Correlations Between the Hollow Fiber Model of Tuberculosis and Therapeutic Events in Tuberculosis Patients: Learn and Confirm

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Background. The hollow fiber system model of tuberculosis (HFS-TB) is designed to perform pharmacokinetics/pharmacodynamics (PK/PD) experiments, and hence the design of optimal doses and dose schedules for the treatment of tuberculosis. To determine if this model is useful for deriving PK/PD data relevant to clinical outcomes, we compared its quantitative output to that from clinical trials.

Methods. We performed a PubMed search to identify clinical studies performed with antituberculosis therapy in which PK/PD data and/or parameters were documented or a dose-scheduling study design was employed. The search period was from January 1943 to December 2012. All clinical studies were published prior to HFS-TB experiments. Bias minimization was done according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses. Clinical publications were scored for quality of evidence, with 1 as the highest score (randomized controlled trials or meta-analyses of such studies), and 4 as the lowest score.

Results. We identified 17 studies that examined the same parameters as in 8 HFS-TB studies. Fifteen of 17 studies had a quality-of-evidence score of 1. The sterilizing and bactericidal effect rates for isoniazid, rifampin, pyrazinamide, and ethambutol were the same in the HFS-TB as in patients. Time to emergence of resistance for monotherapy was the same as in patients. The PK/PD indices associated with efficacy were the same in HFS-TB as in patients for all drugs examined.

Conclusions. The HFS-TB model is highly accurate at identifying optimal drug exposures, doses, and dosing schedules for use in the clinic.

Keywords. clinical correlations; pharmacokinetics/pharmacodynamics; dose-ranging; dose-scheduling; resistance emergence.

In a tongue-in-cheek exposé, Smith and Pell performed a “systematic review of randomized controlled trials” (RCTs) to identify the evidence for the anecdotal belief from the “parachute lobby,” which stated that parachute use prevents death and major trauma “after gravitational challenge, typically in the context of jumping from an aircraft”—after all, there were contrary reports of individuals surviving a 10 000-meter fall [1]. Their point was that there are situations where logic suffices when supported by data, and clinical experiments cannot, and should not, be performed. A logical approach to such clinical conundrums is not to perform RCTs that include suboptimal doses for a fatal disease such as tuberculosis, but to more effectively employ use of validated, translational laboratory models to answer critical questions and design more informed clinical studies [2, 3].

By definition, a pharmacokinetics/pharmacodynamics (PK/PD) model need not be a perfect reproduction of the entire human patient (the system), but should adequately describe the 2 important aspects in a dose response: the pharmacokinetic system and the bacterial response to drug pharmacokinetics (ie, microbial pharmacodynamics). Sound understanding of this PK/PD...
relationship, in turn, enables prediction of bactericidal and sterilizing effect rates of different drug doses to guide selection of optimal clinical doses, dose schedules, and combination regimens [4–6]. Thus, it is not necessary that the whole picture of lung pathological lesions in tuberculosis be reproduced in the model. What is necessary is that the output of the model adequately informs decision making for selecting optimal doses, dose schedules, and drug combination effects in the patient, and that the experimental output can be expressed in mathematical terms that are sufficiently predictive to be projected to the clinic. To summarize Lewis Sheiner’s famous work in 3 words: Learn. Confirm. Apply [7].

The hollow fiber system model of tuberculosis (HFS-TB) was designed to mimic human pharmacokinetics, and to study drug effects on different physiological or metabolic populations of Mycobacterium tuberculosis, believed to be encountered in lesions [8–10]. The idea is to maximize knowledge about dose effect, then confirm and ultimately apply such knowledge to the clinical setting. One way to determine the utility of the HFS-TB, and to understand if it adequately represents the real system, is to determine if the output of the HFS-TB adequately describes what is already known about the performance of drugs and doses that have been measured in tuberculosis patients. The utility of the HFS-TB may then be confirmed in prospective studies of predictive accuracy and the findings applied.

Therefore, we performed a systematic analysis of clinical studies performed prior to HFS-TB studies, and compared the results regarding performance of the drugs and doses to the results subsequently obtained in HFS-TB experiments identified in an earlier systematic analysis [10]. These analyses were part of the Critical Path to TB Drug Regimens (CPTR) initiative for regulatory discussion with the US Food and Drug Administration and qualification opinion from the Committee for Medicinal Products for Human Use at the European Medicines Agency.

METHODS

Objective

Our aim was to determine if published sterilizing and bactericidal effect rates of different doses and exposures, time to emergence of resistance, and PK/PD parameters identified in the HFS-TB correlate with those identified in patients treated for tuberculosis in clinical trials published in the literature. Bactericidal effect is defined as the killing of bacilli in log-phase growth. Sterilizing effect is defined as microbial kill by antibiotics of either semidormant bacilli in an acidic environment or of nonreplicating, persistent bacilli.

Literature Search

The purpose of this study was to identify all tuberculosis studies in humans that could provide relevant data for our analyses examining correlation with output of the HFS-TB. A PubMed search was conducted to identify clinical studies performed with antituberculosis monotherapy or dual therapy in which pharmacokinetic parameters or measures of drug exposure were documented or a dose-scheduling study design was employed. For combination therapy, we also wanted to identify studies that evaluated HFS-TB and Monte Carlo simulations translated to microbial findings in the sputum such as sputum conversion rates. The search period was 1 January 1943 to 31 December 2012 [11–14]. The search terms included either isoniazid or rifampicin or rifampin or pyrazinamide or ethambutol or moxifloxacin or ciprofloxacin or linezolid AND tuberculosis, and the results were filtered to include only clinical trials. The drugs for the search string were chosen based on results from the literature search, which identified 26 HFS-TB studies in the systematic analyses published in the accompanying work [10]. In addition, PK/PD review articles identified using subject headings pharmacokinetics-pharmacodynamics or PK/PD AND tuberculosis were identified and read and their references manually examined.

Quality of Clinical Evidence Scoring

Each clinical study identified was judged for quality of evidence based on a score derived from the Grading of Recommendations Assessment, Development and Evaluation Working Group criteria and the Infectious Diseases Society of America–US Public Health Service Grading System for evidence-based medicine decision making [15, 16]. The quality of evidence was ranked in ascending order, in which the highest-quality source would receive the lowest score of 1 and the lowest-quality source would receive the highest score of 4. The highest quality of evidence (score of 1) was awarded if the study (or studies) was a proper RCT or a meta-analysis of RCTs that followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses recommendations [17]. A score of 2 was assigned if the study (or studies) was a well-designed prospective clinical trial without randomization, or represented a prospective cohort or case-controlled analytic study, or included dramatic experimental study results of uncontrolled clinical studies. An evidence score of 3 was assigned for multiple time-series or dramatic epidemiologic data. The lowest score, 4, was assigned for large retrospective case series in a single center. Consensus statements and agreements by teams of experts were not considered acceptable evidence and were therefore not reviewed.

Parameters Compared Between Clinical Studies and HFS-TB Experiments

The intent was to compare PK/PD outputs such as concentration-effect relationships, dose-scheduling effects, time to resistance emergence, PK/PD factors associated with resistance emergence, and killing effects of standard doses between the HFS-TB experiments and clinical studies. This required the examination of
bactericidal and sterilizing effect rates, and PK/PD indices associated with efficacy and resistance emergence. Specifically, kill rates in sputum of patients, patterns of microbial kill, cessation of effect, and time to emergence of drug resistance were compared between patients and HFS-TB quantitative output, as defined and calculated in the original studies. In other words, we did not recalculate these rates for the current publication, but utilized the rates published in the HFS-TB and clinical studies themselves. This analysis is not considered predictive, but merely descriptive, as the clinical studies were performed prior to the HFS-TB studies as a means to generate hypotheses for a subsequent predictive accuracy analysis.

RESULTS

The literature search identified 17 clinical studies published prior to HFS-TB studies that could be utilized for descriptive correlation with 8 HFS-TB experiments [3, 9, 18–41] (Table 1). In 15 of the 17 studies, the quality-of-evidence score was 1, indicating that most of the clinical studies were of the highest quality of evidence. One clinical study had a score of 2, and the other had a score of 4.

Table 2 shows the PK/PD correlations between the HFS-TB and patients for isoniazid, as published in the original studies. All clinical studies had a quality-of-evidence score of 1. The table shows differences in early bactericidal activity of the standard dose of isoniazid (300 mg), which achieves different concentrations in patients based on pharmacokinetic variability driven by N-acetyltransferase 2 polymorphisms. In the original publication, the 4-parameter inhibitory sigmoid maximal kill \( (E_{\text{max}}) \) relationship for isoniazid area under the concentration-time curve (AUC)/minimum inhibitory concentration (MIC) vs early bactericidal activity derived in the HFS-TB was modeled for the purpose of running Monte Carlo simulations that incorporated the pharmacokinetic variability encountered in Hong Kong, Cape Town, and Chennai, to derive the expected sputum kill rates in patients. These were then compared to those from clinical trials of the same dose performed in the 3 cities. Table 2 shows a good concordance between the HFS-TB and Monte Carlo experiments and the actual clinical trial observations. Table 2 also shows the results of another analysis that compared the inhibitory sigmoid \( E_{\text{max}} \) model parameters derived in patients treated with different doses of

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<tr>
<td>Standard-dose early bactericidal effect study, ( \log_{10} \text{CFU/mL/day} ) (95% CI)</td>
<td>[36]</td>
<td>[18]</td>
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<tr>
<td>Hong Kong (China)</td>
<td>0.40 (.23–.69)</td>
<td>0.37 (.16–.58)</td>
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<tr>
<td>Cape Town (South Africa)</td>
<td>0.60 (.38–.74)</td>
<td>0.65 (.43–.87)</td>
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<tr>
<td>Chennai (India)</td>
<td>0.90 (.40–.93)</td>
<td>0.94 (.45–1.43)</td>
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<tr>
<td>Other parameters</td>
<td></td>
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<td>Maximal kill in inhibitory sigmoid ( E_{\text{max}} ) curve, ( \log_{10} \text{CFU/mL} )</td>
<td>0.9 ± 0.2 [36]</td>
<td>0.6 ± 0.2 [3, 19]</td>
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<tr>
<td>Hill factor or slope in inhibitory sigmoid ( E_{\text{max}} ) curve</td>
<td>0.9 ± 0.4 [36]</td>
<td>1.0 ± 0.41 [3, 19]</td>
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<td>( EC_{50} ) ± SD</td>
<td>AUC/MIC = 62 ± 28</td>
<td>a</td>
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<tr>
<td>Time to cessation of effect</td>
<td>80 h [35, 36]</td>
<td>72 h [21, 22]</td>
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Abbreviations: AUC, area under the concentration-time curve; CFU, colony-forming units; CI, confidence interval; \( EC_{50} \), exposure mediating 50% of maximal kill; HFS-TB, hollow fiber system model of tuberculosis; MIC, minimum inhibitory concentration; PK/PD, pharmacokinetics/pharmacodynamics; SD, standard deviation.

* Calculated as 1.3 mg/kg.
isoniazid to those from a dose-effect study in the HFS-TB in which the dose-response curves were similar. Furthermore, Table 2 also shows that the cessation of isoniazid microbial kill of *M. tuberculosis* occurred in the same amount of time encountered in patients. Finally, the table shows that the isoniazid PK/PD drivers of efficacy were the same in the HFS-TB as in clinical studies. On the other hand, in the HFS-TB study that involved time to emergence of isoniazid resistance and the reason for cessation of effect (efflux pump derived “resistance” in 80 hours), prior clinical studies suggest a longer time to emergence of isoniazid resistance in patients. This difference was discussed in the correspondence following publication of that article [42, 43].

Table 3 shows the correlation between rifampin HFS-TB findings and microbial kill rates and patterns in tuberculosis patients. All clinical studies had a quality-of-evidence score of 1. Table 3 shows the recapitulation by the HFS-TB kill rates of both bactericidal and sterilizing effect rates of the standard doses in the sputum of tuberculosis patients. In addition, the PK/PD driver was both peak/MIC (postantibiotic effect, resistance suppression) and AUC/MIC (microbial kill) in the HFS-TB, similar to what was seen in patients [3, 18, 39]. There were no discrepancies with the clinical studies.

Table 3 also shows the comparison of ethambutol HFS-TB output with clinical studies. All clinical studies had a quality-of-evidence score of 1. The table shows that at standard dose, the bactericidal and sterilizing effect rates in sputum, as defined by kill slopes in first 2–3 days vs after, were similar to those encountered in the HFS-TB. Maximal early bactericidal effect was encountered at ≥25 mg/kg in patients and human equivalent dose of ≥20 mg/kg in HFS-TB [21, 40]. The PK/PD parameter in the HFS-TB was AUC/MIC in some studies, but peak/MIC in others. In the clinical studies, AUC/MIC was the most consistent driver in this analysis.

Similarly, Table 3 also shows the microbial kill rates of pyrazinamide in sputum of patients compared to the HFS-TB. Three clinical studies had a quality-of-evidence score of 1, and 1 study had a score of 2. The sterilizing effect rates were virtually the same in patients as in the HFS-TB, as was time to emergence of resistance on monotherapy. Sterilizing effect in patients was defined based on sputum microbial kill after the first 2–3 days, whereas that in the HFS-TB was defined as microbial kill of semidormant or nonreplicating bacilli [3, 21, 22]. The PK/PD parameter associated with optimal efficacy was AUC/MIC in the HFS-TB and in patients, based on dose-fractionation design in both studies [3, 9, 30]. There were no discrepancies between HFS-TB findings and patients.

With regard to fluoroquinolones, one of the findings from HFS-TB was the rapid emergence of *M. tuberculosis* resistance to ciprofloxacin and moxifloxacin monotherapy [8, 37]. Resistance arose in 10–14 days in the HFS-TB. Ginsburg et al published a retrospective case series (quality-of-evidence score of 4) showing that fluoroquinolone resistance arose within 10–13 days of inadvertent fluoroquinolone therapy prior to tuberculosis diagnosis [31, 41]. Thus, the same timeline for acquired drug resistance (ADR) was observed in the HFS-TB and in patients.

Finally, in terms of combination therapy, pyrazinamide-isoniazid-rifampin was studied in the HFS-TB at standard doses, and both the sterilizing effect and bactericidal effect were examined [38]. The HFS-TB output was then utilized in Monte Carlo simulations of Cape Town patients, based on

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<tr>
<td><strong>Rifampin</strong></td>
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<tr>
<td>Standard-dose early bactericidal effect, log₁₀ CFU/mL/day (95% CI)</td>
<td>0.28 [39]</td>
<td>0.25 (−0.08 to .57) [18]</td>
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<td>Sterilizing effect rate, log₁₀ CFU/mL/day (95% CI)</td>
<td>0.28 [39]</td>
<td>0.27 (.06–.47) [21, 22]</td>
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<tr>
<td>PK/PD parameter associated with optimal effect</td>
<td>Peak/MIC; AUC/MIC [39]</td>
<td>Peak/MIC [3, 28]</td>
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<tr>
<td><strong>Ethambutol</strong></td>
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<tr>
<td>Maximal early bactericidal activity, log₁₀ CFU/mL/day (95% CI or ±SD)</td>
<td>0.22 (1.14–.29) [40]</td>
<td>0.26 ± 0.12 [21, 22]</td>
</tr>
<tr>
<td>Sterilizing effect rate, log₁₀ CFU/mL/day</td>
<td>0.04–0.10 [40]</td>
<td>0.1 [21, 22]</td>
</tr>
<tr>
<td>PK/PD parameter associated with optimal effect</td>
<td>AUC/MIC; peak/MIC [40]</td>
<td>AUC/MIC [3, 29]</td>
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<tr>
<td><strong>Pyrazinamide</strong></td>
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<tr>
<td>Early bactericidal effect rate, log₁₀ CFU/mL/day</td>
<td>−0.1 [9]</td>
<td>−0.1 ± 0.2 [21, 22]</td>
</tr>
<tr>
<td>Sterilizing effect rate, log₁₀ CFU/mL/day</td>
<td>0.09–0.01 [9]</td>
<td>0.12 ± 0.05 [21, 22]</td>
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Abbreviations: AUC, area under the concentration-time curve; CFU, colony-forming units; CI, confidence interval; HFS-TB, hollow fiber system model of tuberculosis; MIC, minimum inhibitory concentration; PK/PD, pharmacokinetics/pharmacodynamics; SD, standard deviation.

* Established by culture methods.
the pharmacokinetic variability encountered for these drugs in Cape Town. The simulations calculated rates of ADR during the first 2 months of therapy assuming 100% adherence. The simulations demonstrated a 58.3% 2-month sputum conversion rate in 10,000 tuberculosis patients in Cape Town. An examination of clinical studies (quality-of-evidence score of 1) from the Western Cape revealed 2-month sputum conversion rates of 51%–60% [32, 33, 38]. Thus, the 2-month sputum conversion rates from the clinical trials were similar to those identified in the HFS-TB and Monte Carlo simulations.

DISCUSSION

The HFS-TB is used to identify the effect of different doses and concentrations on M. tuberculosis microbial kill and ADR. Thus, it could be used as a drug development tool for examining dose responses of monotherapy and for design of combination regimens. Here, we examined if the kill rates of drugs in this model, which uses the same pharmacokinetic profiles of drugs as encountered in patients, were similar to those in patients. We found comparable sterilizing effect and bactericidal effect rates of drugs, based on sputum bacterial kill rates and microbial kill rates in HFS-TB for bactericidal effect (log-phase growth) and for sterilizing effect (semidormant bacteria and nonreplicating bacilli). Indeed, we found the same dose-effect relationships based on the 4 inhibitory sigmoid $E_{\text{max}}$ model parameters of maximal kill rates, Hill slope, and concentrations associated with optimal kill. The patterns of microbial kill were similar. When the HFS-TB quantitative output was utilized for monotherapy and combination therapy in Monte Carlo simulations, early bactericidal activity, sterilizing effect rates, and sputum conversion rates in particular locales could be recapitulated. Unique to the HFS-TB model, as opposed to other preclinical studies, were findings on resistance emergence where concentrations associated with suppression of resistance can be identified and used in designing more effective dosing schema. Thus, for PK/PD of antibiotics, the HFS-TB is a model that adequately represents the dose-effect relationships in patients and captures PK/PD parameters and exposures associated with optimal effect. It is this aspect of the model that is translatable to patients. Thus, the HFS-TB can inform optimal dosing and dosing regimen design for patients and help reduce the testing of suboptimal doses in patients with tuberculosis who would then fail therapy or develop ADR, which could be driven by suboptimal dosing [44–53].

In our review, there was an example in a single HFS-TB study involving time to emergence of isoniazid resistance for which prior clinical studies suggested a longer time to emergence of isoniazid resistance in patients. There are several possible explanations. First, the discrepancy may simply reflect the semantics of drug “resistance” vs “tolerance.” The latter is phenotypic resistance which is transient. The HFS-TB study identified efflux pump induction as one such reason for isoniazid cessation of therapy and “resistance.” Indeed the phenomenon of efflux pump–induced “tolerance” in growing bacteria has now been described for several first-line antituberculous compounds by others [54]. The discrepancy may be, at least in part, due to the fact that efflux pump detection assays were not used in the early clinical studies we examined. In addition, the older clinical studies utilized the standard isoniazid resistance breakpoints, which are up to 10 times higher than the newly proposed ones [55, 56]. Thus, even if resistance would have occurred in patients, standard tests would have had a poor sensitivity to detect this phenomenon. Resolution of this discrepancy will require prospective clinical studies with appropriate susceptibility tests.

There are limitations to our study. First, one common limitation of systematic analyses is that some potentially influential studies could be missed by the search strategy, which would bias the conclusions. Indeed, some insightful studies could be missed because they did not have a PK/PD design. However, we excluded no studies by publication language and also performed a manual search of references in key publications to minimize bias. Second, and closely related to the first, it could be that some HFS-TB experiments that have not been published (eg, as part of examination of proprietary molecules within the purview of pharmaceutical industry) may not have correlated with clinical studies. This limitation would also be common to all systematic analyses, and could bias final conclusions. To minimize that, the CPTR Initiative made an appeal to a number of pharmaceutical industry partners to share their data at the time of the current study. Third, we were interested in examining clinical correlations that had quantitative outputs, given the potential role of the HFS-TB as a drug development tool reliant on quantitative output. Nonquantitative features of drug effect could also be important in determining how well different doses and concentrations perform.

In summary, we demonstrate that for M. tuberculosis and different concentrations of antibiotics, dose effect, and dose scheduling, as well as resistance emergence, there is concordance between the HFS-TB and clinical results. We learned about the utility of this in vitro model. What remains is to confirm the predictive accuracy of the HFS-TB model [57].

Notes

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Potential conflicts of interest. T. G. founded Jacaranda Biomed Inc., and is also a consultant for Astellas Pharma USA for antifungal compounds. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.
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