AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EXPERIMENTAL THERAPEUTICS

Delamanid Central Nervous System Pharmacokinetics in Tuberculous Meningitis in Rabbits and Humans

Elizabeth W. Tucker,[a,b] Lisa Pieterse,[a] Matthew D. Zimmerman,[c]* Zarir F. Udwadia,[d] Charles A. Peloquin,[e,f] Maria Tarcela Gler,[g] Shashank Ganatra,[d] Jeffrey A. Tornheim,[a] Prerna Chawla,[d] Janice C. Caoili,[f] Brittaney Ritchie,[a] Sanjay K. Jain,[a] Véronique Dartois,[c]* Kelly E. Dooley[a]

[a]Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[b]Johns Hopkins All Children's Hospital, St. Petersburg, Florida, USA
[c]Public Health Research Institute, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, New Jersey, USA
[d]P.D. National Hospital and Medical Research Centre, Mumbai, India
[e]University of Florida College of Pharmacy, Gainesville, Florida, USA
[f]Emerging Pathogens Institute, Gainesville, Florida, USA
[g]Makati Medical Center, Makati City, Philippines

ABSTRACT Central nervous system tuberculosis (TB) is devastating and affects vulnerable populations. Multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculous meningitis (TBM) specifically are nearly uniformly fatal, with little information being available to guide the treatment of these patients. Delamanid (DLM), a nitro-dihydro-imidazooxazole, is a new, well-tolerated anti-TB drug with a low MIC (1 to 12 ng/ml) against *Mycobacterium tuberculosis*. It is used for the treatment of pulmonary MDR-TB, but pharmacokinetic (PK) data for DLM in the central nervous system (CNS) of patients with TBM are not available. In the present study, we measured DLM concentrations in the brain and cerebrospinal fluid (CSF) of six rabbits with and without experimentally induced TBM receiving single-dose DLM. We report the steady-state CSF concentrations from three patients receiving DLM as part of multidrug treatment who underwent therapeutic drug monitoring. Drug was quantified using liquid chromatography-tandem mass spectrometry. In rabbits and humans, mean concentrations in CSF (in rabbits, 1.26 ng/ml at 9 h and 0.47 ng/ml at 24 h; in humans, 48 ng/ml at 4 h) were significantly lower than those in plasma (in rabbits, 124 ng/ml at 9 h and 14.5 ng/ml at 24 h; in humans, 726 ng/ml at 4 h), but the estimated free CSF/plasma ratios were generally >1. In rabbits, DLM concentrations in the brain were 5-fold higher than those in plasma (means, 518 ng/ml at 9 h and 74.0 ng/ml at 24 h). All patients with XDR-TBM receiving DLM experienced clinical improvement and survival. Collectively, these results suggest that DLM achieves adequate concentrations in brain tissue. Despite relatively low total CSF drug levels, free drug may be sufficient and DLM may have a role in treating TBM. More studies are needed to develop a fuller understanding of its distribution over time with treatment and clinical effectiveness.

KEYWORDS central nervous system infections, delamanid, drug resistance, meningitis, tuberculosis

Tuberculous meningitis (TBM) is the most devastating form of extrapulmonary tuberculosis (TB) and the most common form of central nervous system (CNS) TB. Vulnerable populations, such as young children and adults with human immunodeficiency virus (HIV) infection, are disproportionately affected (1, 2). With current standard-of-care regimens, mortality in children remains 16 to 23%, with 80 to 100% suffering permanent neurologic injury, such as limb paresis, visual impairment, hearing loss, or

Citation Tucker EW, Pieterse L, Zimmerman MD, Udwadia ZF, Peloquin CA, Gler MT, Ganatra S, Tornheim JA, Chawla P, Caoili JC, Ritchie B, Jain SK, Dartois V, Dooley KE. 2019. Delamanid central nervous system pharmacokinetics in tuberculous meningitis in rabbits and humans. Antimicrob Agents Chemother 63:e00913-19. https://doi.org/10.1128/AAC.00913-19.

Address correspondence to Kelly E. Dooley, kdooley1@jhmi.edu.

* Present address: Matthew D. Zimmerman and Véronique Dartois, Center for Discovery and Innovation, Hackensack Meridian Health, Nutley, New Jersey, USA.

E.W.T. and L.P. contributed equally to this article.

Received 3 May 2019
Returned for modification 25 June 2019
Accepted 28 July 2019
Accepted manuscript posted online 5 August 2019
Published 23 September 2019

neurodevelopmental deficit (3–6). In adults, mortality may be even higher and is about 28% in HIV-uninfected persons and 65% in HIV-infected persons (7).

Historically, treatment for drug-susceptible TBM is extrapolated from pulmonary TB regimens without regard for drug penetration into the CNS, a system which consists of several compartments (e.g., cerebrospinal fluid [CSF] and the brain parenchyma) that are separated from the systemic circulation via the blood-CSF barrier (BCSFB) and the blood-brain barrier (BBB), respectively (8, 9). However, the optimization of TBM treatment is now being investigated in both translational animal models and large clinical trials. For example, high-dose rifampin has shown mixed results in adults with TBM, with one study involving an intravenous dose of 13 mg/kg of body weight improving mortality, albeit a larger trial of a 15-mg/kg oral dose failed to demonstrate a mortality benefit compared to a 10-mg/kg oral dose (10, 11). These doses may still be inadequate, as several studies utilizing pharmacokinetic (PK)-pharmacodynamic (PD) sampling and modeling have suggested that higher rifampin doses (i.e., $>$20 to 30 mg/kg) are needed to penetrate into the brain parenchyma, where the *M. tuberculosis* bacilli are located (12–16).

Although efforts to optimize current treatment for drug-susceptible TBM are needed, the rise of multidrug-resistant (MDR) TB (i.e., resistance to rifampin and isoniazid with or without resistance to other first-line drugs) highlights the urgency to enhance second-line and novel anti-TB medications to treat this epidemic (17). Drug resistance has a significant effect on mortality in patients with TBM. For instance, in HIV-associated TBM, isoniazid resistance increases the risk of death by 1.78-fold, whereas rifampin-resistant TBM is associated with a 94% mortality (18). MDR-TBM, irrespective of HIV infection status, is reported to be uniformly fatal in some studies (19, 20). Antimicrobials, such as fluoroquinolones (e.g., moxifloxacin) and oxazolidinones (e.g., linezolid), that have high CSF penetration have been used in patients with TBM, with linezolid providing a favorable therapeutic effect in some patients with life-threatening disease (10, 21). In addition to optimizing existing drugs for TBM, novel antimycobacterial agents, such as delamanid (DLM), may also provide much-needed options for the treatment of drug-susceptible TBM, MDR TBM, or extensively-drug resistant (XDR) TBM (TBM caused by MDR-TB strains that are additionally resistant to fluoroquinolones and injectable agents).

DLM (Deltyba; formerly OPC-67683) is a new therapeutic agent that demonstrates potent antimycobacterial activity in both *in vivo* and *in vitro* studies (22). First conditionally approved by the European Medicines Agency in 2014, DLM is a nitro-dihydro-imidazooxazole derivative that inhibits the synthesis of essential components of the mycobacterial cell wall, such as methoxy-mycolic acid and keto-mycolic acid (23). DLM exhibits no bactericidal properties against Gram-positive bacteria, Gram-negative bacteria, or the intestinal flora, making the drug clinically advantageous due to its high specificity for mycobacteria (24). In addition, DLM is active against both laboratory and clinical *M. tuberculosis* strains, with MICs ranging from 0.001 to 0.012 μg/ml in protein-containing media (25, 26). With the exception of a dose-related, modest QTc interval prolongation on electrocardiograms, attributable mainly to its major metabolite (DM-6705), and rare hepatotoxicity, DLM exhibits a desirable toxicity profile compared to current second-line anti-TB drugs and is an oral drug (23, 27). The World Health Organization (WHO) included DLM among drugs that can be used to treat drug-resistant TB in adults in 2014, and its use was extended to children ages 6 to 17 years in 2016 and then to children ages $\geq$3 years in 2018 (27–30). International guidelines and studies of DLM up to now have not focused on the CNS biodistribution in TBM, and these limited data on DLM's CNS penetration were highlighted by WHO's 2019 MDR guidelines (23, 27, 30–32).

We designed a study to evaluate the distribution of DLM and its primary metabolite, DM-6705, into the brain and CSF of rabbits with and without CNS TB. We also collected case reports of patients with XDR-TB who received DLM as part of their salvage regimen who also underwent therapeutic drug monitoring (TDM).

TABLE 1 Summary of rabbit plasma PK data

Parameter[a]	Value for the following compound in the indicated rabbits:					
	DLM			DM-6705		
	Uninfected	Infected	*P*	Uninfected	Infected	*P*
C_{max} (ng/ml)	195.6	255.8		3.5	5.5	
Mean concn (ng/ml)						
9 h	96.4	213.3		1.8	9.6	
24 h	50.3	40.1		2.4	2.1	
T_{max} (h)	12	12		12	12	
$t_{1/2}$ (h)	14.1	13.9		14.3	14.1	
AUC_{0-24} (ng·h/ml)	2,785.6	3,811.2	0.01	49.7	84.8	0.29
AUC_{0-48} (ng·h/ml)	3,461.3	4,229	0.01	69.5	97.8	0.31
$AUC_{0-\infty}$ (ng·h/ml)	3,554.5	4,255.3		84.6	97.8	
MRT_{0-48} (h)	15.4	12.2	0.22	56.9	36.8	0.24
$MRT_{0-\infty}$ (h)	16.5	12.5		66.5	36.8	
k_{el}' (h^{-1})	0.07	0.08		0.05	0.07	

[a]AUC_{0-24}, AUC_{0-48}, and $AUC_{0-\infty}$, area under the concentration-time curve from 0 to 24 h, 0 to 48 h, and 0 h to infinity, respectively; MRT_{0-48} and $MRT_{0-\infty}$, mean retention time from 0 to 48 h and 0 h to infinity, respectively.

RESULTS

Plasma PK of DLM, DM-6705, and DM-6717 in rabbits. Plasma PK data for DLM and DM-6705 of uninfected and infected rabbits following a single 5-mg/kg oral dose, approximately equivalent to 100 mg twice daily in human subjects, are provided in Table 1 and Fig. 1. While the time to the maximum concentration (T_{max}) and half-life ($t_{1/2}$) were similar, the area under the concentration-time curve (AUC) and the maximum concentration (C_{max}) were significantly greater in infected rabbits than in uninfected rabbits. The plasma concentrations of DM-6705 were much lower than those of DLM in rabbits (Table 1 and Fig. 1). DM-6705 metabolite formation appeared to be relatively slow, as DM-6705 was only initially detected at an average of 6.3 h postdose in uninfected rabbits and 3.3 h in infected rabbits. Interestingly, DM-6705 had a significantly lower elimination rate (k_{el}') than DLM (mean DLM k_{el}' from 0 to 48 h [$k_{el}'_{0-48}$] = 0.074 h^{-1}, mean DM-6705 $k_{el}'_{0-48}$ = 0.023 h^{-1}; $P < 0.01$). Another DLM metabolite, DM-6717, was also quantified, and results for plasma, CSF, and brain tissue for this metabolite are available in Text S1, Fig. S1, and Table S1 in the supplemental material. Individual rabbit mass spectrometry results for plasma, CSF, and brain tissue for DLM, DM-6705, and DM-6717 are available in Tables S2, S3, and S4.

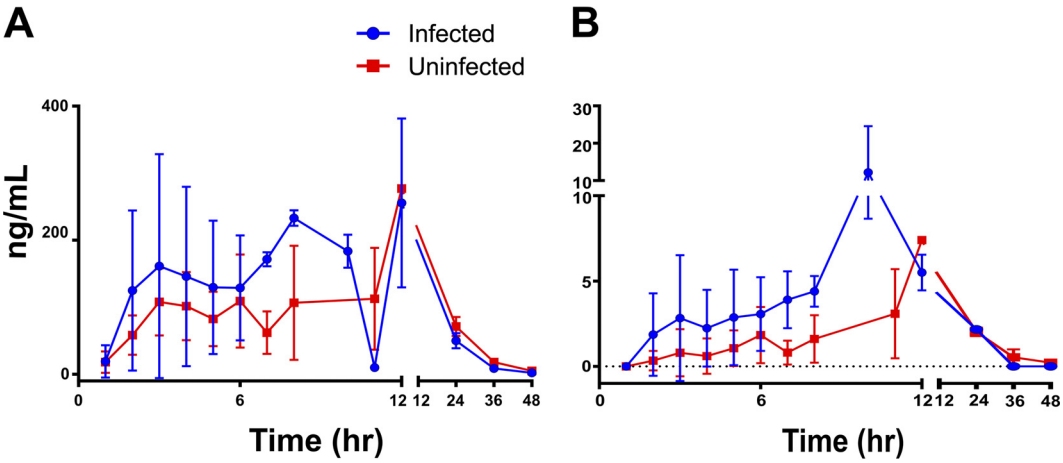

FIG 1 Plasma concentrations of DLM (A) and DM-6705 (B) in infected and uninfected rabbits after a single 5-mg/kg oral dose of DLM. Uninfected and infected rabbits represent 3 rabbits each. Mean ± standard deviation is shown.

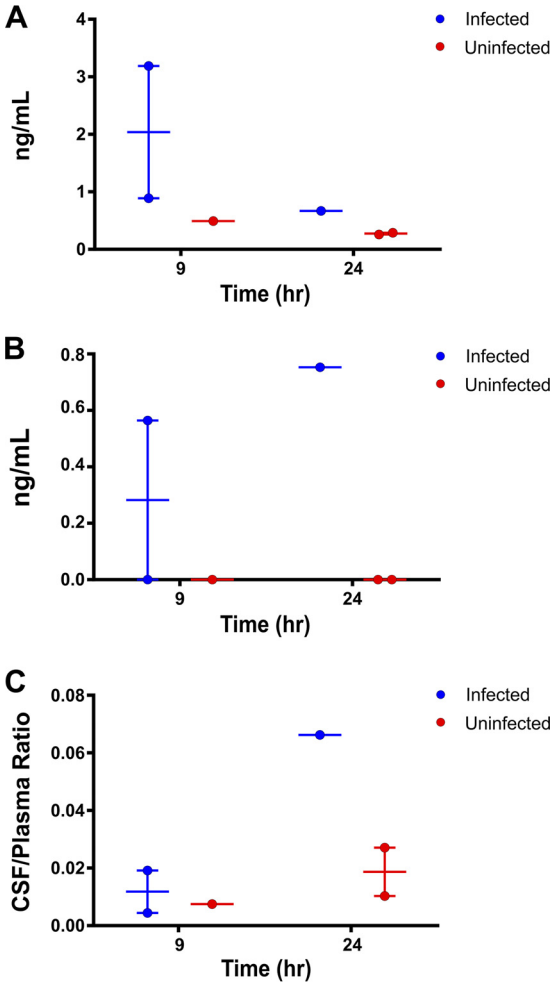

FIG 2 Cerebrospinal fluid (CSF) concentrations of DLM (A) and DM-6705 (B) in infected and uninfected rabbits after a single oral dose of 5 mg/kg of DLM. (C) Total DLM CSF/plasma ratio in uninfected and infected rabbits. Data are for 2 uninfected rabbits euthanized at 24 h, 2 infected rabbits euthanized at 9 h, 1 uninfected rabbit euthanized at 9 h, and 1 infected rabbit euthanized at 24 h. The median ± interquartile range (IQR) is shown.

CSF and brain PK of DLM and DM-6705 in rabbits. DLM was detected in all CSF samples; however, DM-6705 was detected only in infected rabbits (Fig. 2A and B; Table 2; Tables S2 and S3). DLM CSF concentrations in infected rabbits were 4-fold higher (2.04 ng/ml) than those in uninfected rabbits (0.50 ng/ml) at 9 h. Total drug DLM CSF/plasma ratios were extremely low at both time points (Fig. 2C). However, assuming 99.5% protein binding in plasma and 0%, 5%, or 20% binding in CSF, estimates of unbound DLM CSF/plasma ratios were higher (generally >1) (Fig. S2) (33). DLM brain concentrations were considerably higher than plasma concentrations at 9 and 24 h and

TABLE 2 Mean DLM and DM-6705 concentrations in rabbit CSF and brain tissue[a]

Infection category	Time (h)	Mean concn (ng/ml)			
		DLM in CSF	DM-6705 in CSF	DLM in brain tissue	DM-6705 in brain tissue
Uninfected	9	0.49	0	488	160
Uninfected	24	0.27	0	96	286
Infected	9	2.04	0.28	549	425
Infected	24	0.67	0.75	52	221

[a]Data are for 2 uninfected rabbits euthanized at 24 h, 2 infected rabbits euthanized at 9 h, 1 uninfected rabbit euthanized at 9 h, and 1 infected rabbit euthanized at 24 h.

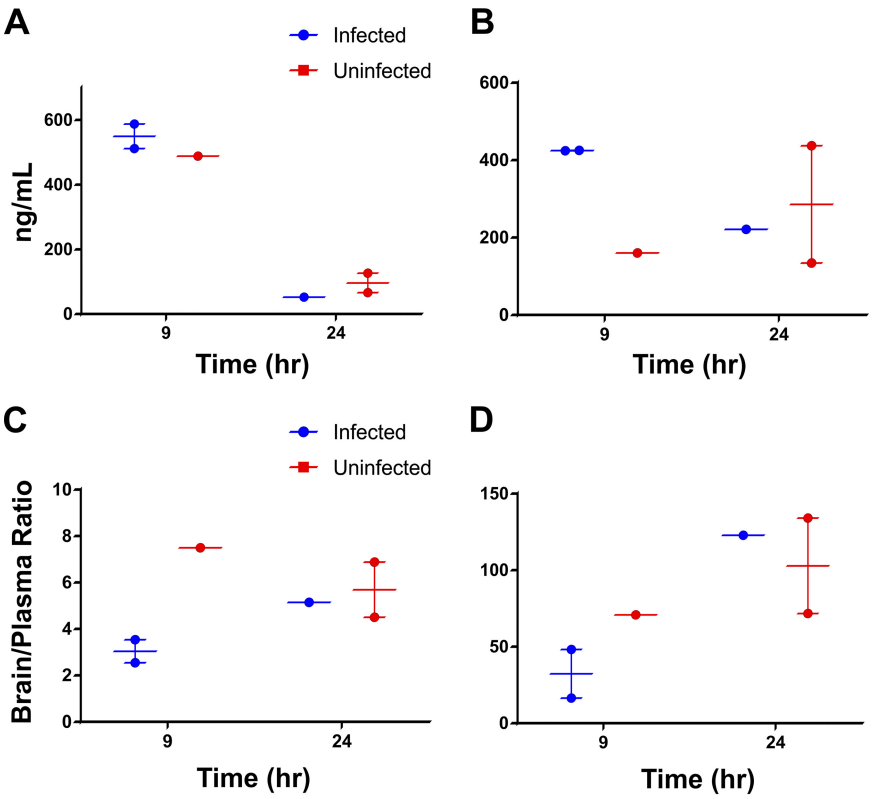

FIG 3 (A and B) Brain tissue concentrations for DLM (A) and DM-6705 (B) in infected and uninfected rabbits after a single oral dose of 5 mg/kg of DLM. (C and D) Brain/plasma ratios for DLM (C) and DM-6705 (D) at the time of brain tissue collection in infected and uninfected rabbits after a single oral dose of 5 mg/kg of DLM. Data are for 2 uninfected rabbits euthanized at 24 h, 2 infected rabbits euthanized at 9 h, 1 uninfected rabbit euthanized at 9 h, and 1 infected rabbit euthanized at 24 h. The median ± interquartile range (IQR) is shown.

were higher in infected rabbits than in uninfected rabbits at the earlier time point (Fig. 3; Tables 1 and 2). DLM and DM-6705 brain/plasma ratios are shown in Fig. 3C and D.

Case reports from patients with drug-resistant TBM. (i) Patient 1. A 26-year-old woman from Mumbai, India, who was otherwise healthy with no known TB exposures presented with cough, fever, headaches, and decreased bilateral vision. Brain magnetic resonance imaging (MRI) demonstrated meningeal enhancement with tuberculomas, and analysis of CSF with a GeneXpert system indicated the presence of rifampin-resistant *M. tuberculosis*. XDR-TBM was confirmed via mycobacteria growth indicator tube (MGIT) analysis. As a result, second-line anti-TB treatment (including kanamycin, moxifloxacin, linezolid, ethionamide, clofazimine, cycloserine, *para*-aminosalicylic acid, and capreomycin) in conjunction with steroids was given. Clinical and radiological improvement was observed; however, relapse occurred 3 months following treatment termination. The patient was placed on a new regimen, which included capreomycin, meropenem, amoxicillin-clavulanate, clofazimine, linezolid, cycloserine, *para*-aminosalicylic acid, and standard-dose DLM (100 mg twice daily), with DLM being provided under compassionate use (27). She completed 24 weeks of DLM treatment and was clinically well with no complaints of headache, vision changes, or fever. Plasma and CSF for TDM measurement were collected at 5 weeks (Table 3).

(ii) Patient 2. A 35-year-old man from the Philippines with HIV infection and neurologic symptoms was found to have MDR-TBM. He was placed on second-line anti-TB treatment that included levofloxacin, amikacin, prothionamide, meropenem, amoxicillin-clavulanate, and linezolid. With treatment, CSF indices were observed to improve, with a decrease in pleocytosis (white blood cell [WBC] count, 147 cells/μl, which decreased to 6 cells/μl) and the total protein concentration (96 mg/dl, which

TABLE 3 Human plasma and CSF MS results for patients 1, 2, and 3 after oral dosing at 100 mg twice daily[a]

	Concn (ng/ml) or ratio						
	Patient 1			Patient 2		Patient 3	
Compartment or parameter	DLM (lab 1)	DLM (lab 2)	DM-6705	DLM (lab 1)	DM-6705	DLM (lab 1)	DM-6705
Plasma (0 h)	550	773.5	109.3	465	NT	450	NT
Plasma (2 h)	450	572.6	86.3	500	NT	580	NT
Plasma (4 h)	750	1,082.8	96.3	473	NT	600	NT
Plasma (7 h)	740	848.3	95	481	NT	510	NT
CSF (4 h)	BLQ	1.8	0.8	33	NT	110	NT
CSF/plasma ratio (4 h)		<0.01	< 0.01	0.07		0.18	

[a]Concentrations were initially measured at lab 1. Samples from patient 1 were also sent to lab 2 so that DM-6705 could be measured. BLQ, below the limit of detection; NT, not tested.

decreased to 64 mg/dl). However, the patient developed worsening right-lower-extremity weakness and was found to have new brain nodules, along with worsening CSF indices (WBC count, 486 cells/μl; protein concentration, 5,319 mg/dl). A new drug regimen that included standard-dose DLM was initiated (along with bedaquiline and linezolid), and plasma and CSF samples for TDM were collected 5 months later (Table 3). Clinically, he completed 68 weeks of DLM and is stable, alert, and interactive with improving ambulation and is now off anti-TB medications.

(iii) Patient 3. A 39-year-old male from the Philippines with HIV infection was admitted for sensorial changes as well as lower-extremity weakness and was suspected to have XDR-TBM. He was initiated on antiretroviral therapy a year prior to his admission but remained nonadherent. As a result, a protease inhibitor-based regimen was reinitiated, albeit 2 months later he presented to the hospital with decreased sensorium. His initial CSF analysis showed cryptococcal meningitis and imaging consistent with transverse myelitis. However, due to high clinical suspicion for TBM, he was started on first-line anti-TB drugs (without rifampin), in addition to amphotericin, with initial improvement. Unfortunately, 2 months later the patient's mental status deteriorated. A repeat CSF protein concentration was still high at 1,903 mg/dl (from 2,082 mg/dl at baseline). Furthermore, while the cryptococcal antigen was still positive, the CSF fungal culture was negative. The patient's brain MRI was concerning for progressive TBM with worsening leptomeningeal enhancement along the sulci, cerebellar gyri, and basal cisterns with perilesional edema in the right parietal lobe, midbrain, dorsal pons, and cerebellum. Therefore, his regimen was switched to include bedaquiline, levofloxacin, DLM, linezolid, and clofazimine, which he received for 35 weeks before transitioning to levofloxacin, linezolid, and clofazimine for continuation-phase treatment. Plasma and CSF for TDM were collected at week 8, with DLM results being shown in Table 3. Clinically, he is receiving his 40th week of treatment and is stable, alert, and coherent, albeit wheelchair bound due to difficulty walking.

DISCUSSION

TBM is the most devastating form of TB, leading to life-long neurologic injury and a high risk of death despite treatment. A major challenge in treating TBM is that drugs not only need to be effective against *M. tuberculosis* but also need to penetrate the BCSFB and BBB to reach the site of bacterial infection. For MDR- and XDR-TBM, both of which are particularly lethal forms of TBM, treatment options are limited and outcomes are nearly universally poor. We used our experimentally induced TBM model in young rabbits due to its ability to reproducibly reflect the heterogeneity of disease (exudative meningitis and tuberculomas) and neurologic deficits in TBM patients (12, 34). In our rabbit study, we found that the concentrations of DLM were almost 5-fold higher in brain than plasma in rabbits with and without CNS TB, while CSF concentrations were low but above the average MIC of DLM against *M. tuberculosis*, and estimates of CSF/plasma ratios of free drug were generally >1. At the same time, three patients with XDR-TB on salvage therapy had detectable but low CSF concentrations and appeared to respond to multidrug DLM-containing regimens.

DLM is a promising antimycobacterial agent approved for the treatment of pulmonary MDR-TB. It has no clinically significant drug-drug interactions with anti-TB and antiretroviral agents due to its lack of an effect on liver metabolism, allowing it to be a safe addition to first- or second-line regimens (35, 36). Although DLM causes modest QTc prolongation, overall it is well tolerated (23, 25). Importantly, recent *in vitro* studies conducted by the National Clinical Center on Tuberculosis (CCTB) of China found that approximately 95% of the XDR-TB strains studied were susceptible to DLM (37). The plasma PK of DLM have been well characterized, but information about drug penetration into the CNS compartment in patients or animals with TBM is lacking (38). To our knowledge, this the first report of CNS PK parameters in rabbits and humans with TBM, which is an important first step as we evaluate the potential utility of DLM for patients with CNS TB.

The dose that we selected for the animal experiments produced DLM and DM-6705 plasma PK that were in the range seen in humans receiving the standard dose of 100 mg twice daily. For example, the mean AUC from 0 to 24 h (AUC_{0-24}) in all rabbits (3,298 ng·h/ml) was in the range of exposures seen in patients with pulmonary TB receiving DLM (mean, 2,500 ± 1,454 ng·h/ml) (38). The mean C_{max} in the rabbits (225 ng/ml) was less than the C_{max} for the average patient (708 ng/ml) but was within the range of plasma C_{max} values previously reported in humans (124 to 1,000 ng/ml). In both rabbits and humans, CSF/plasma ratios of DLM were low. However, the MIC_{95} for DLM against *M. tuberculosis* was 12 ng/ml in protein-containing media, and the levels in CSF exceeded this in 2 of 3 patients but none of the rabbits. Although important PK-PD parameters have not been established for DLM yet, the time above the MIC ($T_{>MIC}$) appears to correlate best with drug activity against *M. tuberculosis* for pretomanid, another nitroimidazole, although the percentage of $T_{>MIC}$ needed to be effective is unknown (39–41). Additionally, DLM is a highly protein-bound drug (99.5%), and so CSF/plasma ratios of unbound drug may be a better measure of the drug's potential activity in the CSF (33, 42). However, our knowledge of protein binding in CSF in patients with meningitis is limited. Figure S2 in the supplemental material shows estimates of CSF/plasma ratios assuming 0%, 5%, and 20% protein binding in CSF and 99.5% protein binding in plasma (33). Interestingly, the concentrations of DLM in the rabbit brain were almost 5-fold higher than those in the plasma. The results of this study are similar to those of a previous study involving radiolabeled DLM in healthy rats without TBM, wherein DLM was found to have high concentrations in tissues, including the CNS (43). When comparing infected and uninfected rabbits, there was a trend of higher DLM concentrations in plasma, CSF, and brain tissue. These results may reflect direct changes to the BBB or BCSFB related to inflammation or the effects of cytokines on drug transporters; this warrants further studies to investigate the pathogenesis (44, 45).

The finding that DLM concentrations within the brain were higher than those in both plasma and CSF is important, as it suggests that DLM may be a better candidate for the treatment of CNS TB, and even TBM, than one might have anticipated if investigations were limited to just measuring total drug levels in the CSF. Rifampin, an essential first-line anti-TB antibiotic, also demonstrates that there are other important discrepancies between the PK of drugs in brain tissue and those in CSF. In animal models, while CSF rifampin levels remain low but constant over the course of treatment with antibiotics, brain penetration (while similarly low) actually decreases with treatment (12). Can a drug with low CSF concentrations but high brain concentrations be used for meningoencephalitis? Isavuconazole is an antifungal with high brain but low CSF concentrations that demonstrates efficacy against cryptococcal meningitis in animal models and also has promising clinical data (46–48). Echinocandins similarly have limited CSF penetration, but brain parenchymal concentrations of anidulafungin, for example, exceed the MIC_{90} of many fungal pathogens, and this drug demonstrates clinical efficacy for neonatal hematogenous candida meningoencephalitis (49–51). The importance of higher brain concentration for improved antimicrobial efficacy and growing evidence that CSF levels are not reflective of brain concentrations demonstrate the importance of assessing antimicrobial levels at the site of infection, in animal

models, and also in humans with clinically translatable technologies, such as positron emission tomography imaging (12). This study highlights the potential utility of translational models to guide dose and drug selection for clinical studies of drugs for TBM treatment (12, 47, 51).

Our study has limitations. Drug distribution data in the rabbit model reflect drug concentration after a single dose, while patient data were obtained at steady state after several weeks of treatment and could be affected by changes in meningeal inflammation and BBB integrity. Additionally, CSF was taken at a single time point in both rabbits and patients and did not provide concentration data over the full dosing interval. Clinical PK data came from clinician case reports and TDM rather than a prospective study focused on evaluating DLM PK and efficacy. The final outcomes of the patients described herein are not yet known, and, given the multidrug regimens used, the outcomes cannot be directly attributed to DLM alone. Lastly, these are reports of the PK of DLM in the CNS and CSF compartments, and so the sample size of rabbits and patients was small, which may limit our power to extrapolate between species and to larger populations. However, cross-species data can provide valuable insights, and we should make every effort to utilize preclinical models to inform clinical studies in the future, particularly in the case of a hard-to-treat, relatively rare diseases, for which trials are challenging to enroll and take years to produce results.

In conclusion, DLM may play a key role in treating patients with nearly untreatable MDR- and XDR-TBM, as brain and meningeal concentrations may be high (if the rabbit, rat, and human drug distributions into these compartments are similar), even if CSF/plasma ratios are low and CSF concentrations just exceed the MIC. Further clinical studies are needed to understand the role of this drug and other new drugs in the treatment of TBM.

MATERIALS AND METHODS

Overall study design. First, this study was designed to assess the drug distribution into the CNS compartments of adolescent rabbits administered DLM. Control rabbits without TB (uninfected) and rabbits with experimentally induced TBM (*M. tuberculosis*-infected [infected] rabbits) were orally administered a single 5-mg/kg dose of DLM on two separate occasions, and samples of plasma, CSF, and brain parenchyma were obtained at prespecified time points. In addition, one patient in India and two patients in the Philippines who were diagnosed with XDR-TBM and who were receiving second-line TB treatment that included DLM underwent steady-state plasma and CSF collection by their treating clinicians for therapeutic drug monitoring following standard dosing (100 mg twice daily). Liquid chromatography (LC)-mass spectrometry (MS) was used to measure the concentration of DLM (in rabbits and humans), the DM-6705 metabolite (in rabbits and humans), and the DM-6717 metabolite (in rabbits only) to gain an understanding of the DLM PK properties in the CNS in the presence of TBM.

Rabbit studies. All rabbit study protocols were approved by the Johns Hopkins University Biosafety and Animal Care and Use Committees according to the *Guide for the Care and Use of Laboratory Animals* (52) for the rabbit studies. All protocols were performed in compliance with biosafety level 3 (BSL-3) containment protocols. Three uninfected and three infected rabbits were used for the rabbit studies.

(i) Animal inoculation. *M. tuberculosis* H37Rv (from the S.K.J. laboratory) was prepared from frozen titrated stock as previously described (12, 34). Female and male New Zealand White (NZW) rabbits (*Oryctolagus cuniculus*; Robinson Services Inc., NC) were anesthetized and injected with approximately 1.42 ± 0.21 $\log_{10}$ CFU for infected rabbits or with phosphate-buffered saline (PBS; Quality Biological, Inc., Gaithersburg, MD) for uninfected rabbits on postnatal day (PND) 5 or 6 using a 28-gauge insulin syringe (28-gauge, 1/2-in. U-100 syringe microfine; BD, Franklin Lakes, NJ). The insulin syringe was used to inject 20 μl of the H37Rv suspension or PBS through the bregma and into the subarachnoid space as described previously (12, 34).

(ii) DLM preparation for rabbit experiments. A 30-ml solution consisting of 275.2 mg DLM powder (DLM; 0.92% [wt/vol]; potency, 218 μg/mg; OPC-67683 powder; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and 3.0 g cycloheximide (10% [wt/vol]; Sigma-Aldrich, St. Louis, MO) was prepared and spun overnight at 4°C. An additional 3.0 g of L-α-phosphatidylcholine lyophilized powder (10% [wt/vol]; Sigma-Aldrich, St. Louis, MO) was added to the homogenized mixture and spun at 4°C for another 24 h. The DLM solution was vigorously vortexed before 5 mg/kg of DLM was administered via an orogastric (OG) tube to each rabbit.

(iii) DLM administration in rabbits. Prior to administration, each rabbit was weighed and then anesthetized on postinfection days 47 to 64 by subcutaneous injection of dexmedetomidine (0.2 μg/g; Zoetis, Florham Park, NJ) and isoflurane inhalation (1.5% isoflurane, USP; Baxter Healthcare Corporation, Deerfield, IL) 30 min prior to the insertion of an OG tube (Argyle Feeding Tube, Medtronic, Fridley, MN). DLM dosing was performed via slow delivery of the viscous DLM mixture directly to the stomach, followed by a 5- to 8-ml sterile water (Sterile Water for Irrigation, USP; Baxter, Deerfield, IL) flush and immediate removal of the OG tube. All rabbits received two, single doses of DLM separated by ~1 week

(6 to 9 days) to allow for comprehensive plasma sampling after the first dose and terminal sampling of plasma, CSF, and brain tissue at euthanization after the second dose (2 infected rabbits and 1 uninfected rabbit were euthanized at 9 h; 1 infected rabbit and 2 uninfected rabbits were euthanized at 24 h).

(iv) Plasma PK sampling in rabbits. Immediately following the first DLM administration, a catheter (5-in. microbore extension set, prepierced T-site with spin collar; Lifeshield, Lake Forest, IL) was placed into the central ear auricular artery for repeated blood sampling. Blood was collected at 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 24, 36, and 48 h using a 1-ml syringe or a 25-gauge needle (25-gauge, 1-in. PrecisionGlide needle; BD, Franklin Lakes, NJ). Following collection, blood samples were immediately placed into Microtainer tubes with K_2EDTA (BD Microtainer tubes with K_2EDTA; BD, Franklin Lakes, NJ). The tubes were centrifuged for 5 min at 4,000 rpm at room temperature, and the supernatant was transferred to a precooled, sterile 2.0-ml microcentrifuge tube (SureSeal screw-cap microcentrifuge tubes; MTC Bio, Metuchen, NJ) to be kept on dry ice until −80.0°C storage.

(v) CSF sample collection in rabbits. Rabbits were euthanized with pentobarbital sodium (120 mg/kg; MWI Veterinary Supply, Boise, ID) 9 and 24 h after the second, single dose of DLM, and CSF was collected via sterile 28-gauge insulin syringe insertion via the cisterna magna. CSF samples were placed in a precooled, sterile microcentrifuge tube containing albumin crystals until −80.0°C storage. Sequentially, the organs of each kit were aseptically harvested after euthanasia and perfusion.

(vi) Brain sample collection in rabbits. Following perfusion, the brains were aseptically harvested, weighed, and placed in 15-ml conical centrifuge tubes (Falcon 15-ml conical centrifuge tubes; Thermo Fisher Scientific, Waltham, MA) and stored at −80.0°C until further analysis. Samples were also obtained with CSF samples at 9 and 24 h post-DLM administration.

Measurement of DLM, DM-6705, and DM-6717 in rabbit samples. Methanol stocks of DLM, DM-6705, and DM-6717 of 1 mg/ml were serially diluted in methanol and subsequently diluted in drug-free control matrices for creation of the standards and quality controls (QCs). All reference powders were received from Otsuka Pharmaceutical Co., Ltd. NZW rabbit control plasma (in K_2EDTA; Bioreclamation IVT, NY) was used to create plasma standard curves and QCs. The control NZW brain homogenate and CSF matrices used for standard curves and QCs were created from in-house-collected tissues. Control and study sample brain tissue was homogenized by adding 4 parts ice-cold PBS buffer with 1% formic acid to 1 part tissue (5× dilution) and shaking the samples using a Fisher bead mill for 1 min at 6,000 rpm with zirconia beads. Formic acid was added to the dilution buffer to stabilize the DLM during homogenization. Control CSF was spiked with 1% (wt/vol) bovine serum albumin (BSA) crystals before spiking with methanol drug stocks. CSF was enriched with BSA to eliminate the nonspecific binding of DLM and metabolites to plastic tubes and other surfaces. All study samples were immediately placed on ice after collection. All standard curves and QCs were created using ice-cold control matrices to stabilize the DLM. Standards, QCs, controls, and study samples were extracted by combining 20 μl of the study sample, QC, standard, or control with 200 μl methanol containing 100 ng/ml of the internal standard (OPC-14714). The extracts were vortexed for 5 min and centrifuged at 4,000 rpm for 5 min. Supernatant (150 μl) was transferred to a 96-well plate for high-performance liquid chromatography (HPLC)-tandem MS (MS/MS) analysis. LC-MS/MS was performed on a Sciex Applied Biosystems Qtrap 6500+ triple-quadrupole mass spectrometer coupled to a Shimadzu 30ACMP HPLC system, and chromatography was performed on an Agilent Zorbax SB-C_8 column (2.1 by 30 mm; particle size, 3.5 μm). Milli-Q-deionized water with 0.1% formic acid was used for the aqueous mobile phase, and 0.1% formic acid in acetonitrile was used for the organic mobile phase. Multiple-reaction monitoring (MRM) of parent/daughter transitions in electrospray positive-ionization mode was used to quantify all molecules. A linear regression with $1/x^2$ weighting was used for standard curve fitting. The coefficient of determination (r^2) was greater than 0.99 for all regressions. The values for all standards and QCs were within 20% of their nominal values. MRM transitions of 535.20/352.20, 466.19/352.30, 481.18/303.30, and 458.18/295.30 were used for DLM, DM-6705, DM-6717, and OPC-14714, respectively.

Clinical case reports. Case reports were provided by the clinicians treating patients with XDR-TBM. Patient samples were collected as part of TDM by their treating physicians and were not part of a research protocol.

(i) Sample collection in humans. All patients had plasma sampled at 0, 2, 4, and 7 h postdose and CSF sampled only at 4 h postdose, after steady state had been achieved, although the duration of treatment differed (5 weeks in patient 1, 5 months in patient 2, 8 weeks in patient 3). Samples were sent to the University of Florida (lab 1) for the quantification of DLM and Rutgers University (lab 2) for the quantification of DLM and DM-6705 (for patient 1 only).

(ii) Measurement of DLM in clinical samples. The Infectious Disease Pharmacokinetics Laboratory at the University of Florida (lab 1) is a Clinical Laboratory Improvement Amendments (CLIA)-licensed and College of American Pathologists (CAP)-certified clinical laboratory. Samples were stored at −80°C until assayed, using a validated LC-MS/MS assay. The plasma standard curves ranged from 10 to 2,000 ng/ml. The standard curve with day precision had a coefficient of variation (CV) 0.45 to 9.78%, and the overall precision had a CV of 3.82 to 8.42%. Overall standard curve accuracy was 91.63 to 109.04%. Samples from patient 1 were also sent to lab 2 so that DM-6705 could be measured. Lab 2 (Rutgers University) used the same machine and technique described above for rabbit samples.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00913-19.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

ACKNOWLEDGMENTS

K.E.D. is supported by a grant from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (grant R01HD074944). E.W.T. is supported by the National Institute of Child Health and Human Development Pediatric Critical Care and Trauma Scientist Development Program (grant K12-HD047349) and the Johns Hopkins All Children's Hospital Foundation Institutional Grant Program. V.D. is supported by the Bill and Melinda Gates Foundation (grant OPP1174780). S.K.J. is supported by a National Institutes of Health Director's Transformative Research Award (award R01-EB020539s). J.T. is supported by the National Institute of Allergy and Infectious Diseases of NIH (grant K23AI135102) and a Johns Hopkins University School of Medicine Clinician Scientist Career Development Award.

The study DLM and DM-6705 were provided by Otsuka Pharmaceutical Co., Ltd.

We thank Michael Urbanowski and Eric Nuermberger for their help optimizing DLM solubility. We also thank Yongee Liu and Jeffrey Hafkin from Otsuka Pharmaceutical Co., Ltd., for providing DLM and commentary on the manuscript.

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

1. Chiang SS, Khan FA, Milstein MB, Tolman AW, Benedetti A, Starke JR, Becerra MC. 2014. Treatment outcomes of childhood tuberculous meningitis: a systematic review and meta-analysis. Lancet Infect Dis 14:947–957. https://doi.org/10.1016/S1473-3099(14)70852-7.
2. Marais S, Pepper DJ, Schutz C, Wilkinson RJ, Meintjes G. 2011. Presentation and outcome of tuberculous meningitis in a high HIV prevalence setting. PLoS One 6:e20077. https://doi.org/10.1371/journal.pone.0020077.
3. Rohlwink UK, Donald K, Gavine B, Padayachy L, Wilmshurst JM, Fieggen GA, Figaji AA. 2016. Clinical characteristics and neurodevelopmental outcomes of children with tuberculous meningitis and hydrocephalus. Dev Med Child Neurol 58:461–468. https://doi.org/10.1111/dmcn.13054.
4. Schoeman J, Wait J, Burger M, van Zyl F, Fertig G, van Rensburg AJ, Springer P, Donald P. 2002. Long-term follow up of childhood tuberculous meningitis. Dev Med Child Neurol 44:522–526.
5. van Well GT, Paes BF, Terwee CB, Springer P, Roord JJ, Donald PR, van Furth AM, Schoeman JF. 2009. Twenty years of pediatric tuberculous meningitis: a retrospective cohort study in the Western Cape of South Africa. Pediatrics 123:e1–e8. https://doi.org/10.1542/peds.2008-1353.
6. Yaramiş A, Gurkan F, Elevli M, Söker M, Haspolat K, Kirbaş G, Taş MA. 1998. Central nervous system tuberculosis in children: a review of 214 cases. Pediatrics 102:E49. https://doi.org/10.1542/peds.102.5.e49.
7. Thwaites GE, Duc Bang N, Huy Dung N, Thi Quy H, Thi Tuong Oanh D, Thi Cam Thoa N, Quang Hien N, Tri Thuc N, Ngoc Hai N, Thi Ngoc Lan N, Ngoc Lan N, Hong Duc N, Ngoc Tuan V, Huu Hiep C, Thi Hong Chau T, Phuong Mai P, Thi Dung N, Stepniewska K, Simmons CP, White NJ, Tinh Hien T, Farrar JJ. 2005. The influence of HIV infection on clinical presentation, response to treatment, and outcome in adults with tuberculous meningitis. J Infect Dis 192:2134–2141. https://doi.org/10.1086/498220.
8. Jain SK, Tobin DM, Tucker EW, Venketaraman V, Ordonez AA, Jayashankar L, Siddiqi OK, Hammoud DA, Prasadarao NV, Sandor M, Hafner R, Fabry Z, NIH Tuberculous Meningitis Writing Group. 2018. Tuberculous meningitis: a roadmap for advancing basic and translational research. Nat Immunol 19:521–525. https://doi.org/10.1038/s41590-018-0119-x.
9. Nau R, Sorgel F, Eiffert H. 2010. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. Clin Microbiol Rev 23:858–883. https://doi.org/10.1128/CMR.00007-10.
10. Ruslami R, Ganiem AR, Dian S, Apriani L, Achmad TH, van der Ven AJ, Borm G, Aarnoutse RE, van Crevel R. 2013. Intensified regimen containing rifampicin and moxifloxacin for tuberculous meningitis: an open-label, randomised controlled phase 2 trial. Lancet Infect Dis 13:27–35. https://doi.org/10.1016/S1473-3099(12)70264-5.
11. Heemskerk AD, Bang ND, Mai NT, Chau TT, Phu NH, Loc PP, Chau NV, Hien TT, Dung NH, Lan NT, Lan NH, Lan NN, Phong LT, Vien NN, Hien NQ, Yen NT, Ha DT, Day JN, Caws M, Merson L, Thinh TT, Wolbers M, Thwaites GE, Farrar JJ. 2016. Intensified antituberculosis therapy in adults with tuberculous meningitis. N Engl J Med 374:124–134. https://doi.org/10.1056/NEJMoa1507062.
12. Tucker EW, Guglieri-Lopez B, Ordonez AA, Ritchie B, Klunk MH, Sharma R, Chang YS, Sanchez-Bautista J, Frey S, Lodge MA, Rowe SP, Holt DP, Gobburu JVS, Peloquin CA, Mathews WB, Dannals RF, Pardo CA, Kannan S, Ivaturi VD, Jain SK. 2018. Noninvasive (11)C-rifampin positron emission tomography reveals drug biodistribution in tuberculous meningitis. Sci Transl Med 10:eaau0965. https://doi.org/10.1126/scitranslmed.aau0965.
13. Savic RM, Ruslami R, Hibma JE, Hesseling A, Ramachandran G, Ganiem AR, Swaminathan S, McIlleron H, Gupta A, Thakur K, van Crevel R, Aarnoutse R, Dooley KE. 2015. Pediatric tuberculous meningitis: model-based approach to determining optimal doses of the anti-tuberculosis drugs rifampin and levofloxacin for children. Clin Pharmacol Ther 98:622–629. https://doi.org/10.1002/cpt.202.
14. Dian S, Yunivita V, Ganiem AR, Pramaesya T, Chaidir L, Wahyudi K, Achmad TH, Colbers A, Te Brake L, van Crevel R, Ruslami R, Aarnoutse R. 2018. Double-blind, randomized, placebo-controlled phase II dose-finding study to evaluate high-dose rifampin for tuberculous meningitis. Antimicrob Agents Chemother 62:e01014-18. https://doi.org/10.1128/AAC.01014-18.
15. Te Brake L, Dian S, Ganiem AR, Ruesen C, Burger D, Donders R, Ruslami R, van Crevel R, Aarnoutse R. 2015. Pharmacokinetic/pharmacodynamic analysis of an intensified regimen containing rifampicin and moxifloxacin for tuberculous meningitis. Int J Antimicrob Agents 45:496–503. https://doi.org/10.1016/j.ijantimicag.2014.12.027.
16. Yunivita V, Dian S, Ganiem AR, Hayati E, Hanggono Achmad T, Purnama Dewi A, Teulen M, Meijerhof-Jager P, van Crevel R, Aarnoutse R, Ruslami R. 2016. Pharmacokinetics and safety/tolerability of higher oral and intravenous doses of rifampicin in adult tuberculous meningitis patients. Int J Antimicrob Agents 48:415–421. https://doi.org/10.1016/j.ijantimicag.2016.06.016.
17. World Health Organization. 2018. Global tuberculosis report 2018. World Health Organization, Geneva, Switzerland.
18. Tho DQ, Torok ME, Yen NT, Bang ND, Lan NT, Kiet VS, van Vinh Chau N, Dung NH, Day J, Farrar J, Wolbers M, Caws M. 2012. Influence of antituberculosis drug resistance and Mycobacterium tuberculosis lineage on outcome in HIV-associated tuberculous meningitis. Antimicrob Agents Chemother 56:3074–3079. https://doi.org/10.1128/AAC.00319-12.
19. Thwaites GE, Lan NT, Dung NH, Quy HT, Oanh DT, Thoa NT, Hien NQ, Thuc NT, Hai NN, Bang ND, Lan NN, Duc NH, Tuan VN, Hiep CH, Chau TT, Mai PP, Dung NT, Stepniewska K, White NJ, Hien TT, Farrar JJ. 2005. Effect of antituberculosis drug resistance on response to treatment and outcome in adults with tuberculous meningitis. J Infect Dis 192:79–88. https://doi.org/10.1086/430616.
20. Vinnard C, King L, Munsiff S, Crossa A, Iwata K, Pasipanodya J, Proops D, Ahuja S. 2017. Long-term mortality of patients with tuberculous men-

ingitis in New York City: a cohort study. Clin Infect Dis 64:401–407. https://doi.org/10.1093/cid/ciw763.
21. Sun F, Ruan Q, Wang J, Chen S, Jin J, Shao L, Zhang Y, Zhang W. 2014. Linezolid manifests a rapid and dramatic therapeutic effect for patients with life-threatening tuberculous meningitis. Antimicrob Agents Chemother 58:6297–6301. https://doi.org/10.1128/AAC.02784-14.
22. Chen X, Hashizume H, Tomishige T, Nakamura I, Matsuba M, Fujiwara M, Kitamoto R, Hanaki E, Ohba Y, Matsumoto M. 2017. Delamanid kills dormant mycobacteria in vitro and in a guinea pig model of tuberculosis. Antimicrob Agents Chemother 61:e02402-16. https://doi.org/10.1128/AAC.02402-16.
23. Ryan NJ, Lo JH. 2014. Delamanid: first global approval. Drugs 74:1041–1045. https://doi.org/10.1007/s40265-014-0241-5.
24. Lewis JM, Sloan DJ. 2015. The role of delamanid in the treatment of drug-resistant tuberculosis. Ther Clin Risk Manag 11:779–791. https://doi.org/10.2147/TCRM.S71076.
25. Matsumoto M, Hashizume H, Tomishige T, Kawasaki M, Tsubouchi H, Sasaki H, Shimokawa Y, Komatsu M. 2006. OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis in vitro and in mice. PLoS Med 3:e466. https://doi.org/10.1371/journal.pmed.0030466.
26. Stinson K, Kurepina N, Venter A, Fujiwara M, Kawasaki M, Timm J, Shashkina E, Kreiswirth BN, Liu Y, Matsumoto M, Geiter L. 2016. MIC of delamanid (OPC-67683) against Mycobacterium tuberculosis clinical isolates and a proposed critical concentration. Antimicrob Agents Chemother 60:3316–3322. https://doi.org/10.1128/AAC.03014-15.
27. World Health Organization. 2014. The use of delamanid in the treatment of multidrug-resistant tuberculosis: interim policy guidance. World Health Organization, Geneva, Switzerland.
28. World Health Organization. 2016. WHO treatment guidelines for drug-resistant tuberculosis: 2016 update. World Health Organization, Geneva, Switzerland.
29. World Health Organization. 2018. Rapid communication: key changes to treatment of multidrug-and rifampicin-resistant tuberculosis (MDR/RR-TB). World Health Organization, Geneva, Switzerland.
30. World Health Organization. 2016. The use of delamanid in the treatment of multidrug-resistant tuberculosis in children and adolescents: interim policy guidance. World Health Organization, Geneva, Switzerland.
31. von Groote-Bidlingmaier F, Patientia R, Sanchez E, Balanag V, Jr, Ticona E, Segura P, Cadena E, Yu C, Cirule A, Lizarbe V, Davidaviciene E, Domente L, Variava E, Caoili J, Danilovits M, Bielskiene V, Staples S, Hittel N, Petersen C, Wells C, Hafkin J, Geiter LJ, Gupta R. 2019. Efficacy and safety of delamanid in combination with an optimised background regimen for treatment of multidrug-resistant tuberculosis: a multicentre, randomised, double-blind, placebo-controlled, parallel group phase 3 trial. Lancet Respir Med 7:249–259. https://doi.org/10.1016/S2213-2600(18)30426-0.
32. World Health Organization. 2019. WHO consolidated guidelines on drug-resistant tuberculosis treatment. World Health Organization, Geneva, Switzerland.
33. Sasahara K, Shimokawa Y, Hirao Y, Koyama N, Kitano K, Shibata M, Umehara K. 2015. Pharmacokinetics and metabolism of delamanid, a novel anti-tuberculosis drug, in animals and humans: importance of albumin metabolism in vivo. Drug Metab Dispos 43:1267–1276. https://doi.org/10.1124/dmd.115.064527.
34. Tucker EW, Pokkali S, Zhang Z, DeMarco VP, Klunk M, Smith ES, Ordonez AA, Penet MF, Bhujwalla Z, Jain SK, Kannan S. 2016. Microglia activation in a pediatric rabbit model of tuberculous meningitis. Dis Model Mech 9:1497–1506. https://doi.org/10.1242/dmm.027326.
35. Shimokawa Y, Sasahara K, Yoda N, Mizuno K, Umehara K. 2014. Delamanid does not inhibit or induce cytochrome P450 enzymes in vitro. Biol Pharm Bull 37:1727–1735. https://doi.org/10.1248/bpb.b14-00311.
36. Mallikaarjun S, Wells C, Petersen C, Paccaly A, Shoaf SE, Patil S, Geiter L. 2016. Delamanid co-administered with antiretroviral drugs or anti-TB drugs shows no clinically relevant drug-drug interactions in healthy subjects. Antimicrob Agents Chemother 60:5976–5985. https://doi.org/10.1128/AAC.00509-16.
37. Pang Y, Zong Z, Huo F, Jing W, Ma Y, Dong L, Li Y, Zhao L, Fu Y, Huang H. 2017. In vitro drug susceptibility of bedaquiline, delamanid, linezolid, clofazimine, moxifloxacin, and gatifloxacin against extensively drug-resistant tuberculosis from Beijing, China. Antimicrob Agents Chemother 61:e00900-17. https://doi.org/10.1128/AAC.00900-17.
38. Diacon A, Dawson R, Hanekom M, Narunsky K, Venter A, Hittel N, Geiter L, Wells C, Paccaly A, Donald P. 2011. Early bactericidal activity of delamanid (OPC-67683) in smear-positive pulmonary tuberculosis patients. Int J Tuberc Lung Dis 15:949–954. https://doi.org/10.5588/ijtld.10.0616.
39. Ahmad Z, Peloquin CA, Singh RP, Derendorf H, Tyagi S, Ginsberg A, Grosset JH, Nuermberger EL. 2011. PA-824 exhibits time-dependent activity in a murine model of tuberculosis. Antimicrob Agents Chemother 55:239–245. https://doi.org/10.1128/AAC.00849-10.
40. Diacon AH, Dawson R, Hanekom M, Narunsky K, Maritz SJ, Venter A, Donald PR, van Niekerk C, Whitney K, Rouse DJ, Laurenzi MW, Ginsberg AM, Spigelman MK. 2010. Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive tuberculosis patients. Antimicrob Agents Chemother 54:3402–3407. https://doi.org/10.1128/AAC.01354-09.
41. Diacon AH, Dawson R, Du Bois J, Narunsky K, Venter A, Donald PR, van Niekerk C, Erondu N, Ginsberg AM, Becker P, Spigelman MK. 2012. Phase II dose-ranging trial of the early bactericidal activity of PA-824. Antimicrob Agents Chemother 56:3027–3031. https://doi.org/10.1128/AAC.06125-11.
42. Marais BJ, Heemskerk AD, Marais SS, van Crevel R, Rohlwink U, Caws M, Meintjes G, Misra UK, Mai NT, Ruslami R, Seddon JA, Solomons R, van Toorn R, Figaji A, McIlleron H, Aarnoutse R, Schoeman JF, Wilkinson RJ, Thwaites GE, Tuberculous Meningitis International Research Consortium. 2016. Standardized methods for enhanced quality and comparability of tuberculous meningitis studies. Clin Infect Dis 64:501–509. https://doi.org/10.1093/cid/ciw757.
43. Shibata M, Shimokawa Y, Sasahara K, Yoda N, Sasabe H, Suzuki M, Umehara K. 2017. Absorption, distribution and excretion of the anti-tuberculosis drug delamanid in rats: extensive tissue distribution suggests potential therapeutic value for extrapulmonary tuberculosis. Biopharm Drug Dispos 38:301–312. https://doi.org/10.1002/bdd.2064.
44. Doorduin J, de Vries EF, Dierckx RA, Klein HC. 2014. P-glycoprotein activity in the blood–brain barrier is affected by virus-induced neuroinflammation and antipsychotic treatment. Neuropharmacology 85:548–553. https://doi.org/10.1016/j.neuropharm.2014.06.017.
45. Miller DS. 2010. Regulation of P-glycoprotein and other ABC drug transporters at the blood–brain barrier. Trends Pharmacol Sci 31:246–254. https://doi.org/10.1016/j.tips.2010.03.003.
46. Thompson GR, III, Rendon A, Ribeiro Dos Santos R, Queiroz-Telles F, Ostrosky-Zeichner L, Azie N, Maher R, Lee M, Kovanda L, Engelhardt M, Vazquez JA, Cornely OA, Perfect JR. 2016. Isavuconazole treatment of cryptococcosis and dimorphic mycoses. Clin Infect Dis 63:356–362. https://doi.org/10.1093/cid/ciw305.
47. Wiederhold NP, Kovanda L, Najvar LK, Bocanegra R, Olivo M, Kirkpatrick WR, Patterson TF. 2016. Isavuconazole is effective for the treatment of experimental cryptococcal meningitis. Antimicrob Agents Chemother 60:5600–5603. https://doi.org/10.1128/AAC.00229-16.
48. Falci DR, Pasqualotto AC. 2013. Profile of isavuconazole and its potential in the treatment of severe invasive fungal infections. Infect Drug Resist 6:163–174. https://doi.org/10.2147/IDR.S51340.
49. Groll AH, Mickiene D, Petraitis V, Petraitiene R, Ibrahim KH, Piscitelli SC, Bekersky I, Walsh TJ. 2001. Compartmental pharmacokinetics and tissue distribution of the antifungal echinocandin lipopeptide micafungin (FK463) in rabbits. Antimicrob Agents Chemother 45:3322–3327. https://doi.org/10.1128/AAC.45.12.3322-3327.2001.
50. Kethireddy S, Andes D. 2007. CNS pharmacokinetics of antifungal agents. Expert Opin Drug Metab Toxicol 3:573–581. https://doi.org/10.1517/17425225.3.4.573.
51. Warn PA, Livermore J, Howard S, Felton TW, Sharp A, Gregson L, Goodwin J, Petraitiene R, Petraitis V, Cohen-Wolkowiez M, Walsh TJ, Benjamin DK, Jr, Hope WW. 2012. Anidulafungin for neonatal hematogenous Candida meningoencephalitis: identification of candidate regimens for humans using a translational pharmacological approach. Antimicrob Agents Chemother 56:708–714. https://doi.org/10.1128/AAC.05826-11.
52. National Research Council. 2011. Guide for the care and use of laboratory animals, 8th ed. National Academies Press, Washington, DC.