Pharmacokinetics of Antimony in Patients Treated with Sodium Stibogluconate for Cutaneous Leishmaniasis

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The pharmacokinetics of Sb was examined in 29 patients with cutaneous leishmaniasis following the intramuscular administration of a dose of sodium stibogluconate equivalent to 600 mg of Sb. Blood was sampled at different time intervals from each patient and Sb was measured in whole blood by electrothermal atomic absorption spectrophotometry after an appropriate dilution with Triton X-100. The 24-hr urine was also collected and analyzed similarly. The blood concentration-time data conformed to the one-compartment open model with mean and (SEM) of the apparent first-order rate constants for absorption (k_a) and elimination (k_d) of 1.71 (0.15) and 0.391 (0.016) hr^{-1}, respectively. The maximum concentration of Sb achieved was 8.77 (0.39) mg/L and the peak time was 1.34 (0.09) hr. The total body clearance (TBC) and the volume of distribution (V_d) were 17.67 (1.38) L/hr and 45.7 (2.6) L, respectively, assuming a complete absorption. The fraction of dose of Sb excreted in the urine was 0.80 (0.07) and the renal clearance was 12.7 (1.16) L/hr. The frequency distribution pattern of the area-under-the-curve (AUC) appears to be bimodal and separates patients into those with low exposure to Sb (AUC = 11.7-29.04 mg.hr/L) (i.e., rapid eliminators) and those with high exposure to Sb (AUC = 31.5-49.1 mg.hr/L) (i.e., slow eliminators). This may explain the variability observed in the response to treatment of leishmaniasis with sodium stibogluconate.

KEY WORDS: antimony; sodium stibogluconate; pentavalent antimonials; pharmacokinetics; cutaneous leishmaniasis; antimony in whole blood; urinary excretion of antimony; interpatient variability.

INTRODUCTION

Leishmaniasis consists of a spectrum of diseases ranging from the simple, self-healing, single cutaneous lesion to the fatal visceral form known as kala-azar. Within this spectrum, there is a mucocutaneous form of the disease characterized, in severe cases, by nasal cartilage destruction. Leishmaniasis is caused by protozoan parasites belonging to the genus *leishmania* that are transmitted by the female blood-sucking sand fly which feeds on mammalian blood for egg production.

Pentavalent antimonials in the form of sodium stibogluconate (Pentostam®) or meglumine antimonate (Glucontime®) are the drugs of choice for treatment of all forms of leishmaniasis. However, marked variabilities in the patients response to these agents have long been documented (1-5). Although these drugs have been in use for more than four decades (6-7), little is known about their pharmacokinetics, particularly, in regards to its impact on these variabilities. Indeed, to the best of our knowledge, some of the pharmacokinetic parameters for Sb have not been previously reported, and others were determined in a small number of patients.

The present study was conducted to examine the pharmacokinetics of Sb in a larger number of patients with cutaneous leishmaniasis following the intramuscular administration of a dose of sodium stibogluconate equivalent to 600 mg of Sb. Further, the frequency distribution pattern of the area-under-the-curve was investigated in relation to the variability of the patients response to treatment with this drug.

MATERIALS AND METHODS

Patients

Twenty nine male patients with cutaneous leishmaniasis who were attending the Dermatology Clinic at King Khaled Hospital in Al-Kharj, east of Riyadh in the central region of Saudi Arabia, entered this study after giving an informed written consent. The clinical characteristics of these patients are presented in Table I. A week before drug administration, the patients who weighed 60-75 kg underwent a full medical examination including liver, kidney and heart function tests, a complete blood profile, etc. They were also tested for blood carried diseases, i.e., hepatitis B and C, and AIDS. Apart from the skin disease, they were all found in good health. The patients were admitted into the hospital 24 hours prior to drug administration where they were placed under a careful medical supervision.

Drug Administration

Pentavalent antimony was administered intramuscularly to the patients in the form of sodium stibogluconate [Pentostam (10 mg of Sb/ml injection), The Welcome Foundation Ltd, Berkhamstead, England] at a dose equivalent to 600 mg of Sb per day which was repeated for 10 consecutive days.

Specimens Collection

After the first dose was administered, blood (5 ml each) was sampled from each patient via a heparin lock into special, heparinized tubes recommended for trace elements work [Becton Dickinson Vacutainer Systems, Rutherford, New Jersey] at different time intervals, i.e., 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours. An additional sample was collected immediately prior to drug administration to serve as a blank. The urine excreted during the 24 hr after the first dose was also collected in 4-liter plastic container to which 2 gm of sodium metabisulfite was added as an antioxidant. Both blood and urine specimens were kept at 4°C until they were transferred to the laboratory at King Faisal Specialist Hospital and Research Centre where they were placed in the refrigerator (i.e., 4°C). The volume of the urine was then measured and an adequate aliquot (10 ml) was stored at

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−20° until analysis. Urine from four patients were disregarded because of suspicion of incomplete collection.

Analysis of Antimony in Whole Blood and Urine

Antimony was measured in blood and urine by flameless atomic absorption spectrophotometry. The instrument used was a 975 model equipped with a graphite tube atomizer (GTA-95) and an autosampler (Varian Techtron Pty., Ltd. Milgrave, Victoria, Australia). The instrument parameters (lamp, lamp current, spectral bandwidth, wavelength) were those recommended by the manufacturer (8). The furnace operating conditions included three drying steps (75, 95, 140°C for 2, 15, and 10 sec, respectively), 5 ashing steps (300, 450, 480, 600, and 600°C for 8, 15, 15, 10, and 2 sec, respectively), and 3 atomization steps (all at 2600°C for 1.1, 1.1, and 2 sec). Nitrogen gas was used for the analysis except during the first three ashing steps where air was employed. A background correction with potassium dihydrogen sulfate solution (1%, W/V) as a chemical modifier were used for this analysis.

A solution (2%, V/V) of Triton X-100 (Serva Feinblochemica, Heidelberg, New York) was used for dilution of blood samples prior to injection into the furnace. Dependent upon the expected concentration of Sb, the dilution ratio was either 1:20 (i.e., 0.05 ml blood with 1 ml Triton) for samples collected within the first 12 hours of drug administration or 1:2 (i.e., 0.2 ml blood with 0.4 ml Triton) for the remaining samples. After the mixture was vortex-mixed for 30 seconds and centrifuged at 1,000 g for 2 minutes, 0.5 ml of the clear solution was transferred into an atomic absorption plastic sample cup. The autosampler was programmed to inject 5 μl of the diluted sample and 3 μl of the chemical modifier.

The concentration of Sb in the blood or urine samples collected from each patient was calculated by dividing the absorbance value obtained for the sample by the slope of the absorbance *versus* concentration standard curve prepared daily in whole blood or urine under conditions identical to those used for the patient samples. The standard curve included a blank blood or urine sample to which no Sb was added. All analyses were performed in at least quadruplicate runs.

The analysis was validated daily by examining the linearity and precision of the assay at the beginning and end of the day of analysis. The absorbance was linearly related to concentration [correlation coefficient (r) > 0.9927, (blood) and r > 0.9953 (urine)], and the coefficient of variation (CV) was consistently < 9.6% at different concentrations of Sb.

Pharmacokinetics Calculations

The concentration-time data obtained for each patient were analyzed according to the one-compartment open model with a first-order absorption phase (9) using a nonlinear least-square regression analysis [STATGRAPHICS Statistical Graphic System package, Statistical Graphics Co., Rockville, Maryland], and the apparent first-order rate constants for absorption (k_a) and elimination (k_d) were computed.

The total body clearance (TBC) of Sb was calculated by dividing the dose (D) by the area-under-the-curve (AUC) assuming a complete absorption (9). This parameter was estimated by adding the area-under-the-curve up to the last sample collected (i.e. 24 hr) (AUC_f) to the area-under-the-tail (AUC_{tail}) which was calculated by dividing the concentration of the last sample collected by k_d. The volume of distribution of Sb was computed by dividing TBC by k_d.

The fraction of the dose of Sb excreted in urine (f_u) was calculated according to the following equation assuming a complete absorption:

$$f_u = A_u/D$$

where A_u is the amount of Sb excreted in urine in 24 hours. The renal clearance of Sb (RC) was computed as follows:

$$RC = f_u . TBC$$

Statistical Evaluation

The data generated were subjected to appropriate statistical analyses using the STATGRAPHICS Statistical Graphic System package (Statistical Graphics Co., Rockville, Maryland), and various statistical parameters were computed.

RESULTS

Table I summarizes the clinical characteristics of the patients included in this study. The mean age of these 29 male, Arab (i.e., 22 Egyptians, 5 Sudanese, and 1 Syrian) farm workers was 32.3 years and the mean duration of the disease was 8.3 weeks.

A representative plot of the blood concentration of antimony as function of time obtained in one of the patients is presented in Fig. 1. As demonstrated in this model-predicted curve, the data conformed well to the one-compartment open model with first-order absorption; the observed *minus* predicted residuals were small and randomly distributed with r^2 ranging between 0.9159 and 0.9968 and mean = 0.9643. The mean and standard error of the mean (SEM) of the rate constants for absorption (k_a) and elimination (k_d) generated from this fit are presented in Table II along with other pharmacokinetic parameters.

As shown in Table II, the mean (SEM) values of the absorption ($t_{1/2a}$) and elimination ($t_{1/2}$) half-lives of Sb in these patients were 0.479 (0.035) and 1.85 (0.07) hr, respectively, and the maximum blood concentration [(i.e., C_{max} = 8.77 (0.39) mg/L] was reached at 1.34 (0.09) hr. The model-independent parameters, area-under-the-curve (AUC) and total body clearance (TBC), were 37.01 (1.57) mg.hr/L and 17.67 (1.38) L/hr, respectively, and the volume of distribution (V_d) was 45.68 (2.62) L. A frequency distribution histogram of the area-under-the-curve (Fig. 2) is apparently bimodal and separates the patients into rapid (AUC = 11.7-

Table I. Summary of the Clinical Characteristics of the Patients

Parameter	Age (year)	Sex (M/F)	Number of lesions	Duration of lesions (weeks)
Mean	32.3	29/0	10	8.3
SD	7.3		12	7.5
Range	24–56		1–57	1–40

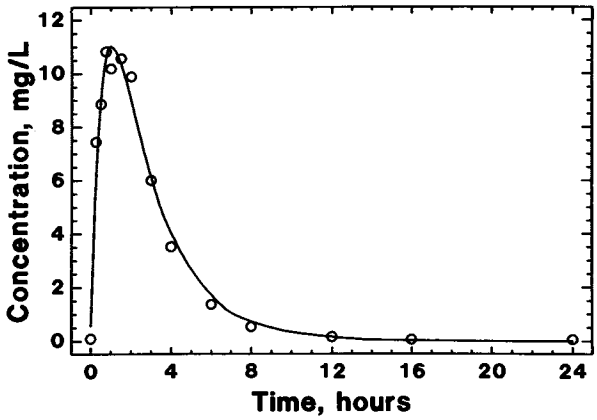

Figure 1. Representative fit of antimony blood concentration-time data to the one-compartment open model with first-order absorption following intramuscular injection of a dose of sodium stibogluconate equivalent to 600 mg of antimony in one of the patients. Key: actual data (○); model-predicted (——).

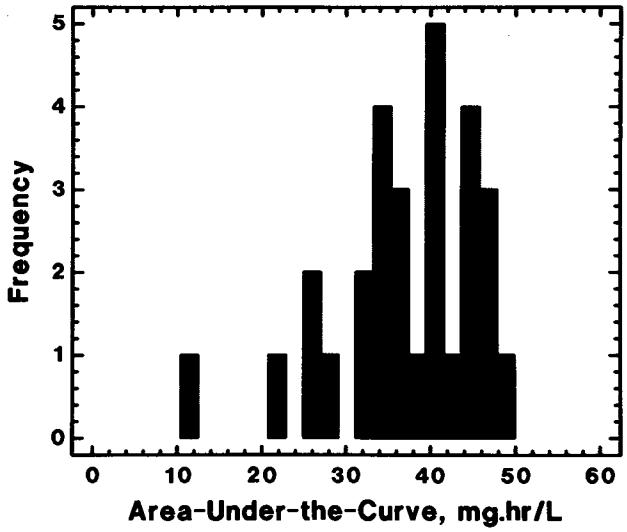

Figure 2. A frequency distribution histogram of the area-under-the-curve of blood concentration of antimony *versus* time in patients treated with sodium stibogluconate for cutaneous leishmaniasis.

29.0 mg.hr/L) and slow eliminators (AUC = 31.5-49.1 mg.hr/L) of Sb. Indeed, there was a highly significant difference ($p \leq 6.7 \times 10^{-7}$ by unpaired t-test and $p \leq 9.6 \times 10^{-6}$ by Wilcox rank test) in AUC between the rapid [mean (SEM) = 22.83 (3.42) mg.hr/L] and slow [mean (SEM) = 39.96 (1.07) mg.hr/L] eliminators. The fraction of dose excreted in urine (f_u) was 0.8 (0.07) and the renal clearance (RC) was 12.67 (1.16) L/hr.

DISCUSSION

In contrast to earlier reports with sodium stibogluconate where the three-compartment (10) or two-compartment (11) model was used to describe the blood concentration of Sb as function of time, the data generated in this study which is by far the largest to date (i.e. 29 patients), fitted the one-compartment model quite well (Fig. 1) with $r^2 > 0.91$. It is noteworthy that the absorption half-life values reported by Pamplin *et al.* (10) (i.e., mean ± SD = 0.39 ± 0.9 hr, n = 10 patients) and by Chulay *et al.* (11) (i.e., mean ± SD = 0.76 ± 0.13 hr, n = 2 patients) are similar to our value for this model-dependent parameter (i.e., mean ± SD = 0.48 ± 0.19 hr, n = 29 patients). Further, the peak concentration of Sb and the peak time as reported by Chulay *et al.* (i.e., 9.35 mg/L and 2 hr, respectively) are in agreement with our values for these parameters (i.e., 8.77 ± 2.1 mg/L and 1.336 ± 0.47 hr, respectively). Also, the fraction of Sb dose recovered by Rees *et al.* (12) in urine of patients with visceral leishmaniasis within 24 hr after sodium stibogluconate administration (i.e., 40% - 120%) and the renal clearance (i.e., 7.2 L/hr) coincide with our results (i.e., 80 ± 32% and 12.66 ± 5.17 L/hr, respectively). Unfortunately, the area-under-the-curve, total body clearance, and volume of distribution of Sb following sodium stibogluconate administration have never been previously determined, thus, making our values the first to be reported for these parameters. It should be noted that based on the value obtained for the volume of distribution (i.e. 45.7 L), antimony appears to be distributed in the total body water.

Table II. Pharmacokinetic Parameters of Antimony in Patients with Leishmaniasis Treated with Intramuscular Injection of a Dose of Sodium Stibogluconate Equivalent to 600 mg of Antimony

Parameter	Mean	SEM	Range
k_a (hr^{-1})	1.711	0.149	0.729–4.691
$t_{1/2a}$ (hr)	0.479	0.035	0.148–0.874
k_d (hr^{-1})	0.391	0.016	0.248–0.622
$t_{1/2}$ (hr)	1.854	0.072	1.114–2.792
t_{max} (hr)	1.336	0.087	0.500–2.000
C_{max} (mg/L)	8.771	0.390	4.510–12.50
AUC (mg · hr/L)	37.01	1.572	11.719–49.09
TBC (L/hr)	17.67	1.377	12.224–51.20
V_d (L)	45.68	2.62	30.50–86.79
f_u	0.804	0.072	0.181–1.41
RC (L/hr)	12.67	1.157	3.438–21.04

k_a = first-order rate constant for absorption, $t_{1/2a}$ = half-life of absorption, k_d = first-order rate constant for elimination, $t_{1/2}$ = half-life of elimination, t_{max} = peak time, C_{max} = maximum concentration, AUC = area-under-the-curve, TBC = total body clearance, V_d = volume of distribution, f_u = fraction of antimony excreted in the urine, RC = renal clearance.

The large variabilities in the patients response to treatment of leishmaniasis with sodium stibogluconate may be ascribed to various factors including the ability of Sb to reach the infected sites at a sufficiently high concentration (13,14). This indeed depends primarily upon the pharmacokinetic characteristics of Sb in the patient. In a study of the pharmacokinetics of sodium stibogluconate in hamsters, Berman *et al.* (15) suggested that for eradicating the parasites *in vivo*, the peak concentration of Sb is more important than the area-under-the-curve (AUC). However, this may not be the case since a short exposure of the parasites to a high concentration of Sb followed by a sharp decline (i.e. smaller AUC) would not ensure abating the parasites to the same extent as if a sufficiently high concentration is maintained for a reasonable length of time (i.e., larger AUC). The frequency distribution pattern of the AUC separates the patients into those with high exposure to Sb (AUC > 31.5 mg

hr/L) who may also be called slow eliminators (TBC < 19.05 L/hr) and those with low exposure to Sb (AUC < 29.04 mg.hr/L) who may also be called rapid eliminators (TBC > 20.7 L/hr). The saparation of the patients into slow and rapid eliminators of Sb is supported by the highly significant difference in AUC between these two groups. This may explain the difference in response to treatment with sodium stibogluconate frequently observed among patients with cutaneous leishmaniasis.

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