Comparison of ertapenem, ampicillin, and meropenem against the intracellular forms of *Listeria monocytogenes* in human THP-1 macrophages

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ABSTRACT

Objectives : Ertapenem (ETP) is a new carbapenem with prolonged half-life (approx. 4h), which could make it more suitable than meropenem (MEM) or ampicillin (AMP) for the treatment of listeriosis. However, *L. monocytogenes* is largely intracellular, and the activity of ETP against these forms has not been investigated. We, therefore, compared ETP to AMP and MEM in a model of human macrophages where AMP and MEM are cidal over a 24h incubation (approx. 2 log CFU decrease; Carryn et al., J Antimicrob Chemother. 2003, 51:1051-2).

Methods : MIC (arithmetic dilutions) and MBC (geometric dilutions) were determined in TSB by standard methods. Activity against extracellular and intracellular forms of *L. monocytogenes* was examined in THP-1 macrophages incubated with extracellular concentrations (ETP,155 mg/L; AMP and MEM, 50 mg/L) equivalent to the Cmax achievable in human serum after conventional dosing. The stability of the drugs in the culture medium under our experimental conditions was checked by HPLC.

Results : Activities in broth and in the cellular model are shown in the Table. (change in log CFU)

	Broth		THP-1 model	
	MIC (mg/L)	MBC (mg/L	extracellular	intracellular
ETP	0.48±0.03	>64	-0.57±0.05	0.96±0.23
AMP	0.37±0.23	>64	-0.46±0.03	-1.81±0.01
MEM	0.05±0.00	>64	-0.38±0.05	-1.82±0.01

means +/- SEM (n=3 independent experiments)

Thus, whereas AMP, MEM and ETP have similar activities against *L. monocytogenes* in broth, AMPand MEM were cidal but ETP unable to control the growth of intracellular *L. monocytogenes*. Yet, assay of cell-associated ETP showed that its apparent cellular concentration exceeded the MIC. Decreasing the serum concentration in the culture medium (from 10 to 2%) did not change the results. Stability studies showed a lower degree of degradation for ETP than for MEM.

Conclusions: In this model, ETP did not eradicate intraphagocytic *L. monocytogenes*. It is possible that intracellular conditions (e.g. binding to cytoplasmic proteins) hinder ETP intracellular activity in comparison with AMP or MEM.

INTRODUCTION

L. monocytogenes is an intracellular bacteria responsible for life-threatening infections in immunocompromised patients and pregnant women. The current therapeutic treatment consists in the combination of ampicillin (or meropenem) and an aminoglycoside. Because of the intracellular character of this infection, appropriate treatment options need to be assessed in models of infected cells.

Using infected human THP-1 macrophages, we recently showed that ampicillin and meropenem display a marked bactericidal activity against intracellular *L. monocytogenes*, provided that they are maintained for a prolonged time (24 h) in contact with the infected cells (1). These results suggest that beta-lactam activity develops on a time-dependent manner against intracellular bacteria as they do for extracellular organisms.

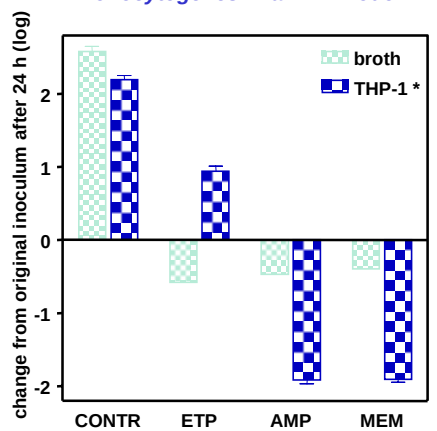
Inn this context, ertapenem appeared to us as an interesting alternative to meropenem because of its prolonged half-life in vivo, allowing for less frequent administrations (2).

AIM OF THE STUDY

- To compare the extracellular (broth) and intraphagocytic (infected macrophages THP-1) activity of ertapenem with that of meropenem and ampicillin, at a concentration mimicking the Cmax reached in patients undergoing conventional therapy.
- To study the cellular accumulation and the stability of ETP in the experimental conditions used for determining activity and to evaluate the potential influence of drug binding to serum proteins on activity.

RESULTS

1. comparative extracellular and intracellular activity of beta-lactams against *L. monocytogenes* in a 24 h model



MIC (mg/L)	0.48±0.03	0.37±0.23	0.05±0.00
MBC (mg/L)	> 64	> 64	> 64

Change in the number of viable *L. monocytogenes* in broth or in infected macrophages after 24 h incubation in the absence of antibiotic (CONTR) or with ertapenem (ETP), ampicillin (AMP) or meropenem (MEM). The drugs were added at an extracellular concentration corresponding to the Cmax measured in the serum of patients (50 mg/L for AMP and MEM; 155 mg/L for ETP). For experiments with cells, the culture medium was added by 10 % decomplemented calf serum. Data are the mean ± SEM of 3 independent experiments.

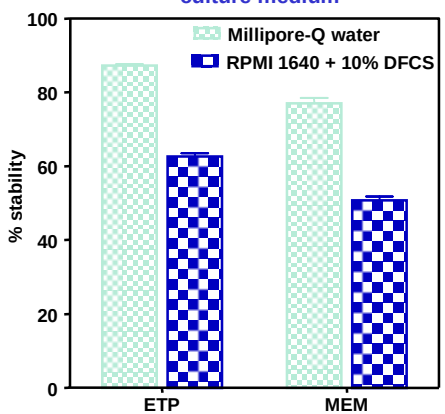
* Reducing the serum content of the culture to 2 % does not significantly alter the results.

2. accumulation of ertapenem in THP-1 macrophages after 24 h incubation

	cellular concentration (mg/L cell vol)	accumulation factor
non infected cells	27.9 ± 9.3	0.18 ± 0.06
infected cells	37.2 ± 10.9	0.24 ± 0.07

Extracellular concentration, 155 mg/L;
data are mean ± SEM of 3 independent experiments

3. comparative stability of carbapenems after 24 h incubation in water or in the culture medium



Stability of ertapenem (ETP) and meropenem (MEM) after 24 h incubation at 37°C of the drug at a concentration mimiking its Cmax in the serum of patients (155 mg/L for ertapenem; 50 mg/L for meropenem) in water or in the culture medium (RPMI + 10 % decomplemented calf serum). Data are the mean ± SEM of 3 independent experiments.

METHODS

- Minimal Inhibitory Concentration and Minimal Bactericidal Concentration were determined in Tryptic Soy Broth (TSB) respectively by arithmetic and geometric dilution (3).
- Extracellular activity was assayed by CFUs counting after 24h exposition to the antibiotic in TSB (1,3) .
- Intracellular activity was measured after 24 h of incubation of THP-1 human macrophages infected with an initial inoculum 5 bacteria/cell. The number of CFUs in cell lysates was determined and the results were expressed by reference to the sample protein content (1,3).
- Ertapenem cellular accumulation was determined in non-infected or infected THP-1 after 24 h incubation. Ertapenem concentration was determined by microbiological assay (*E. coli*, lowest limit of detection : 0,25 mg/L; linearity from 0.25 mg/L to 16 mg/L, r^2:0,9921); the apparent cellular concentration (Cc) was calculated by using a conversion factor of 5 µl of cell volume per mg of cell protein.
- Stability of ertapenem and meropenem was evaluated over a 24 h incubation period in water or in RPMI+10% calf serum. The drugs were assayed by RP-HPLC (4,5) (Lichrospher 100 RP-18, 25 x 4 cm, injection volume : 50 µl ; elution buffer : 25 mM phosphate buffer pH 6.5/acetonitrile [vol/vol : 93: 7] for ertapenem and sodium acetate 10.53 mM pH 4/acetonitrile [vol/vol: 90:10]) for meropenem; flow rate : 1 ml/min; UV detector : 300 nm; linearity from 0,09 mg/L to 200 mg/L [r^2=0,9999]). The extraction procedure from culture medium involved 3 successive cycles of precipation of proteins in acetonitrile and centrifugation, the addition of dichloromethane to the supernatant and the injection of the sample in a Waters 2690 system.

CONCLUSIONS

- Against extracellular *L. monocytogenes*, ertapenem shows, as ampicillin and meropenem, only a bacteriostatic activity.
- Against intracellular *L. monocytogenes*, ertapenem is unable to control bacterial growth, in sharp contrast to ampicillin and meropenem, which are bactericidal.
- The cellular accumulation of ertapenem is low, but its cellular concentration nevertheless reaches values ~ 75 X higher than the MIC.
- The lack of intracellular activity of ertapenem cannot be ascribed to an unsufficient cellular concentration, nor to the presence of serum in the culture medium, nor to a larger extracellular degradation (in comparison with meropenem).
- Further studies are needed to elucidate the contrasting behavior of ertapenem and ampicillin or meropenem.

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