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Sterilizing Effect of Ertapenem-Clavulanate in a Hollow-Fiber Model of Tuberculosis and Implications on Clinical Dosing

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ABSTRACT Carbapenems are now being explored for treatment of multidrug-resistant tuberculosis (MDR-TB), especially in conjunction with clavulanate. Clinical use is constrained by the need for multiple parenteral doses per day and the lack of knowledge of the optimal dose for sterilizing effect. Our objective was to identify the ertapenem exposure associated with optimal sterilizing effect and then design a once-a-day dose for clinical use. We utilized the hollow-fiber system model of tuberculosis in a 28-day exposure-response study of 8 different ertapenem doses in combination with clavulanate. The systems were sampled at predetermined time points to verify the concentration-time profile and identify the total bacterial burden. Inhibitory sigmoid maximum-effect (E_{max}) modeling was used to identify the relationship between total bacterial burden and the drug exposure and to identify optimal exposures. Contrary to the literature, ertapenem-clavulanate combination demonstrated good microbial kill and sterilizing effect. In a dose fractionation hollow-fiber study, efficacy was linked to percentage of the 24-h dosing interval of ertapenem concentration persisting above MIC (%T_{MIC}). We performed 10,000 MDR-TB patient computer-aided clinical trial simulations, based on Monte Carlo methods, to identify the doses and schedule that would achieve or exceed a %T_{MIC} of ≥40%. We identified an intravenous dosage of 2 g once per day as achieving the target in 96% of patients. An ertapenem susceptibility breakpoint MIC of 2 mg/liter was identified for that dose. An ertapenem dosage of 2 g once daily is the most suitable to be tested in a phase II study of sterilizing effect in MDR-TB patients.

KEYWORDS *Mycobacterium tuberculosis*, ertapenem, hollow-fiber infection model, MDR-TB, pharmacodynamics, pharmacokinetics

The emergence of drug-resistant tuberculosis (TB), especially multidrug-resistant TB (MDR-TB), extensively drug-resistant TB (XDR-TB), and virtually incurable TB (termed totally drug-resistant TB by some), is a global emergency that threatens to undermine many gains of chemotherapy (1–4). As a result, there is currently a four-pronged effort to combat this problem: (i) identification of new small molecules to kill drug-resistant *Mycobacterium tuberculosis*, (ii) repurposing of antimicrobial drugs not currently used to treat TB into TB therapeutics, (iii) host-directed therapy, and (iv) use of pharmacokinetics/pharmacodynamics (PK/PD) science to optimize efficacy while suppressing emergence of acquired drug resistance (5–8). Carbapenems, extensively used to treat Gram-negative bacteria over the last 30 years, have also been shown to be effective

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against *M. tuberculosis* in *vitro* and *in vivo* when in the presence of a β-lactamase inhibitor (6, 9).

Several initiatives are ongoing to explore the added value of carbapenems given as part of a multidrug regimen for M/XDR-TB (10, 11). In murine TB, efficacy has been demonstrated for meropenem and imipenem with clavulanate; however, ertapenem was no better than nontreatment (9). In addition, ertapenem demonstrated high MICs, suggesting possible natural resistance. However, ertapenem degrades rapidly in *in vitro* growth media at incubation temperatures used to measure MICs with conventional methods (12). We have since developed an MIC assay that corrects for this degradation, which has demonstrated much lower MICs (12). The main advantage of ertapenem to patients could be its half-life of 4 h, which could allow a once-a-day schedule, as opposed to 0.6 to 0.7 h for meropenem and imipenem, which necessitates multiple and prolonged intravenous infusions per day (13). The multiple infusions per day with meropenem and imipenem make it rather difficult to administer long-duration therapy in M/XDR-TB. Recently, the first TB clinical data with ertapenem showed that it was well tolerated as part of a salvage regimen for MDR-TB patients (13, 14). Unfortunately, the efficacy of the drug could not be assessed, as it was used in a multidrug regimen; moreover, its sterilizing effect is unknown.

The hollow-fiber system model of TB (HFS-TB) has been used to examine the sterilizing effect of anti-TB agents, defined as the ability to kill either semidormant *M. tuberculosis* under acidic conditions or nonreplicating persisters under hypoxia (15–17). It was qualified by the European Medicines Agency and editorially endorsed by the U.S. Food and Drug Administration (http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2015/02/WC500181899.pdf). The HFS-TB in tandem with computer-aided clinical trial simulations was found to have a forecasting accuracy of >94% of observed optimal exposures and doses in TB patients in the clinic (18–20). This makes this model ideal to identify optimal doses for treatment of M/XDR-TB, which can directly be translated into clinical use. Our objective was to use these models to identify the optimal sterilizing-effect dose of ertapenem for treatment of MDR-TB.

RESULTS

Dose-effect HFS-TB study for sterilizing effect. In the first HFS-TB, which was mainly a dose ranging study, we cultured *M. tuberculosis* H37Ra under acidic conditions to a semidormant state and then use it to inoculate HFS-TB units with circulating Middlebrook 7H9 acidified to a pH of 5.8, as described previously (15, 21). Different ertapenem exposures, based on human-equivalent doses of 0.25, 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 g, were administered into the central compartment of duplicate HFS-TB units via a computer-controlled syringe pump over 30 min, as in patients; drug concentrations achieved in each of the 16 HFS-TB were measured at 8 different time points over the first 24 h. Clavulanate was also dispensed via syringe pump to achieve a peak of 3 mg/liter at the end of 30 min of infusion. Pharmacokinetic modeling of the measured drug concentrations revealed that the lowest Akaike information criterion scores (22) were for a one-compartment model. The ertapenem total clearance (±standard deviation) was 4.11 ± 1.83 liters, and the volume was 22.55 ± 4.0 liters, which translates to a half-life of 3.80 h. The regression for observed concentrations versus pharmacokinetic model predicted concentrations had an r^2 of 0.997 and the slope was 0.996 ± 0.006, which is close to unity. Thus, the one-compartment model described the data well, with no bias.

Figure 1 shows that ertapenem achieved a good sterilizing effect. The bacterial burden at the start of therapy was 4.0 $\log_{10}$ CFU/ml. The data are presented as inhibitory sigmoid maximum-effect (E_{max}) models between "nominal" human-equivalent dose and microbial burden. In Fig. 1, there was no model convergence on day 3, while on day 28, at the end of the experiment, all ertapenem-treated systems had bacterial burdens below limits of detection. All systems achieved percent time above MIC (%T_{MIC}) of 100% of the dosing interval; the trough at 23.5 h was >4 mg/liter in all systems, and all achieved the same microbial kill on day 28.

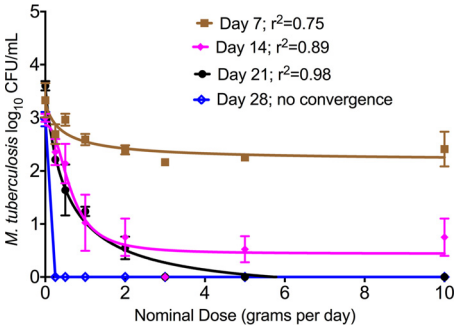

FIG 1 Ertapenem-clavulanate dose-effect sterilizing effect in the hollow-fiber model. Drug treatments are depicted as "nominal" human-equivalent doses. On day 3, inhibitory sigmoid E_{max} modeling demonstrated no model convergence, and there was very little kill; thus, regressions for day 3 were left out. However, by day 7 there was already good microbial kill, characterized by maximal kill (E_{max}) of 1.13 ± 0.34 $\log_{10}$ CFU/ml. By day 28, all ertapenem-treated HFS-TB units completely sterilized the bacteria.

Ertapenem dose fractionation study in the HFS-TB. Next, we performed a new HFS-TB, this time using *M. tuberculosis* H37Rv and a dose fractionation design, for a treatment duration of 14 days. On measurement of ertapenem concentrations, similar to the first study, the concentrations were also best described using a one-compartment model; the observed versus predicted concentrations revealed a slope of 0.995 ± 0.002 ($r^2 > 0.999$). The concentration-time profiles achieved with each dose are shown by dosing schedule in Fig. 2A to C, together with the ertapenem (plus clavulanate at 2.5 mg/liter) MIC of 4 mg/liter. Inhibitory sigmoid E_{max} model fitting by exposure, expressed as either maximum concentration (C_{max})/MIC, area under the concentration-time curve from 0 to 24 h (AUC_{0-24})/MIC, or $\%T_{MIC}$, revealed Akaike information criterion scores shown in Table 1. The lowest scores were for $\%T_{MIC}$, which means that this is the PK/PD index linked to microbial kill. Figure 2D shows the inhibitory sigmoid E_{max} MIC curves for each sampling day based on $\%T_{MIC}$. Based on day 10, which had the highest r^2 (0.94), the relationship between $\%T_{MIC}$ and bacterial burden was as follows: $\log_{10}$ CFU/ml $= 5.68 - \%T_{MIC}^{2.56}/[23.52^{2.56} + \%T_{MIC}^{2.56}]$. From this relationship, we calculated the 80% effective concentration (EC_{80}) as a $\%T_{MIC}$ of 40.41% of the dosing interval. Indeed, this can be read off Fig. 2D as well, which shows that one gets the same exposure for optimal kill whichever sampling day is examined.

Monte Carlo simulations to identify optimal ertapenem dose. In TB patients, pharmacokinetic variability is one of the most important drivers of sterilizing effect (23–29). Therefore, in order to identify the optimal ertapenem dose for pulmonary TB, we performed Monte Carlo simulations of 10,000 patients with pulmonary TB, using the pharmacokinetic parameter estimates and between-patient variability indices shown in Table 2 based on previous studies (30–32). We also accounted for the ertapenem penetration into epithelial lining fluid (ELF) of 7.48% ± 8.17% (which mirrors the non-protein-bound concentration of 5 to 15%), and that in lung tissue of 23.6% ± 12.3% (33). We performed simulations to determine how much 1.0 g once a day, 1.0 g twice a day, 2.0 g once a day, 2.0 g twice a day, or 3.0 g once a day would achieve or exceed the target exposure, which is a $\%T_{MIC}$ of 40.41%, associated with optimal sterilizing effect in ELF of patients. For internal validation, we compared the pharmacokinetic parameters in the 10,000 simulated patients to those of Burkhardt et al. (30) in Table 2, which shows that the simulations faithfully recapitulated the pharmacokinetic parameters and variability. As an extra external validation step, we compared the pharmacokinetic parameters in the simulations to those we actually observed in our MDR-TB patients in The Netherlands, as shown in Table 2 (13). Table 2 shows that the pharmacokinetic parameters and variance in our simulations were virtually identical to those we observed in patients. Therefore, the simulations were accurate in reproducing what is identified in the clinic.

Figure 3A shows the target attainment probability (TAP) for each dose and dosing schedule as the MIC changes. On one extreme, the dosage of 1 g once a day had a TAP

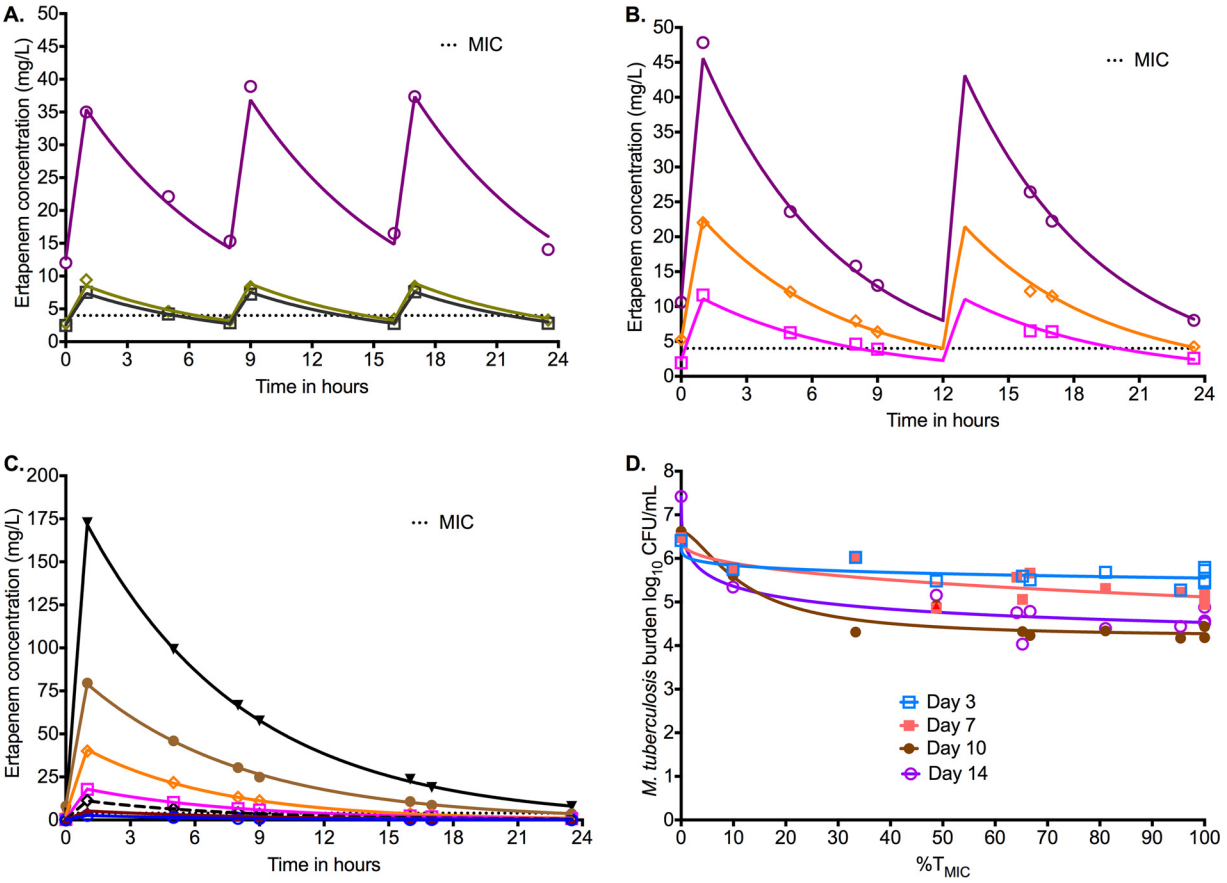

FIG 2 Dose fractionation study to determine PK/PD index linked to ertapenem efficacy. The concentration time profiles are shown relative to the MIC. Symbols indicate measured concentrations and the lines modeled profile. (A) Concentration-time profiles of ertapenem identified in the HFS-TB with a dosing schedule of every 8 h. (B) Concentration-time profiles of ertapenem identified in the HFS-TB with a dosing schedule of every 12 h. (C) Concentration-time profiles of ertapenem identified in the HFS-TB with a dosing schedule of once a day. Given the concentration range, the scale obscures the time that concentrations persisted above MIC for some doses. For the blue open circles, the lowest concentration, the time above MIC was 0 h. For the dose shown by cayenne triangles the time was 3 h, for the black open diamonds it was 8.32 h, and for the open magenta squares it was 11.7 h. The rest can be read off the graph. (D) Inhibitory sigmoid E_{max} model for $\%T_{MIC}$ versus bacterial burden. On day 7, the maximal kill (E_{max}) was 1.14 $\log_{10}$ CFU/ml, consistent with findings in the first HFS-TB dose-effect study. The study was carried out for only 14 days. Examination of the curves on each day shows that 80% of maximal kill occurs around a $\%T_{MIC}$ of 40% on all sampling days except day 3, when it occurs with lower exposures.

less than 90% once the MIC was 1 mg/liter, while the dosage of 3 g twice a day achieved a high TAP until 8 mg/liter and then fell precipitously at 16 mg/liter. For 2 g a day, the TAP fell at an MIC of 2 mg/liter. This means that the susceptibility breakpoint for ertapenem plus clavalunate will fall between MIC of 1 and 16 mg/liter and will depend on the final dose chosen.

Since MIC variability is also an important determinant of therapy response in TB patients (25, 34–36), we also took into account the MIC distribution. Figure 3B shows the ertapenem MIC distribution from 33 MDR-TB patients isolates in The Netherlands, in the presence of clavulanate. Figure 3B shows that all isolates would have MICs between greater than 1 mg/liter and below 128 mg/liter. However, in the past we have

TABLE 1 Akaike information criterion scores for PK/PD index versus ertapenem sterilizing effect

	Score on:			
Parameter	Day 3	Day 7	Day 10	Day 14
AUC_{0-24}/MIC	−30.26	−18.99	−31.41	−5.112
C_{max}/MIC	−30.63	−17.19	−31.55	−2.144
$\%T_{MIC}$	−60.39	−62.96	−49.39	−45.06

TABLE 2 Comparison of pharmacokinetic parameter and concentration estimates and ranges in 10,000 simulated patients to those actually observed in patients treated with 1 g

Parameter	Value		
	Subroutine prior based on literature, mean ± SD	For 10,000 simulated TB patients, mean (range)	Observed in MDR-TB patients, mean (range) (13)
Total clearance, liters/h	2.63 ± 0.83	2.6 (0.02–6.00)	2.1 (0.09–3.23)
Vol, liters	10.6 ± 2.51	11 (1.2–19)	7.3 (2.61–11.10)
Half-life, h		2.8 (2.20–3.70)	2.4 (2.05–3.53)
AUC_{0-24}, mg · h/liter		448 (166–4,255)	545 (309–1,130)

shown that ertapenem degrades during the MIC testing, and if one accounts for the degradation, there is a 4-tube dilution decrease in MICs; if clavulanate is added and ertapenem is supplemented, there is a 7-tube dilution difference (12). Thus, we transformed the MICs for the 33 clinical isolates down by 4 tube dilutions as well, as shown in Fig. 3B. In that scenario, only 6.5% of isolates had an MIC greater than 1 mg/liter.

Summation of all TAPs to account for distribution of MICs gives the proportion of 10,000 TB patients who would achieve the target exposure of $\%T_{MIC}$ of 40%, termed the cumulative fraction of response (CFR). Figure 3C shows the CFRs for the once-a-day dosing schedule for both observed MICs and transformed MICs. For the transformed MICs, the dosage of 2 g a day had a CFR of 96%. We also determined the MICs of

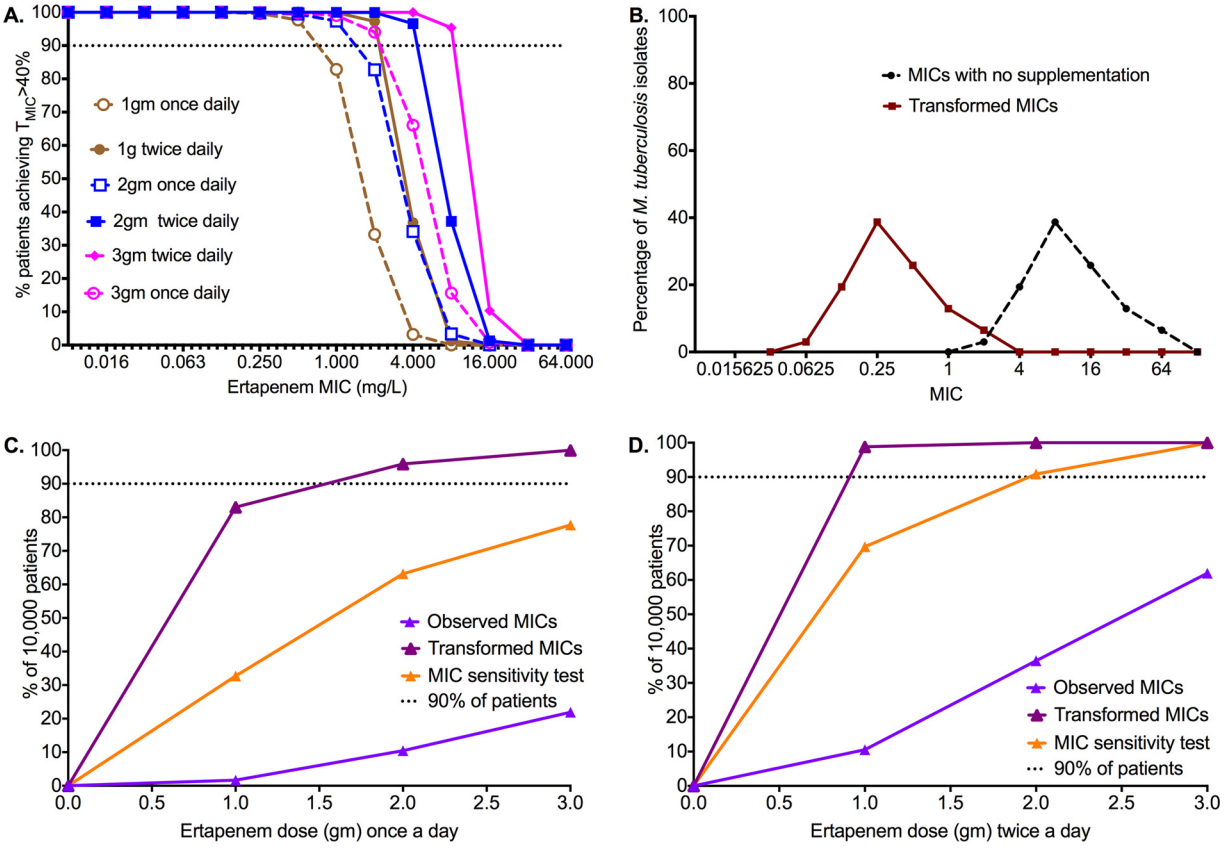

FIG 3 Target attainment probability and cumulative fraction of response for various ertapenem doses. (A) Target attainment probability for $\%T_{MIC}$ of 40% as *M. tuberculosis* MIC changes. No dose or dosing schedule is effective once MICs are 16 mg/liter. (B) Ertapenem MIC distribution in isolates from The Netherlands, with and without transformation to account for ertapenem degradation. (C) Proportion of 10,000 patients who achieved or exceeded a $\%T_{MIC}$ of 40% with once-a-day dosing. The proportion is highly sensitive to the MIC and fell on sensitivity analysis, a worst-case scenario. (D) Proportion of 10,000 patients who achieved or exceeded a $\%T_{MIC}$ of 40% with twice-a-day dosing. The twice-a-day dosing schedule achieved the target in higher proportions of patients, even on sensitivity analysis. However, given the hardship of twice a day administration of therapy in TB, we chose the dosage of 2 g once a day as being most practical.

ertapenem plus clavalunate for 4 clinical isolates incubated at 4°C versus 37°C to try and slow down drug degradation: MICs were lower at 4°C by 4, 2, 3, and 2 tube dilutions. Therefore, we performed sensitivity testing by examining CFR if MIC transformation was only 2 tube dilutions lower (worst-case scenario). Figure 3C shows that the dosage of 2 g once a day would not achieve the target in 90% of patients; nevertheless, it would achieve this in 63% of patients, which is still reasonable. Figure 3D shows the results of a twice-a-day dosing schedule; as would be expected from a $\%T_{MIC}$-driven drug, this dosing schedule performed better. The dosage of 1.0 g twice a day would achieve target exposure in 99% of patients and on sensitivity testing would still achieve this in 70% of patients. The dosage of 2 g twice a day would achieve >90% even on sensitivity testing.

DISCUSSION

This is the first study that showed the efficacy and sterilizing effect of ertapenem-clavulanate, unlike findings in the murine model, likely because the HFS-TB mimicked the half-life of 4 h encountered in patients, in contrast to 1.0 h in mice. We were able to recapitulate ertapenem's pharmacokinetics, and its half-life of 4 h, as encountered in TB patients, which likely explains the better efficacy in this model than that encountered in mice, in which the ertapenem half-life is 1 h. Moreover, dosages simulated in the model were in a range that would likely be tolerable in patients. This study showed the advantage of the hollow-fiber system, namely, a better recapitulation of human-like pharmacokinetics, and of microbial sterilizing-effect conditions. The Monte Carlo simulations then introduced the variability that would be encountered for pharmacokinetic parameters between patients and MICs between *M. tuberculosis* strains. Our two-step external validation approach in the simulations ensured that our simulations reflected clinical reality; sensitivity testing accounted for any uncertainty in MIC distribution. This allowed us to perform dose-effect studies that take into account the exposure-effect relationship as described for the hollow-fiber model, the essential aspects of drug behavior in patients, such as pharmacokinetic variability and the ratios for drug penetration to lungs, that are important in determining efficacy, and susceptibility of *M. tuberculosis* isolates encountered in hospitals. This approach, in many experiments based on the same *M. tuberculosis* isolate as we used in the current study (*M. tuberculosis* H37Rv), has been found to be >94% accurate in identifying clinical doses that are optimal in TB patients based on recent presentation for regulatory approval (19).

Ertapenem-clavulanate may play an important role in the intensive phase of TB treatment due to its sterilizing effect. In addition, intravenous administration may be more suitable for the intensive phase, in which M/XDR-TB patients are likely to be administered therapy in a TB clinic. As carbapenems are already part of the WHO list of TB drugs for M/XDR-TB, the next step is to explore the use of ertapenem-clavulanate in patients, using the dosage of 2 g once a day that we identified. Recently, it has been shown that meropenem-clavulanate has promising activity against MDR-TB *in vitro* (37, 38). Indeed, imipenem-clavulanate and meropenem-clavulanate were associated with a treatment success of >57% and culture conversion of >60% in a recent systemic analysis of five studies (39). However, since clavulanate is administered as oral amoxicillin-clavulanate, gastrointestinal side effects may become a problem if this formulation is administered for a prolonged duration multiple times a day with meropenem or imipenem, which would compromise absorption of other oral drugs. Unfortunately, the current suppliers of carbapenems are not interested in developing an infused combination of carbapenem and clavulanate. The main advantage of ertapenem is its long half-life, enabling once-daily dosing, which would also allow a once-a-day clavulanate dose, potentially reducing side effects. This may even facilitate dosing in an outpatient setting. Patients may present at the clinic once a day for their drug administration as part of directly observed treatment, or they could receive treatment as a once-a-day infusion at home when sputum culture negative in those countries where the drug is already part of home care for treatment of other chronic infections. Ertapenem has a

labeled infusion time of only 30 min, which facilitates a relatively short stay at an outpatient clinic. Even more rapid infusion has been explored and showed similar drug exposure and tolerability (40). We show that a dosage of 2 g given once daily could contribute to an effective regimen. Ertapenem up to a dose of 3 g has been administered to healthy volunteers (41). Moreover, doses up to 2 g have been administered in 30 min without any additional complications (42). However, there is a need for a prospective phase II study exploring the safety and efficacy of 2 g of ertapenem with clavulanate once a day in MDR-TB patients.

On the other hand, the amount of time clavulanate has to be around to keep potentiating ertapenem is still unclear. Thus, the target concentration to aim with dosing is unclear. Clavulanate has a shorter half-life than does ertapenem. However, penetration into the bronchial mucosa is 118%, and its protein binding is minimal at 20%, and likely an effective concentration remains at the site of effect even when dosed once. Since clavulanate is renally eliminated, between-patient variability in systemic clearance, which is about 58%, is driven mainly by renal function: the lower the creatinine clearance, the less the drug is cleared (43). Separate dose-effect studies on the role of clavulanate will need to be conducted, after which simulations similar to the ones described here can be performed.

Finally, pharmacokinetics/pharmacodynamics-based susceptibility breakpoints in TB, mostly derived from hollow-fiber model monotherapy studies, have been shown to be highly accurate in delineating TB patients who fail or respond to combination therapy (25, 34, 35). The 2-mg/liter ertapenem susceptibility breakpoint we identified for the dosage of 2 g a day should thus be used by clinicians as decision-making tool to determine if a patient will respond to ertapenem therapy. This breakpoint will differ from the epidemiological cutoff value, which may be more useful for epidemiological tracking of acquired ertapenem resistance, as opposed to clinical decision-making.

There are some limitations to our study. First, we used two isolates *M. tuberculosis* for the sterilizing-effect experiments. Inclusion of a larger number of isolates could change the final target exposure associated with optimal efficacy. However, hollow-fiber studies in the past with these isolates were found to be predictive of the optimal exposure targets in patients for sterilizing effect (15, 24, 25, 44–46). A second limitation is that we used pharmacokinetic data from critically ill patients as prior data for our Monte Carlo simulations. The type of disease that a patient has can alter the pharmacokinetic parameters, so TB patients could have different pharmacokinetics. However, as shown in Table 2, the pharmacokinetic parameter estimates in simulated patients, and the AUC_{0-24} achieved with 1-g doses, were virtually identical to those we have identified in TB patients in The Netherlands as part of therapeutic drug monitoring. This validates that simulated patients had pharmacokinetic parameters and concentrations similar to those encountered in TB patients.

In conclusion, we have shown by simulation of human drug exposure of different dosages in an *in vitro* infection model of *M. tuberculosis* that ertapenem-clavunalate may be a valuable asset to TB treatment. Based on available pharmacokinetic data, we have identified that the dosage of ertapenem most suitable to be tested in a phase II study is 2 g once daily. An MIC of 2 mg/liter should be used to define resistance to this drug.

MATERIALS AND METHODS

We used *M. tuberculosis* H37Ra (ATCC 25177) and H37Rv (ATCC 27294) for our experiments, with growth and storage conditions described before (15). These isolates have been used in the HFS-TB before, with good forecasting accuracy. Ertapenem was purchased from Merck Sharp & Dohme. Clavulanate was purchased from Sigma-Aldrich. Drugs were dissolved in sterile water and syringe filtered for further use. Hollow-fiber cartridges were purchased from FiberCell (Frederick, MD).

Hollow-fiber system model of TB. Construction of the HFS-TB to measure the sterilizing effect has been described in detail previously (15). The system recapitulates concentration-time profiles of drugs encountered in patients, taking into account the penetration into lungs. In the sterilizing-effect studies, semidormant *M. tuberculosis* growing in Middlebrook 7H9 broth acidified using acetic acid to a pH of 5.8 was used; this isolate grows at a rate 8- to 10-fold lower than that of log-phase *M. tuberculosis* (47). The HFS-TB in this model use acidified Middlebrook 7H9 broth without oleic acid, albumin, or catalase but

with 20% dextrose. The peripheral compartment of each of 16 HFS-TB units with circulating acidified Middlebrook 7H9 broth was inoculated with *M. tuberculosis*. All HFS-TB units were incubated at 37°C under 5% CO_2 for the entirety of the study. Different ertapenem exposures, based on human-equivalent doses of 0.25, 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 g, were administered into the central compartment via a computer-controlled syringe pump over 30 min, as in patients. The concentrations achieved with the doses were AUC_{0-24}s of 0, 25, 50, 100, 200, 250, 500, and 1,000 mg · h/liter. There were two replicate hollow-fiber systems for each dose or AUC_{0-24}. Clavulanate was also dispensed via syringe pump to achieve a peak of 3 mg/liter at the end of 30 min of infusion. Medium inflow and outflow were set to mimic the ertapenem half-life of 4 h encountered in patients; we took into account the degradation rate of the drug that we have identified in the past. We recapitulated pharmacokinetics as described in the package insert for INVANZ (ertapenem for injection) for intravenous or intramuscular administration.

The central compartments of each HFS-TB was sampled six times during the first 24 h, and ertapenem concentrations were measured using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method as described previously (48) in order to verify that human-like pharmacokinetics had been achieved. Ertapenem concentrations were modeled using a one-compartment pharmacokinetic model with first-order input and elimination, using ADAPT 5 software, as described previously (15, 24, 25, 44–46). These actual exposures achieved in the HFS-TB were subsequently used in the PK/PD analyses. In order to enumerate the *M. tuberculosis* burden as CFU per milliliter, the peripheral compartment of each HFS-TB unit was sampled on days 0, 3, 7, 14, 21, and 28. Samples were washed and processed as described previously (15) and spread on Middlebrook 7H10 agar supplemented with 10% oleic acid-dextrose-catalase. The cultures were incubated for 21 days at 37°C with 5% CO_2 before the colonies were counted.

Identification of optimal ertapenem dose using computer-aided clinical trial simulations. For the domain of input, we utilized the pharmacokinetic parameter estimates and between-patient variability indices identified by Burkhardt et al. (30). We performed simulations to determine how much 1.0 g once a day, 1.0 g twice a day, 2.0 g once a day, 2.0 g twice a day, 3.0 g once a day, or 3.0 g twice a day would achieve or exceed the target exposure, which is the $\%T_{MIC}$ associated with optimal sterilizing effect in lung tissue of patients.

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