Pharmacokinetic–pharmacodynamic modelling of meropenem against VIM-producing *Klebsiella pneumoniae* isolates: clinical implications

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VIM-producing *Klebsiella pneumoniae* isolates are usually associated with high MICs to carbapenems. Preclinical studies investigating the pharmacokinetic–pharmacodynamic (PK-PD) characteristics of carbapenems against these isolates are lacking. The *in vitro* antibacterial activity of meropenem against one WT and three VIM-producing *K. pneumoniae* clinical isolates (median MICs 0.031, 8, 16 and 128 mg l⁻¹) was studied in a dialysis-diffusion PK-PD model and verified in a thigh infection neutropenic animal model by testing selected strains and exposures. The *in vitro* PK-PD target associated with bactericidal activity was estimated and the target attainment for different dosing regimens was calculated with Monte Carlo analysis. The *in vitro* model was correlated with the *in vivo* data, with log₁₀ CFU/ml reduction of <1 for the VIM-producing (MIC 16 mg l⁻¹) and >2 for the WT (MIC 0.031 mg l⁻¹) isolates, with %/T >MIC 25 and 100 %, respectively. The *in vitro* bactericidal activity for all isolates was associated with 40 % fT>MIC and attained in >90 % of cases with the standard 1 g q8 h 0.5 h infusion dosing regimen only for isolates with MICs up to 1 mg l⁻¹. For isolates with MICs of 2–8 mg l⁻¹, prolonged infusion regimens (4 h infusion q8 or 2 h infusion q4) of standard (1 g) and higher (2 g) doses or continuous infusion regimens (3–6 g) are required. For isolates with a MIC of 16 mg l⁻¹ the unconventional dosing regimen of 2 g as 2 h infusion q4 or 12 g continuous infusion will be required. Prolonged and continuous infusion regimens of meropenem may increase efficacy against VIM-producing *K. pneumoniae* isolates.

**INTRODUCTION**

*Klebsiella pneumoniae* is associated with a wide range of infections, including urinary tract infections, pneumonia, surgical-site infections and soft-tissue infections (Podschun & Ullmann, 1998). Carbapenems can be used to treat these infections, particularly against ESBL-producing strains, although their efficacy is limited against carbapenemase-producing strains (CP-Kp). The clinically important carbapenamases belong to three classes: the class A enzymes of *K. pneumoniae* carbapenemase (KPC) type, the class B enzymes, which are zinc-dependent, represented by VIM, IMP and NDM types, and the class D enzymes of the OXA-48 type (Tzouvelekis et al., 2012). These infections are associated with high mortality, whereas the emergence of multidrug-resistance phenotypes limits further the therapeutic options.

Meropenem is a carbapenem with broad-spectrum activity that demonstrates a time-dependent activity with a 40 %
fT > MIC required for therapeutic efficacy (Mattoes et al., 2004). Although CP-Kp strains have MICs above the susceptibility breakpoint of meropenem (1 mg l\(^{-1}\) for CLSI, 2 mg l\(^{-1}\) for EUCAST), administration of prolonged infusion and high-dose regimens have been used to treat these infections (Markogiannakis et al., 2013; Mattoes et al., 2004). The standard dosing regimen is 0.5–1 g q8 as 0.5 h infusion. However, population pharmacokinetic models showed that the probability of pharmacodynamic target attainment with this regimen is low for isolates with elevated MICs (Li et al., 2006). Therefore, alternative, more effective, regimens are sought mainly via preclinical models.

Pharmacodynamic and animal infection models assessing activity of carbapenems against CP-Kps have so far resulted in interesting and, at the same time, intriguing findings. In vitro pharmacodynamic models using KPC-producing K. pneumoniae isolates showed that a meropenem dosing regimen of 2 g q8 as 3 h infusion was variably effective for isolates with a MIC of 8 mg l\(^{-1}\), possibly owing to differences in specific or co-existent resistance mechanisms (Bulik et al., 2010). Most interestingly, animal studies (mouse thigh infection) showed that both doripenem and ertapenem were effective against the metallo-\(\beta\)-lactamase (MBL) NDM-producing but not against KPC-producing K. pneumoniae isolates exhibiting similar levels of resistance to the latter drug (Wiskirchen et al., 2013, 2014). Moreover, using the same animal model, increased doses of imipenem exhibited measurable bactericidal activity against K. pneumoniae resistant to the latter drug owing to production of another clinically important MBL, VIM-1 (Daikos et al., 2007). It is also of note that a trend to higher survival rates for infections by VIM-producing K. pneumoniae isolates compared with mixed infections by VIM- and KPC-producing isolates (71 vs 57 %) has been reported (Daikos et al., 2014). Therefore, apart from the MIC, the type of carbapenemase in CP-Kps may affect the pharmacokinetic–pharmacodynamic (PK-PD) characteristics and consequently the therapeutic efficacy of carbapenems.

Few preclinical PK-PD studies with meropenem have been performed against K. pneumoniae, and none with VIM-producing isolates. Given the absence of preclinical full PK-PD data of meropenem against VIM-producing K. pneumoniae isolates, we studied the in vitro and in vivo antibacterial activity of meropenem against VIM-1-producing clinical isolates of K. pneumoniae exhibiting various levels of resistance to meropenem in a closed dialysis-diffusion PK-PD model and a neutropenic thigh infection animal model. Furthermore, the probability of pharmacodynamic target attainment was calculated for different dosing regimens and the required trough levels were estimated for the standard dosing regimen.

**METHODS**

**Bacterial strains.** One WT (TZAN59) and three \(\beta\)-lactamase-producing (SEC2, SEC4 and 6/100) K. pneumoniae clinical isolates were used. The SEC2, SEC4 and 6/100 isolates produce only VIM-1 carbapenemases and belong to the same PFGE type (Giakkoupi et al., 2003; Loli et al., 2006). In particular, SEC4 is porin-deficient and does not carry any of the major outer membrane porins of OMPK35 and OMPK36. Meropenem MICs were determined at least four times for all isolates by broth microdilution according to Clinical Laboratory Standards Institute methodology using Escherichia coli ATCC 25922 as the quality-control strain. The WT isolate TZAN59 had a median (range) MIC of 0.031 (0.031–0.062) mg l\(^{-1}\) whereas the three VIM-producing isolates, 6/100, SEC2 and SEC4, had MICs of 8 (2–16), 16 (8–16) and 128 (128–256) mg l\(^{-1}\), respectively. For the calculation of the PK-PD indices, we employed the MIC determined on the day of the experiments using the same inoculum as in the in vitro or animal experiments. These isolates were chosen in order to cover a broad range of MICs of VIM-producing isolates that are resistant to meropenem. Inocula were prepared in saline from overnight cultures of all isolates in Mueller–Hinton agar and adjusted to obtain a final inoculum of 10\(^{6}\) c.f.u. ml\(^{-1}\).

**Antibiotics and medium.** Meropenem for intravenous injection (50 mg ml\(^{-1}\); Astra-Zeneca Pharmaceuticals) was reconstituted according to the manufacturer’s instructions. In preliminary experiments, we compared the in vitro activity of the clinical formulation and the pure powder in MIC and time-kill assays, and no differences were found. We therefore used the clinical formulation in all further experiments. Cation-adjusted Mueller–Hinton broth (CAMHB) was used as the bacterial growth medium throughout the in vitro experiments.

**In vitro PK-PD model.** The in vitro closed diffusion-dialysis PK-PD model consisted of two compartments, the internal and the external compartment, as previously described (Meletiadis et al., 2012). The external compartment consisted of a 750 ml flask placed on a 37 °C magnetic heating stirrer, whereas the internal compartment consisted of a 10 ml dialysis tube made of semi-permeable cellulose with membrane molecular weight cutoff of 1000 kDa (Float-A-Lyzer; Spectrum Labs). The external compartment contained CAMHB whereas the internal compartment contained CAMHB with 10\(^{5}\) c.f.u. ml\(^{-1}\) of each isolate. The dialysis tube enabled bidirectional penetration of antibacterial drugs and nutrients, while preventing the escape of bacteria. Magnetic stirrer bar in the external compartment provided sufficient mixing of the content.

**In vitro pharmacokinetics.** Meropenem was added to both compartments at peak concentrations of 30 and 15 mg l\(^{-1}\), as previously observed in patients with standard dosing regimens of 1 and 0.5 g, respectively (Binder et al., 2013). Various dosing regimens were simulated in order to obtain different values of %fT > MIC attained in clinical practice with different prolonged infusion regimens. Therefore, meropenem concentrations were kept constant in the in vitro model for 1.5, 3, 6 and 12 h. At the end of these periods, the internal compartment was transferred within 1 h twice to a new external compartment with fresh drug-free CAMHB. Meropenem was diffused from the internal compartment to the external compartment, resulting in a biphasic decrease of its concentration in the internal compartment. This was repeated every 24 h for 3 days. Drug concentrations in the internal compartment were determined with a microbiological diffusion assay using Escherichia coli ATCC 25922 in Mueller–Hinton agar (Oxoid) (Tam et al., 2005).

**In vitro pharmacodynamics.** Bacterial counts were assessed by sampling 20 pl from the internal compartment at regular time points and performing quantitative cultures. Time-kill curves were constructed by plotting log\(_{10}\) c.f.u. ml\(^{-1}\) against time. Pharmacodynamic effects were assessed based on c.f.u. reduction at 72 h compared to the drug-free control for each dosing regimen and isolate.

**In vitro PK-PD modelling.** The 72 h log\(_{10}\) c.f.u. ml\(^{-1}\) reduction over %fT > MIC was analysed with nonlinear regression analysis using the
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RESULTS

In vitro data
Different %fT>MIC were obtained with the 1.5, 3, 6 and 12 h prolonged-infusion regimens targeting fCmax of 30 and 15 mg L⁻¹. The time-concentration profiles of the two simulated doses were characterized by mean ± SD fCmax of 24.6 ± 3.6 and 14.2 ± 2.2 mg L⁻¹ and a t½ of both phases of <2 h (Fig. 1). For some strains and infusion regimens, meropenem concentration in the internal compartment was decreased by up to 25% during incubation. The susceptible isolate TZAN59 was completely killed by all four infusion regimens, whereas the resistant isolate SEC4 was not killed by any of the four infusion regimens (Fig. 2). The isolates 6/100 and SEC2, with MICs of 8–16 mg L⁻¹, were killed only by 12 h prolonged infusion with fCmax 30 mg L⁻¹ (Figs 3 and 4). The log10(CFU/ml⁻¹) reduction over %fT>MIC followed a sigmoidal pattern (R²=0.95), with the mean (95% CI) maximal bactericidal activity at 40% (37–45) %fT>MIC and a bacteriostatic effect of 25% (22–30).

In vivo data
The serum Cmax, Cmin and t½ values were 15.2 mg L⁻¹, 0.10 mg L⁻¹ and 18.2 min for the dose of 30 mg kg⁻¹, and 28.5 mg L⁻¹, 0.15 mg L⁻¹ and 14.1 min for the dose of 60 mg kg⁻¹, respectively (Fig. 5). The %fT>MIC for the dosing interval for the WT isolate TZAN59 and the VIM-producing SEC2 isolate was 100 and 15% for the 30 mg kg⁻¹ dose and 100 and 23% for the 60 kg kg⁻¹ dose, respectively. The log10(c.f.u.ml⁻¹) reduction for the 30 and 60 mg kg⁻¹ dosages, respectively, was −1.94 and −2.78 for the TZAN59 isolate and −0.62 and −0.83 for the SEC2 isolate, at 24 h (Fig. 6a, b).

In vitro–in vivo correlation
In vitro 4 and 6 h prolonged infusion every 24 h resulted in %fT>MIC values very similar to those obtained in animals with the dosages of 30 and 60 mg kg⁻¹ i.p. In particular, the %fT>MIC for TZAN59 was 100% at both dosages whereas for the SEC2 isolate the %fT>MIC values were 18 and 25%, respectively (Fig. 5). In agreement with the in vivo data, where the log10(CFU/ml⁻¹) reduction was larger for TZAN59 and smaller for SEC2, a bactericidal activity was found for TZAN9 whereas a bacteriostatic effect was found for the isolate SEC2. Note that the log10(CFU/ml⁻¹) reduction of the WT strain was larger in vitro (~4log10[c.f.u.ml⁻¹] reduction) than in vivo(~2log10[c.f.u.ml⁻¹] reduction) despite the fact that both in vitro and in vivo dosing regimens resulted in 100% %fT>MIC. This may be related to the lower free tissue levels compared with serum levels of meropenem, as previously reported, or other host-related factors that minimized the in vivo efficacy of meropenem (Nicolaou, 2008). Interestingly, a significant c.f.u. reduction was observed in vitro for SEC2 after drug addition, indicating that dosing regimen optimization may enhance killing.

Bridging to human data
The simulated patient population had very similar characteristics to the target population, with mean ± SD age, weight and serum creatinine of 39 ± 18 years, 72.8 ± 16.11 kg and

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1.12 ± 0.78 mg dl⁻¹, respectively. The probability rates of target attainment for different dosing regimens and MIC are shown in Table 1. More than 90 % of target attainment was found with 1 g q8 as 0.5 h infusion for isolates with MIC up to 1 mg l⁻¹, whereas for isolates with MICs of 2–4 mg l⁻¹, the target was attained with 3 and 4 h infusion of 1 g q8 or 0.5 h infusion of 1 g q4, or 3 g continuous infusion. Isolates with MIC 8 mg l⁻¹ will require 2 g q8 as 3–4 h infusion, 2 g q4 as 0.5 h infusion, 1 g q4 as 2 h infusion or 6 g continuous infusion. Isolates with MIC 16 mg l⁻¹ will

![Fig. 1. In vitro pharmacokinetics of meropenem for 1.5 (a), 3 (b), 6 (c) and 12 h (d) prolonged infusion q24 dosing regimens with fCmax of 15 (V) and 30 mg l⁻¹ (Δ). Horizontal dotted line represents the limit of detection.](image)

![Fig. 2. Time–kill curves in the in vitro model for the susceptible WT isolate TZAN59 (MIC 0.031 mg l⁻¹) (a) and the VIM-1-producing isolate SEC4 with high-level resistance to meropenem (MIC 256 mg l⁻¹) (b). TZAN59 but not SEC4 was killed at all of the simulated infusion dosing regimens. Here the time-kill curves for 1.5 (a) and 12 h (b) prolonged-infusion q24 are presented. The dotted line represents the limit of detection. o, Drug-free control; V, Cmax 15 mg l⁻¹; Δ, Cmax 30 mg l⁻¹.](image)
require 2 g q4 as 2 h infusion or 12 g continuous infusion. The standard dosing regimen of 1 g as 0.5 h infusion q8 will not attain the pharmacodynamic target for most patients infected with isolates with MICs >1 mg l⁻¹. Monte Carlo analysis showed that 85, 63, 33 and 5 % of those patients will attain the pharmacodynamic target for isolates with MIC 2, 4, 8 and 16 mg l⁻¹, with trough levels higher than 0.02, 0.06, 0.23, 0.95, and 3.87 mg l⁻¹, respectively.

DISCUSSION

The in vitro and in vivo findings of the present study showed that meropenem exposures of 25 % fT>MIC and >40 % fT>MIC were associated with a bacteriostatic and a bactericidal effect of meropenem against VIM-1-producing K. pneumoniae isolates. Based on the 40 % fT>MIC pharmacodynamic target, high target attainment rates were found with 1 g q8 as 0.5 h infusion dosing regimen for isolates with MICs up to 1 mg l⁻¹. Isolates with MICs of 2–4 mg l⁻¹ will require 1 g q8 as 3 or 4 h infusion, 1 g q4 as 0.5 h infusion or 3 g continuous infusion. Isolates with MIC 8 mg l⁻¹ will require 1 g q4 as 2 h infusion, 2 g q8 as 3 or 4 h infusion, 2 g q4 as 0.5 h infusion or 6 g continuous infusion. Finally, for isolates with MIC 16 mg l⁻¹, 2 g q4 as 2 h infusion or 12 g continuous infusion will be required to attain the pharmacodynamic target.

In a previous in vitro PK-PD study, a high-dose prolonged-infusion regimen of meropenem (2 g q8 as 3 h infusion)
was able to kill KPC-type isolates with a MIC of 2 mg l\(^{-1}\) but not with MICs \(\geq\)16 mg l\(^{-1}\), whereas variable activity was found for isolates with a MIC of 8 mg l\(^{-1}\) (Bulik et al., 2010). Although a 40 % \(f/T > \text{MIC}\) was targeted, this was not achieved for the entire period because of the hydrolysis that took place, resulting in lower meropenem exposure. Such a phenomenon was observed in some experiments in our in vitro model when up to 25 % reduction of meropenem concentration was observed inside the dialysis tube during incubation. Interestingly, in the previous study, regrowth was observed of an isolate with a MIC of 8 mg l\(^{-1}\) even when the \%\(f/T > \text{MIC}\) remained above 40 % (50–69 %), leading to the conclusion that specific or co-existent resistance mechanisms may alter the pharmacodynamics of meropenem. However, in vivo studies demonstrated that, regardless of the production of \(\beta\)-lactamas and other meropenem resistance mechanisms, the desired bactericidal effect can still be achieved as long as the drug exposure is 40 % \(f/T > \text{MIC}\), as also found with the VIM-1-producing isolates used in the present study (Maglio et al., 2004). Our in vivo data are in line with previous data in a neutropenic animal model of thigh infection by extended-spectrum \(\beta\)-lactamase-producing K. pneumoniae isolates treated with meropenem, where c.f.u. reduction was minimal for isolates with a MIC of 8 mg l\(^{-1}\), and around 2 log\(_{10}\) for isolates with MICs of 0.03–0.06 mg l\(^{-1}\) (DeLyke et al., 2007).

By bridging the in vitro pharmacodynamic target with human pharmacokinetic data from patients treated with meropenem, we found that the standard dosing regimen of meropenem (1 g q8 as 0.5 h infusion) is sufficient to attain the pharmacodynamic target of a bactericidal effect for isolates with MICs \(\leq\)1 mg l\(^{-1}\). This is in agreement with the current CLSI susceptibility breakpoint of \(\leq\)1 mg l\(^{-1}\) (CLSI, 2010) and with previous simulation studies (Li et al., 2006). Higher exposures will be required for isolates with higher MICs. Prolonged infusion dosing regimens of up to 4 h (50 % of the dosing interval) have been used to treat bacterial infections (Fehér et al., 2014; Jaruratanasirikul et al., 2005). Our analysis indicated that a dosing regimen of 2 g q8 as 4 h infusion will result in target attainment rates of 100, 100, 100 and 71 % for VIM-producing isolates with MICs 2, 4, 8 and 16 mg l\(^{-1}\), respectively. In a retrospective observational study of febrile neutropenic patients treated with meropenem, the 4 h infusion dosing regimen of 1 g q8 resulted in better clinical outcome than the standard 0.5 h infusion regimen (Fehér et al., 2014). Monte Carlo simulation analysis in the present study showed that 4 h infusion regimens of standard (1 g) or higher (2 g) doses could be used in order to attain the pharmacodynamic target for infections caused by isolates with MICs 2 and 4 mg l\(^{-1}\), whereas for isolates with MIC 8 mg l\(^{-1}\), the dosing regimen of 2 g as 3–4 h infusion q8, or as 2 h infusion q4, 1 g as 2 h infusion q4, or 6 g as continuous infusion will be required. For isolates with a MIC of 16 mg l\(^{-1}\), the pharmacodynamic target will be attained with 2 g as 2 h infusion q4 or with 12 g continuous infusion. These findings merit further clinical investigation.

The PK-PD target of 40 % \(f/T > \text{MIC}\) may be valid only for specific patient populations and types of infections, since higher PK-PD targets were found for neutropenic patients with bacteraemia and elderly Chinese patients with lower respiratory infections by different Gram-negative bacteria (Ariano et al., 2005; Zhou et al., 2011). On the same lines, a trough level/MIC ratio of 4–6 was suggested to maximize effectiveness of meropenem in critically ill patients and adult patients with lower respiratory tract infections, although few infections (<10 %) were due to K. pneumoniae and even fewer to
non-WT *K. pneumoniae* isolates (Li et al., 2007; Pea et al., 2012). Reduced tissue penetration, emergence of resistance, immunosuppression and other comorbidities may require higher serum exposures for a therapeutic outcome.

In conclusion, a simple *in vitro* PK-PD model well-correlated *in vivo* outcome was developed in order to study the pharmacodynamics of meropenem against *K. pneumoniae* isolates. Using a three-stage pharmacodynamic approach (extensive *in vitro* experiments--limited animal experimentation--bridging with human pharmacokinetics), clinically valuable pharmacodynamic data were obtained. The exposure index associated with bactericidal action was 40 % \(fT > \text{MIC}\) for VIM-producing *K. pneumoniae* isolates. Simulation analysis indicated that high doses of up to 2 g and prolonged infusion for up to 4 h q8 or 3–12 g continuous infusion regimens are required in order to attain the this pharmacodynamic target for isolates with MICs up to 16 mg l\(^{-1}\).

### REFERENCES


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