Supplementary material: Population Pharmacokinetic Analysis of Rifampicin in Plasma, Cerebrospinal Fluid, and Brain Extracellular Fluid in South African Children with Tuberculous Meningitis

Sample collection details

Plasma: Three serial blood samples (0.6 mL each) were sampled on a single day in the first and second week, and where possible at 2-, 4-, and 6-hours post-dose. Whole blood was drawn through an indwelling peripheral venous catheter in patients who did not have a routine arterial line. The venous line was inserted to coincide with clinically indicated routine sampling to ensure that the patient does not endure additional veno-punctures. Whole blood samples were collected in a vacutainer EDTA blood collection tube and kept on ice. These were immediately processed and biobanked at -80 °C within 30 minutes, pending bioanalysis.

Lumbar and Ventricular CSF: Clinically indicated CSF samples were collected at random time points up to 24 hours post-dose into sterile 15 mL tubes and kept on ice. The samples were immediately spun at 1400 rpm for 10 minutes to separate CSF from any tissue pellets or blood and stored at -80°C within 30 minutes of sampling until analysis. Briefly, LCSF was collected for standard diagnostic and/or therapeutic procedures. Repeat lumbar punctures were performed to treat raised intracranial pressure (ICP) in patients with communicating HCP (CommHC) (1). The frequency of lumbar punctures depends on the persistence or resolution of raised ICP. In addition, VCSF was obtained from 1) ventriculoperitoneal shunt (VPS) placement as a definitive treatment for non-communicating HCP (NCHC) or failed medical treatment of communicating hydrocephalus (CommHC), 2) external ventricular drain (EVD) insertion, routine testing for bacteriology, and during air encephalograms and/or column tests to distinguish between CommHC and NCHC, or 3) endoscopic third ventriculostomy (ETV)

in a selection of patients with NCHC. Our standard protocol for treating HCP in TBM is previously published (2).

Brain ECF using microdialysis (MD) technique: The MD catheter was placed concurrently with the EVD and monitor into the same region (usually right frontal white matter) or sometimes on the side where hypodensity was more prominent on the initial head computed tomography scan. The MD probe in a standard manner: it was perfused with a physiological solution (CNS Perfusion fluid, MDialysis) at a constant flow rate of 0.3 μL/min using an infusion pump (CMA 107, MDialysis, Stockholm, Sweden). Molecules in the interstitium of the brain diffuse through a 100 KDa pore size (MDialysis, Stockholm, Sweden) semipermeable membrane at the tip of the catheter, along their concentration gradient in the brain and were collected into a vial. MD vials were changed at hourly intervals for bedside monitoring of brain extracellular fluid and analyzed at the bedside (ISCUSFlex, MDialysis) for clinical purposes to observe changes in glucose, lactate, pyruvate, glycerol and glutamate using an automated system. Briefly, glucose and the lactate/pyruvate ratio are used to monitor the energy status of the brain with respect primarily to the adequacy of substrate delivery for aerobic metabolism. Patients with TBM are at high risk of stroke due to perfusion-limiting vasculitis. Evidence of brain ischemia can be addressed by more aggressive treatment of intracranial pressure, increasing blood pressure, and increasing systemic oxygenation. Glycerol is a measure of ongoing cellular breakdown. Glutamate is a measure of excitotoxicity. Remnant fluid after bedside analysis was kept on ice and biobanked at -80°C within 6 hours of collection. As MD volumes are typically small, hourly samples were pooled over 2–3-hour epochs to ensure sufficient volumes for liquid chromatography tandem mass spectrometry analysis (>20 μL). Concentrations of substances in the microdialysate are a percentage of the true concentrations in the ECF and not absolute concentrations. This percentage is termed relative recovery.

Analytical method details

Rifampicin was analyzed in all samples: plasma, lumbar CSF, ventricular CSF, and brain ECF. Additionally, rifampicin's primary metabolite, 25-desacetyl rifampicin, was analyzed in the plasma samples. Liquid chromatography tandem mass spectrometry assays were developed and validated at the Division of Clinical Pharmacology, University of Cape Town. Plasma samples were processed with a protein precipitation extraction method using rifampicin-d3 and 25-desacetyl-rifampicin-d3 as internal standards, followed by high performance liquid chromatography with tandem mass spectrometry detection using an AB SCIEX API 3000 instrument. Isocratic chromatography was performed on a Discovery C18 (5 µm, 50 mm x 4.6 mm) analytical column. The analyte, metabolite and internal standards were monitored at mass transitions of the protonated precursor ions 823.4, 781.5, 826.5, and 784.5 to the product ions 791.4, 749.4, 794.4, and 752.4 for rifampicin, 25-desacetyl-rifampicin, rifampicin-d3 and 25-desacetyl-rifampicin-d3, respectively. The calibration curves both fit a quadratic (weighted by $1/x^2$) regression over the ranges 0.117–30.0 µg/mL for rifampicin and 0.0391–10.0 µg/mL for 25-desacetyl-rifampicin. The combined accuracy and precision statistics of the limit of quantification, low, medium, and high-quality controls (three validation batches [n=18]) were between 101% and 107% and between 2.7% and 3.7% for rifampicin and 25-desacetyl-rifampicin, respectively.

CSF samples were processed with a protein precipitation extraction method using acidified acetonitrile and ascorbic acid, followed by high performance liquid chromatography with tandem mass spectrometry detection using an AB SCIEX API 5500Q instrument. Liquid chromatography was performed on a Poroshell 120 EC-C18 (2.7 µm, 4.6 mm x 50 mm) analytical column. Rifampicin and rifampicin-d3 were monitored at mass transitions of the protonated precursor ions 823.4 and 826.5 to the product ions 791.4 and 794.4, respectively. The calibration curve fit a quadratic (weighted by $1/x^2$) regression over the range 5 to 2500

ng/mL. The combined accuracy and precision statistics of the limit of quantification, low, medium, and high-quality controls (three validation batches [n=18]) were between 88.9% and 102.9% and between 3.8% and 8.3% for rifampicin.

Population pharmacokinetic analysis

Residual unexplained variability was described using a combined proportional and additive error model, with the additive error for all samples set to be at least 20% of the LLOQ. Concentrations below the lower limit of quantification (BLQ) were censored according to Beal's M6 method, in which the last censored value in a series during the absorption phase and the first censored value in a series in the terminal phase were replaced with LLOQ/2 and the other censored values in a series were discarded (3). To account for the larger level of uncertainty in the imputed censored values, their additive error was inflated by LLOQ/2.

Model development and covariate inclusion were guided by physiological plausibility, model fit diagnostics including the drop in the objective function value (OFV) and inspection of diagnostic plots, including visual predictive checks (VPCs). Comparison between nested models was done using the likelihood ratio test for the drop in OFV, assumed to be approximately χ^2 distributed with n degrees of freedom, where n is the number of additional estimated parameters. Covariates were added in a stepwise manner in order of importance determined by the largest significant drop in the OFV. A p-value of 0.05 was generally used for inclusion and 0.01 for retention.

References

1. Figaji AA, Fieggen AG. 2010. The neurosurgical and acute care management of tuberculous meningitis: Evidence and current practice. Tuberculosis 90:393–400.

2. Figaji a a, Fieggen a G, Peter JC. 2003. Endoscopic third ventriculostomy in tuberculous meningitis. Child's Nerv Syst 19:217–225.

3. Beal SL. 2001. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn 28:481–504.

Supplementary Tables

Table S1: Summary of albumin and total protein values in plasma, lumbar and ventricular cerebrospinal fluid (CSF), and brain extracellular fluid (ECF)

	Median (min - max) of all samples			
	Albumin (g/L)		Total protein (g/L)	
	N	Median (IQR)	N	Median (IQR)
Lumbar CSF	191	1.97 (2.83)	47	2.73 (3.46)
Ventricular CSF	193	0.489 (0.448)	31	0.890 (0.835)
Plasma	147	32.0 (7.00)	-	-
LCSF : plasma	68	0.052 (0.098)	-	-
VCSF : plasma	82	0.017 (0.011)	-	-

Supplementary Figures

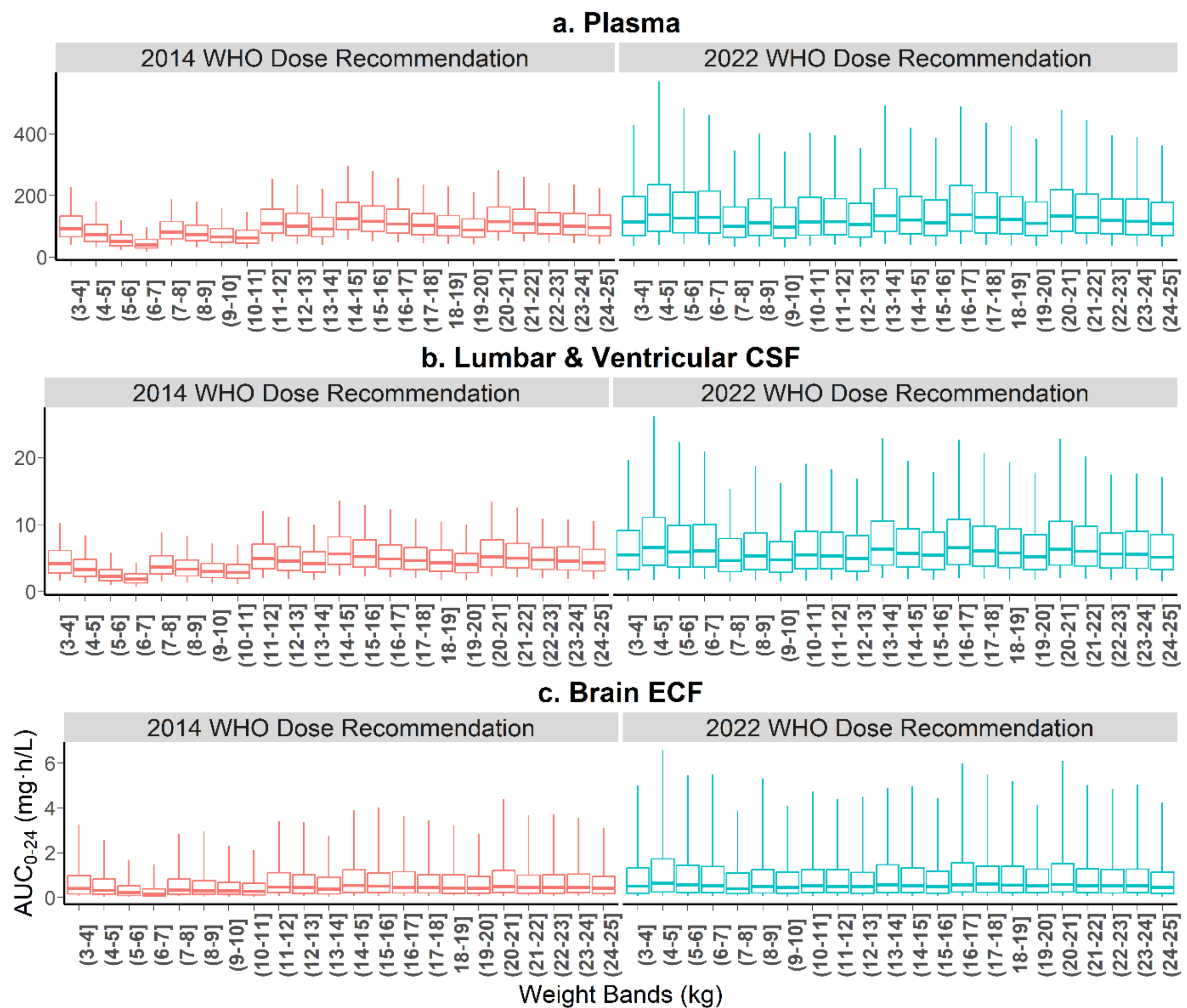

Figure S1 Exposures at steady-state as area under the curve 24h (AUC_{0-24h}) across all dosing weight bands in plasma, cerebrospinal fluid (CSF) (lumbar and ventricular), and brain extracellular fluid (ECF) shown for both the 2014 WHO dose regimen recommendation and the 2022 WHO short intensive regimen