Bioanalysis

African plasma samples were assayed for paromomycin concentrations using HPLC-UV , based on a previously reported method [1] , which was further validated at the Clinical Pharmacology Section, African Centre for Clinical Trials, Nairobi, Kenya. Aliquots (50-300 µl) of plasma samples from study subjects were processed up to a final step involving pre-column derivatization to convert paromomycin, an aminoglycoside that does not absorb UV light into a derivative that absorbs UV light, thus allowing quantification by high performance liquid chromatography (HPLC). Paromomycin and internal standard (kanamycin) were derivatized with 2, 4 – dinitrofluorobenzene at 65°C, followed by extraction with tert-butyl methyl ether (TBME). The organic phase (TBME) was then evaporated in a water bath (37°C) under a gentle flow of white spot nitrogen. The resulting residue was reconstituted in a mixture of acetonitrile and water, and injected into the chromatography system. Separation was performed on a Thermohypersil reverse phase C18 column (4.6 x 150 mm) coupled to a guard column. The mobile phase was a mixture of water/acetonitrile /methanol, adjusted to pH 3.0 with phosphoric acid. The mobile phase was degassed for 20 minutes in an ultra-sonic water bath before use, and delivered in isocratic mode at a flow rate of 1.2 ml/min. Column effluent was monitored at 350 nm. Calibration curves were prepared in duplicate by spiking blank plasma with varying known amounts of paromomycin sulphate (0.2-25 µg of base equivalents) and a fixed amount (5 µg) of kanamycin sulphate, followed by derivatization and extraction as described above. Quantification of paromomycin in samples from patients was done with reference to the linear portion of the calibration curve. Quality control (QC) samples representing high, medium and low concentrations of paromomycin were analyzed simultaneously with the patient samples, demonstrating good assay precision with intra- and inter-day coefficient of variation below 10%. Accuracy as determined from assay of QC samples was within acceptable ranges for low, medium and high QC values for both urine and plasma samples. The % recovery was over 80% for all QC concentrations. The limit of detection of paromomycin was 0.05 µg/mL and the lower limit of quantification 0.1 µg/mL.

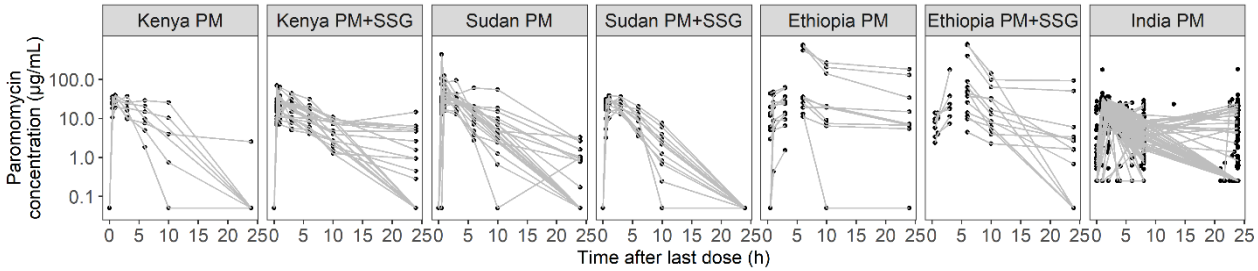
In Indian plasma samples, paromomycin concentrations were determined by the use of a sensitive and specific liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) method, performed at the Analytical Division of the Drug Studies Unit (DSU) at the University of California, San Francisco, CA, USA. Human plasma samples (200 µl) were analyzed for paromomycin with a LC/MS/MS procedure on a Micromass Quattro Ultima LC system equipped with an Allure PFP propyl column (4.6 x 50 mm, 5 µm particle size), a methanol/water (v/v) containing 20 mM ammonium acetate and 0.14% trifluoroacetic acid, pH 3.3 mobile phase, and mass spectrometric detection with positive ionization by electrospray and mass scanning by MRM (Multiple Reaction Monitoring) analysis. Sample preparation consisted of the addition of internal standard into 200 µl of plasma samples, precipitation of the samples with 30% trifluoroacetic acid, centrifugation, transfer of the supernatant and addition of 50% methanol to each sample prior to separation by LC/MS/MS. Calibration standards and QC samples were generated by spiking blank (interference-free) human plasma with paromomycin. Calibration standards at ten concentrations, excluding blanks, and QC samples at three concentrations within the calibration range of the assay were analyzed routinely with study samples. The QC samples were used to monitor the performance of the assay. All human plasma concentrations were calculated from a paromomycin human plasma calibration curve. The range for the assay was 0.5 to 240 µg/ml with inter-day precision of 2.93 to 5.04% and intra-day precision of 1.09 to 2.53%. Plasma concentrations of paromomycin less than 0.5 µg/ml were reported as below the limit of quantitation (BLQ). Assay validation procedures and criteria were in accordance with the US Food and Drug Administration (FDA) “Guidance for Industry: Bioanalytical Method Validation” (May, 2001).

Reference

1. Kanyok TP, Killian AD, Rodvold KA, Danziger LH. Pharmacokinetics of intramuscularly administered aminosidine in healthy subjects. Antimicrob Agents Chemother. 1997;41:982–6.

Figure S1. Paromomycin observations stratified by country and treatment group. Panel A: All observations; Panel B: Observations included in pharmacokinetic analysis. BLQ observations are fixed to half BLQ (0.05 µg/mL for Eastern Africa, 0.25 µg/mL for India).

A



B

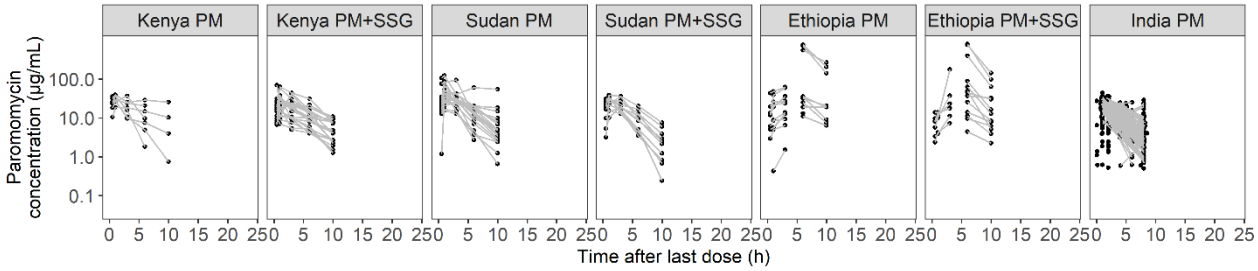
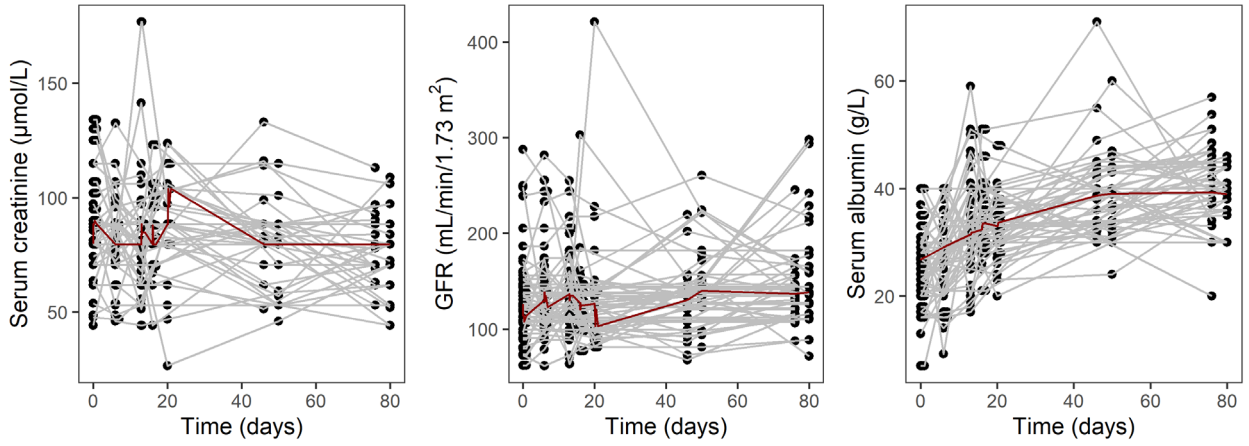


Figure S2. Serum creatinine levels and glomerular filtration rate in patients from Kenya and Sudan; serum albumin in patients from Kenya, Sudan, and Ethiopia.



NONMEM control stream

; Description: Final pooled paromomycin PK model Eastern Africa and India

; Author: L. Verrest

$PROBLEM PK

$INPUT ID DOSE DOSEBASE DV TIME CMT AMT II ADDL WTB AGE CNTRY FLAG EVID MDV BLQ

;;;; Dataset description ;;;;

; ID = Original subject ID

; DOSE = Daily PM sulfate dose (mg)

; DOSEBASE = PM base (mg): PM sulfate * 0.7554

; DV = PM plasma concentration (ug/mL)

; TIME = Time after first dose (h)

; CMT = Compartment. 1 = dose, 2 = PM plasma concentration

; AMT = PM base dose amount (mg)

; II = Inter-dose interval

; ADDL = Additional doses

; WTB = Body weight at baseline (kg)

; AGE = Age (years)

; CNTRY = Country. 1 = Kenya, 2 = Sudan, 3 = Ethiopia, 4 = India

; FLAG = Samples to be excluded. 0= include, 1 = exclude

; EVID = Event ID. 0 = DV, 1 = dose, 2 = no dose or DV. EVID = 2 for DVs at T=0 and T=24.

; MDV = Missing DV. = DV, 1 = dose. MDV = 1 for DVs at T=0 and T=24.

; BLQ = DV below limit of quantification (0.1 ug/mL for East-Africa, 0.5 ug/mL for India). 0 = no, 1 = yes.

```
$DATA     nm.paromomycinPooled.final.csv IGNORE=@

IGNORE(BLQ.EQ.1) ; no BLQ samples

IGNORE(FLAG.EQ.1) ; remove excluded samples

$SUBROUTINE ADVAN13 TOL=6

$MODEL

COMP = (DEPOT)

COMP = (CENTRAL)

COMP = (AUC)

$ABB COMRES=2

$PK

TVCL = THETA(1)

TVV2  = THETA(2)

TVKA = THETA(3)

TVF1 = THETA(4)

;; Covariates

CLAGE = ( 1 + THETA(5)*(AGE - 20)) ; 20 = median population age

CLTIME = EXP(THETA(6)*TIME)

CL = TVCL *(WTB/39)**0.75 * EXP(ETA(1)) * CLAGE * CLTIME  ; 39 = median population baseline

body weight

IF(CNTRY.EQ.4) CL = TVCL *(WTB/39)**0.75 * EXP(ETA(1)) * CLAGE

V2  = TVV2 *(WTB/39)**1.00* EXP(ETA(2))

IF(CNTRY.EQ.4) V2  = TVV2 *(WTB/39)**1.00

KA = TVKA

IF(CNTRY.EQ.3) KA = THETA(3) * THETA(7)
```

```
F1 = TVF1

IF(CNTRY.EQ.3) F1 =  1 * THETA(8) * EXP(ETA(3))  ; Ethiopia

IF(CNTRY.LT.3) F1 =  1 * THETA(9)                ; Kenya and Sudan

K20   = CL/V2

S2    = V2

AUCtau = F1*DOSEBASE/CL

IF(NEWIND.LE.1) THEN

COM(1)=0

COM(2)=0

ENDIF
```

$DES

```
;; Derive paromomycin Cmax, Tmax, and AUC

CONC = A(2)/V2

IF(CONC.GT.COM(1)) THEN

COM(1)=CONC

COM(2)=T

ENDIF

CC = A(2)/V2

DADT(1)= -KA*A(1)

DADT(2)=  KA*A(1)-K20*A(2)

DADT(3)= CC

AUC = A(3)
```

$ERROR

```
CMAX=COM(1)

TMAX=COM(2)

IPRED = F

IF(IPRED.LT.1E-6) IPRED=1E-6

IF(CNTRY.LT.4) W = SQRT(THETA(10)**2*IPRED**2 + THETA(12)**2)   ; Eastern Africa

IF(CNTRY.EQ.4) W = SQRT(THETA(11)**2*IPRED**2 + THETA(13)**2) ; India

Y = IPRED + W*EPS(1)

IRES = DV-IPRED

IWRES = IRES/W
```

$THETA

```
(0, 4.38)       ; 1 CL

(0, 15.6)       ; 2 V2

(0, 1.99)       ; 3 KA

(1 FIX)         ; 4 F1

(-0.000782)  ; 5 COV(Cl,time)

(-0.0125)       ; 6 COV(Cl,age)

(0, 0.224)      ; 7 ka(Ethiopia)

(0, 2.46)       ; 8 F1(Ethiopia)

(0, 1.17)       ; 9 F1(Kenya,Sudan)

(0, 0.418)      ; 10 Prop. RE Eastern Africa

(0, 0.315)      ; 11 Prop. RE India

(0 FIX)         ; 12 Add. RE Eastern Africa

(0 FIX)         ; 13 Add. RE India
```

$OMEGA

0.216843 ; 1 CL

0.2 ; 2 V2

0.8 ; 3 F1(Ethiopia)

$SIGMA

(1 FIX)

$ESTIMATION METHOD=1 INTER MAXEVAL=2000 NOABORT PRINT=10 POSTHOC ATOL=6

SIGL=6 NSIG=2 ETASTYPE=1

$COV PRINT=E

$TABLE ID DOSE DOSEBASE DV TIME CMT AMT II ADDL WTB AGE CNTRY FLAG BLQ

EVID IPRED CWRES CL V2 KA F1 ETA1 ETA2 ETA3 CMAX TMAX AUCtau AUC CC

ONEHEADER NOPRINT FILE=sdtab0017