; [b] change (log) from original inoculum (24h incubation); [c] Cmax in patients. (Means ± SEM of three independent experiments)

Thus, in contrast to MEM, ETP was not active against intracellular L.m. Both drugs, however, showed similar activities against intracellular S.a.

Conclusion : The contrasting behaviour of ETP, in comparison with MEM, suggests that both MIC and local environment modulate the intracellular activities of b-lactams.

INTRODUCTION

Intracellular infections remain a medical challenge due to (i) their recurrent character and (ii) the difficulty for antibiotics to gain access to the infected compartment (*L. monocytogenes*, cytosol; *S. aureus*, phagolysosomes).

Beta-lactams often constitute a first choice in our therapeutic armamentarium because of their diverse spectrum of activity and high therapeutic index. We recently showed that they also display bactericidal activity against the intracellular forms of infections localized either in the cytosol (L.m.)[2] or in the phagolysosomes (S.a, poster A1488). Because of their short half-life and time-dependent activity, they need, however, repeated administrations. In this respect, molecules with prolonged half-life like ertapenem may potentially offer a therapeutic advantage.

AIM OF THE STUDY

To compare the extracellular (broth) and intracellular (THP-1 infected cells) activity of ETP (long half-life penem) and MEM (short half-life penem) :

- against *Listeria monocytogenes* and *Staphylococcus aureus*,
- using a concentration corresponding to the peak observed in human receiving conventional doses of these antibiotics[3,4].

RESULTS

Intrinsic activity

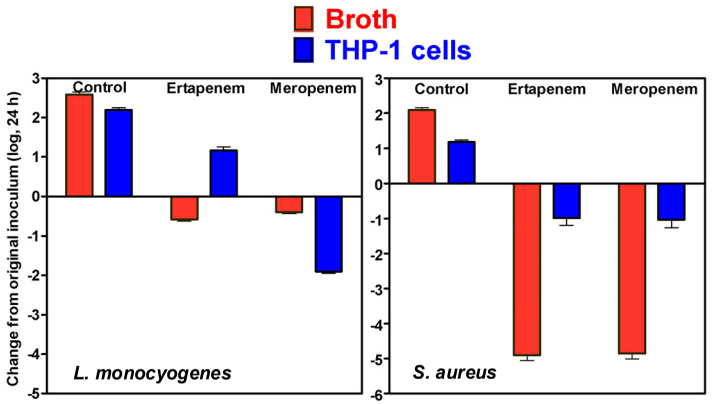
Intrinsic activity	Drug	*L. monocytogenes*	*S. aureus*
MIC (mg/L)[a]	ETP	0.48	0.11
	MEM	0.05	0.15
MBC (mg/L)[b]	ETP	> 64	0.25
	MEM	> 64	0.25

[a] arithmetic dilutions ; [b] geometric dilutions

ETP and MEM display similar activity against S.a. but MEM is more active than ETP against L.m.
MBC values suggest that both drugs are bacteriostatic against L.m. and bactericidal against S.a.

Pharmacodynamic studies

Extracellular and intraphagocytic activities againts *L. monocytogenes* and *S. aureus* in a 24h model



Change in the number of extracellular or intracellular *L. m.* or *S. a.* after 24 h incubation in control conditions or with ETP (155 mg/L) or MEM (50 mg/L). Data are mean ± SEM of 3 independent experiments.

L. m. : both penems are bacteriostatic against extracellular bacteria; ETP fails to control the intracellular growth of *L.m.*, in sharp contrast with meropenem, which causes a 2 log decrease as compared to the initial inoculum.

S.a. : both penems are highly bactericidal in broth and are also active against intracellular forms. However, intracellular activity is lower than extracellular activity.

METHODS

- Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentrations (MBC) were determined in Tryptic Soy Broth (TSB) by arithmetic and geometric dilutions respectively[1].
- Extracellular activity were determined by CFUs counting after a 24 h incubation with the antibiotic in TSB[2,5]
- Activity against intracellular bacteria (*L. monocytogenes* EGD, serotype ½ a; opsonized-*S.aureus* ATCC 25923) : cells were infected (L.m., 5 bacteria/cell; S.a., 4 bacteria/cell) during 1 h at 37°C and washed with PBS (for S.a, the washing was preceded by a 45 min. incubation in PBS added with 50 mg/L of gentamicin), to remove non-phagocytosed and non-firmly adherent bacteria. Cells were then incubated for up to 24 h with ertapenem, meropenem or in control conditions (i.e. medium added by gentamicin at its MIC [L.m., 1 mg/L; S.a., 0.5 mg/L] to avoid extracellular contamination over prolonged incubation)[2,5].

CONCLUSIONS

- Both penems show a similar behavior against extracellular bacteria but are far more active against S.a. than against L.m., which can be related to their static effect against the latter organisms (MBC > 64 mg/L)
- Penems differ in their intracellular activity:
  - MEM is more active against L.m. than S.a.
  - ETP is inactive on L.m. but as active as MEM on S.a.

The reasons for these discrepancies need to be further explored but could be related to

- ✓the higher intrinsic activity of MEM against L.m. (viz MIC values)
- ✓an insufficient intracellular bioavailability of ETP (binding to cytosolic constituents ?)

The data suggests that ETP would be an appropriate alternative for MEM for S.a. infections but not for L.m. infections.

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