Comparative activity of ertapenem and piperacillin–tazobactam in a murine systemic infection model with Bacteroides fragilis and Escherichia coli

It is now well-recognized that most intra-abdominal infections involve multiple types of bacteria. The most frequently encountered organisms in intra-abdominal infections are Escherichia coli and Bacteroides fragilis. These two micro-organisms are indigenous components of faecal microbiota, but can cause intra-abdominal abscess following surgery (Goldstein & Snydman, 2004). Therefore, an effective antimicrobial agent for the treatment of intra-abdominal infections requires a broad spectrum of activity against both aerobic and anaerobic bacteria.

Ertapenem is a new carbapenem with a broad spectrum of activity against Gram-negative pathogens, including extended-spectrum-β-lactamase and AmpC-producing Enterobacteriaceae, as well as against Gram-positive aerobic and anaerobic pathogens (Goldstein & Snydman, 2004; Weder, 2004). Clinical trials involving complicated intra-abdominal infections demonstrated that ertapenem has equivalent efficacy and safety when compared with piperacillin–tazobactam (Solomkin et al., 2003; Dela Pena et al., 2006). However, in the setting of mixed infections, antimicrobial therapy may result in clinical improvement and a decrease in bacterial burden without, necessarily, eradicating the infection. There are no in vivo time-kill studies regarding ertapenem and piperacillin–tazobactam pharmacodynamics. Thereby, in the present study, we compared the in vivo activity of ertapenem with that of the piperacillin–tazobactam combination in a murine systemic mixed infection model.

A piperacillin–tazobactam preparation (Tazocin; Lederle Piperacillin) and ertapenem sodium (Invanz; Merck Sharp & Dohme) were purchased commercially and solubilized in sterile saline (0.9 % NaCl) for injection. For MIC determinations, ertapenem sodium was kindly provided as standard powder by Merck Sharp & Dohme. B. fragilis (ATCC 25285) and E. coli (ATCC 25922) were used throughout the experiments. The obligate anaerobe B. fragilis was incubated anaerobically at 37 °C in an anaerobic chamber (Forma Scientific) containing an atmosphere of 85 % N2, 10 % H2 and 5 % CO2, and the facultative anaerobe E. coli was incubated aerobically or anaerobically at 37 °C.

MICs of the test drugs were determined before and after antimicrobial therapy by the agar dilution method, according to the recommendations of the Clinical Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) (NCCLS, 2003, 2004).

All experimental procedures with animals were approved by the ethics committee in animal experimentation of the Universidade Federal de Minas Gerais (CETEA/UFMG). Female Swiss mice (Animal Care Center, Universidade Federal de Minas Gerais, Brazil), 3 weeks old and weighing 15 to 18 g, were inoculated intraperitoneally with 0.1 ml of a mixed culture containing about $10^7$ c.f.u. of each bacteria per mouse. The bacterial inoculum was prepared by suspending overnight cultures of B. fragilis and E. coli in 3 % porcine gastric mucin (Sigma-Aldrich), which were then mixed together in a volume ratio of 1:1.

In pharmacokinetic studies, infected mice were treated 3 h after bacterial challenge with a single subcutaneous (sc) injection of either ertapenem (25 mg kg$^{-1}$) or piperacillin–tazobactam (340 mg kg$^{-1}$). Antibiotic concentrations were determined in quadruplicate by an agar diffusion bioassay (Chapin-Robertson & Edberg, 1991). E. coli ATCC 25922 was used as the indicator organism for both drugs. Standard curves were determined using mouse plasma. Zones of inhibition were measured to the nearest 0.01 mm by a digital caliper (digtimate caliper; Mitutoyo). The linearity of the standard curves was assessed with a regression coefficient of $\geq 0.995$, and intra-plate and inter-plate variations were $\leq 11 \%$. The limits of detection of the assay were 0.12 μg ertapenem ml$^{-1}$ and 4 μg piperacillin–tazobactam ml$^{-1}$.

For bacterial titre studies, infected animals were administered four doses of ertapenem (25 mg kg$^{-1}$, sc) or piperacillin–tazobactam (340 mg kg$^{-1}$, sc) at 6 h intervals, starting 3 h after infection. A separate group of control animals received saline (0.9 % NaCl) following the same schedule as for mice treated with antibiotics. Just prior to antibiotic initiation, and at 3, 6, 9 and 21 h after the first injection, groups ($n=4$) of control and drug-treated animals were sacrificed by cervical dislocation. Subsequently, samples of blood and peritoneal fluid from each animal were harvested. In all specimens collected, bacterial counts were determined by performing a 1:10 serial dilution in sterile PBS and then 0.1 ml aliquots of each dilution were plated onto selective medium: MacConkey agar (Difco) for E. coli and Bacteroides bile esculin agar for B. fragilis. Viable colonies of E. coli were counted after overnight incubation at 37 °C, and B. fragilis colonies were counted after incubation for 48 h at 37 °C in an anaerobic chamber. Dilutions were performed to eliminate potential
carryover effects. The limit of quantification was 300 c.f.u. ml⁻¹.

For each drug studied, analysis of variance (two-way ANOVA) was used to compare the effects between the different groups, followed by use of the Holm–Sidak method to compare treated and untreated groups two by two. P values of <0.05 were considered significant.

The MICs of ertapenem and piperacillin–tazobactam for E. coli ATCC 25922 and B. fragilis ATCC 25285 are summarized in Table 1. No change in drug susceptibility was observed among any of the post-therapy bacterial samples.

Following the first 6 h sc administration into infected mice, ertapenem (25 mg kg⁻¹) and piperacillin–tazobactam (340 mg kg⁻¹) had Cₘₐₓ values of 117.9 and 179.8 µg ml⁻¹ and half-lives of 1.0 and 0.5 h, respectively. The projected exposures for a single dose of ertapenem and piperacillin–tazobactam in comparison with human exposures to both antibiotics are shown in Table 2. The time proportions of the dosing interval where the plasma concentration exceeded the MIC (% t>MIC) were 100 and 46% for ertapenem and piperacillin–tazobactam, respectively (Table 1).

The pre-treatment E. coli counts were 6.7 and 6.1 log₁₀ c.f.u. ml⁻¹ in the peritoneal fluid and blood, respectively. Analysis of the kill curves revealed that ertapenem exhibited significantly higher killing activity than piperacillin–tazobactam (P<0.05, two-way ANOVA) (Fig. 1a, b). Until 6 h after the first dose, the E. coli counts in the peritoneal fluid and blood were similar for both treatments (P>0.05). However, at 9 h after the first dose, the E. coli counts in the peritoneal fluid and blood were reduced to a higher extent by ertapenem (−3.8 and −4.2 log₁₀ c.f.u. ml⁻¹, respectively) compared with piperacillin–tazobactam (−1.5 and −1.8 log₁₀ c.f.u. ml⁻¹, respectively). Ertapenem produced sustained suppression of counts, and eventually resolved the infection in the peritoneal fluid and blood by 21 and 9 h, respectively.

The pre-treatment B. fragilis counts were 4.6 and 4.1 log₁₀ c.f.u. ml⁻¹ in the peritoneal fluid and blood, respectively. Analysis of the kill curves revealed that both drugs resolved the infection in the blood and peritoneal fluid by 3 and 6 h, respectively, although ertapenem exhibited significantly higher killing activity than piperacillin–tazobactam in the peritoneal fluid (P<0.05, two-way ANOVA) (Fig. 1c, d). Nine hours after the first dose, the B. fragilis counts in the peritoneal fluid and blood were reduced by ertapenem to −3.7 and −2.6 log₁₀ c.f.u. ml⁻¹, respectively. At the same time, piperacillin–tazobactam reduced the B. fragilis counts in the peritoneal fluid and blood to −2.3 and −1.8 log₁₀ c.f.u. ml⁻¹, respectively.

In the therapeutic concentration range, ertapenem is highly bound to plasma protein (95%) and this phenomenon probably contributes to its long plasma half-life (Majumdar et al., 2002). The main pharmacokinetic–pharmacodynamic parameter of carbapenems and penicillins, which are antibiotics with time-dependent killing activities, is the % t>MIC (Nix et al., 2004). In the present model of systemic infection, the superior activity of ertapenem, mainly against E. coli, can be attributed, at least in part, to its longer plasma half-life in mice (resulting in a longer % t>MIC when compared to piperacillin–tazobactam (Tables 1 and 2).

Both drugs exhibited similar activity against B. fragilis, probably due to a lower inoculum (−4.7 log₁₀ c.f.u. ml⁻¹) detected just prior to the first dose. The influence of inoculum size of E. coli and B. fragilis on piperacillin–tazobactam activity has been described in other studies (Goldstein et al., 1991; König et al., 1998; Stearne et al., 2004). König et al. (1998) demonstrated that piperacillin activity was diminished at least 128-fold when high inocula of E. coli ATCC 25922 were used. In a recent in vitro study we also demonstrated a bacteriostatic activity of

| Table 1. Pharmacodynamic parameters of ertapenem and piperacillin–tazobactam |
|---|---|---|
| Antibiotic | MIC* (µg ml⁻¹) for: | % t>MIC in infected mice |
| * | E. coli ATCC 25922 | B. fragilis ATCC 25285 |
| E. coli | Piperacillin–tazobactam | Piperacillin–tazobactam |
| E. coli† | 0.016 | 0.12 | 100 |
| Piperacillin–tazobactam‡ | 2.0–0.25 | 0.5–0.06 | 46 |

*MIC values determined before and after the antimicrobial therapy are the same.
†Susceptibility breakpoints for ertapenem: B. fragilis ≤4.0 µg ml⁻¹ and E. coli ≤2.0 µg ml⁻¹.
‡Susceptibility breakpoints for piperacillin–tazobactam: B. fragilis ≤32.0/4.0 µg ml⁻¹ and E. coli ≤16.0/4.0 µg ml⁻¹.

| Table 2. Comparative pharmacokinetic parameters of ertapenem and piperacillin–tazobactam in humans and infected mice serum |
|---|---|---|
| | Ertapenem | Piperacillin–tazobactam |
| | Mouse | Human* | Mouse | Human‡ |
| Dose | 25 mg kg⁻¹ sc, 1.0 g iv once daily four doses‡ | 340 mg kg⁻¹ sc, 3.375 g iv, every four doses‡ | 179.8±23.6 | 122.2±30.37 |
| Cₘₐₓ (µg ml⁻¹) | 117.9±19.5 | 154.9±22.0 | 179.8±23.6 | 122.2±30.37 |
| t ½ (h) | 1.0 | 3.8 | 0.5 | 1.24 |

*Data from Majumdar et al. (2002).
†Data from Li et al. (2005).
‡Total dose of 100 mg kg⁻¹ day⁻¹ and 1.35 g kg⁻¹ day⁻¹ for ertapenem and piperacillin–tazobactam, respectively.
piperacillin–tazobactam against high inocula of E. coli ATCC 25922, as well as an increase in MIC (Santos et al., 2007). Thus, this inoculum effect can be an alternative explanation for the higher activity of ertapenem against E. coli (starting inoculum of ~6.4 log10 c.f.u. ml−1) compared with piperacillin–tazobactam. A caveat of the present study is that single strains of B. fragilis and E. coli were examined, and it remains to be determined whether the findings of this investigation extend to other strains.

In conclusion, the data from the present study demonstrated that although both drugs have been effective in the treatment of this model of mixed infection, ertapenem exhibited a higher activity against E. coli relative to piperacillin–tazobactam, in agreement with a previous time-kill study in vitro (Santos et al., 2007).

Acknowledgements

The study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Merck Sharp & Dohme. The authors are grateful to Luzia Rosa Resende, José Sérgio Barros de Souza, João Paulo Lopes de Andrade and to all undergraduate students of the Oral Microbiology and Anaerobes Laboratory for technical support.

Kênia Valéria dos Santos,1 Jacques Robert Nicoli,1 Wanderlany Amancio Martins,1 Simone Cristina Coutinho,1 Ana Carolina Morais Apolônio,1 Cláudio Galuppo Diniz,2 Maria Auxiliadora Roque de Carvalho1 and Luiz de Macêdo Farias1

1Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
2Departamento de Parasitologia, Microbiologia e Imunologia da Universidade Federal de Juiz de Fora, Minas Gerais, Brazil

Correspondence: Luiz de Macêdo Farias (macedo@icb.ufmg.br)

References


