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Skin Uptake, Distribution, and Elimination of Antimony following Administration of Sodium Stibogluconate to Patients with Cutaneous Leishmaniasis

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We examined in this study the pharmacokinetics of Sb in the affected skin and normal skin of patients treated with sodium stibogluconate for cutaneous leishmaniasis and compared the results with those for the blood. The procedure was fully explained, and a written consent was obtained from each of nine patients. After a dose of sodium stibogluconate equivalent to 600 mg of Sb was administered intramuscularly, small skin biopsies were collected under local anesthesia at different time intervals from the circumferences of the lesions and simultaneously from normal skin. Antimony was measured in these biopsies after suitable ashing and processing by flameless atomic absorption spectrophotometry. The means (with standard errors of the means in parentheses) of the peak concentration, time to peak concentration, area under the curve, half-life, and mean residence time in lesions were 5.02 (1.43) μg/g, 2.1 (0.4) h, 32.8 (6.1) μg · h/g, 6.88 (0.54) h, and 10.4 (1.2) h, respectively, and those in normal skin were 6.56 (2.01) μg/g, 2.6 (0.8) h, 44.0 (15.8) μg · h/g, 5.44 (0.83) h, and 8.08 (1.34) h, respectively. There was no significant difference in any of these parameters between lesions and normal skin, whereas the differences in peak concentration, half-life, and mean residence time between lesions and whole blood were significant ($P \leq 0.05$). The penetration of Sb into skin, either affected or normal, as measured by the skin/blood area under the curve ratio appears to be complete, but the disposition is slow compared with that from the blood.

The best treatment for leishmaniasis is currently achieved with pentavalent antimonials in the form of sodium stibogluconate (SSG) or meglumine antimonate. However, because of the rapid blood clearance of these drugs (5, 13, 14, 17), an increase in dose and dosing frequency has been recommended by the World Health Organization (18). The amount of exposure of the infectious parasite to pentavalent antimony is believed to be an important factor in eradicating the cutaneous leishmania disease. Thus, delivering the dose by intralesion injections of SSG has recently been advocated (8, 10, 15). When the drug is administered intramuscularly, the contact with the parasite is undoubtedly controlled by the rate and extent at which this metal reaches and leaves the lesions following the administration of SSG. Therefore, knowledge of the kinetics of the uptake and disposition of this metal in affected skin is vital for optimization of the dosage regimens of these drugs in the treatment of cutaneous leishmaniasis. Although these drugs have been in use for more than four decades (11, 16), no such study has yet been reported for SSG. Plasma kinetic data are clinically relevant to the extent that they reflect those of the site of action.

We undertook to examine in this study the kinetics of Sb in the affected skin and normal skin of patients treated intramuscularly with sodium stibogluconate for cutaneous leishmaniasis and to compare the results with those for the blood samples of these patients. The impact of the leishmania infection on the kinetics of antimony in skin was also addressed.

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MATERIALS AND METHODS

Patient selection. Nine patients from a group of 29 with cutaneous leishmaniasis who participated in a larger study on the pharmacokinetics of Sb in whole blood (1) volunteered to take part in this investigation. The procedure was fully and individually explained to the patients, and a written consent was obtained from each. Also, they were given the choice of withdrawing at any given point during the study. The patients were all seen at the Dermatology Clinic, King Khaled Hospital, in Al-Kharj, near Riyadh, in the central region of Saudi Arabia. The diagnosis of cutaneous leishmaniasis was based on clinical and parasitological examinations. Table 1 summarizes the clinical characteristics of these patients. Prior to the study, the patients who weighed 60 to 75 kg were examined in the clinic, and several laboratory tests for the liver, kidney, heart, complete blood profile, hepatitides B and C, and AIDS, etc., were also performed. Except for the skin disease, all patients were healthy.

Drug administration. A dose of sodium stibogluconate (Pentostam [10 mg of Sb per ml injected]; Welcome Foundation Ltd., Berkhamstead, England) equivalent to 600 mg of pentavalent antimony was administered intramuscularly to the patients who were admitted into the hospital 24 h earlier. This dose was repeated for 10 consecutive days.

Specimen collection. Skin biopsies were collected under local anesthesia at different times (0, 0.5, 1, 2, 4, 8, 12, and 24 h) from the circumferences of the lesions and simultaneously from normal skin by a qualified dermatologist with the assistance of two nurses using a 4-mm skin biopsy punch. The local anesthesia was induced by injecting 0.5 ml of a lidocaine hydrochloride solution (2%) (Abbott Laboratories, North Chicago, Ill.) into the sampling site 1 min before the biopsy was excised. One of the patients (patient 9) elected to limit the sampling to the affected skin only. The sampling sites were chosen so as to avoid sensitive areas of the skin. They were washed with soap and water, and hair was shaved. The samples were first immersed in liquid nitrogen and placed in small biopsy vials that were properly labeled. These vials were then put in a small portable liquid nitrogen container and later transferred to the laboratory in King Faisal Specialist Hospital and Research Centre at the end of the 24-h sampling period.

Analysis of antimony in skin. Each of the biopsy samples collected was accurately weighed after being thawed and was digested in a crucible with 3 ml of a mixture of concentrated nitric and sulfuric acids (1:1, vol/vol) by heating for 3 h. The residue was ashed in an ashing oven for 6 h, and the white ash was dissolved in 0.5 ml of a 1% HCl solution by sonication for 2 min. The solution was then

TABLE 1. Clinical characteristics of patients[a]

Patient	Age (yr) [29.4 ± 2.1]	Lesions	
		No. [12.9 ± 5.6]	Duration (wk) [5.3 ± 0.9]
1	30	4	4
2	42	4	6
3	36	10	4
4	24	7	4
5	24	12	4
6	25	4	4
7	25	57	10
8	27	13	10
9	32	5	2

[a] All are male. Means ± SEMs are given in brackets.

transferred to small plastic cups, and antimony was measured by electrothermal atomic absorption spectrophotometry.

We used a spectrophotometer (model 975) equipped with a graphite tube atomizer (GTA-95) and an autosampler (Varian Techtron Pty., Ltd., Milgrave, Australia) for the analysis. The lamp, lamp current, spectral bandwidth, and wavelength employed were those recommended by the manufacturer (12), and the operating conditions of the furnace were presented earlier (1). A potassium dihydrogen sulfate solution (1%, wt/vol) was used as a chemical modifier, and the autosampler was programmed to inject 5 μl of the diluted sample and 3 μl of the modifier. All analyses were performed in at least quadruplicate runs with a background correction.

We calculated the concentration of Sb in the biopsy samples collected from the patients by dividing the absorbance value obtained for the sample by the slope of the absorbance-versus-concentration standard curve prepared daily by using intact normal skin (from amputated limbs) under conditions identical to those used for the patient samples. A normal skin sample to which no Sb was added was also prepared to serve as a blank.

To validate the analysis, the linearity and precision were examined at the beginning and end of each day of analysis. There was a highly linear ($r > 0.9947$) relationship between the absorbance and concentration of antimony in skin, and the coefficients of variation at 0.3, 1.8, and 5 μg of Sb/g of skin were 14.8, 4.95, and 2.77%, respectively. The minimum quantitation limit of the assay is 0.02 μg/g.

Calculation of pharmacokinetic parameters. The concentration-time data generated for each patient were subjected to compartmental independent analysis by using the Model-PK personal computer package (McPherson Scientific, Rosanna, Australia), and various pharmacokinetic parameters (maximum concentration [C_{max}], time to maximum concentration [T_{max}], area under the curve from 0 to infinity [AUC], disposition half-life [$t_{1/2}$], and mean residence time [MRT] in skin) were determined.

Statistical analysis. We analyzed the data obtained according to parametric and nonparametric tests by using the STATGRAPHICS statistical graphic system package (Statistical Graphics Co., Rockville, Md.) and considered the difference significant at $P \leq 0.05$. The specific test used is indicated where appropriate in the text.

RESULTS

Nine male Arab farm workers were included in this study. As demonstrated in Table 1, the mean age of these patients was 29.4 years (standard error of the mean [SEM], 2.1), the mean number of lesions was 12.9 (SEM, 5.6), and the mean duration of these lesions was 5.3 weeks (SEM, 0.9).

Figure 1 depicts a plot of the mean concentration of antimony in leishmania lesions, normal skin, and whole blood of the patients as a function of time. As shown in this figure, for the first 4 h after the administration of SSG, the mean concentrations in lesions were smaller than those in normal skin or whole blood, where antimony exhibited the highest level. However, because of the more rapid declines of the concentrations in blood and normal skin after 4 h, the difference in AUC between the lesions (32.8 μg · h/g [SEM, 6.1]) and normal skin (44 μg · h/g [SEM, 15.8]) or blood (37.9 μg · h/ml [SEM, 2.5]) was not significant according to matched-pair *t*-test statistics or Wilcox sign or rank tests.

The various model independent parameters obtained for antimony in affected skin and normal skin in each of the patients are presented in Tables 2 and 3, respectively. Table 2 also presents the values obtained previously (1) for the volume of distribution in and total clearance of Sb from blood for each of these patients. As shown in these tables, the mean C_{max}, T_{max}, $t_{1/2}$, and MRT in lesions were 5.02 μg/g (SEM, 1.44), 2.1 h (SEM, 0.4), 6.88 h (SEM, (0.54), and 10.4 h (SEM, 1.2), respectively, and those in normal skin were 6.56 μg/g (SEM, 2.01), 2.6 h (SEM, 0.8), 5.44 h (SEM, 0.83), and 8.08 h (SEM, 1.34), respectively. There was no significant difference in any of

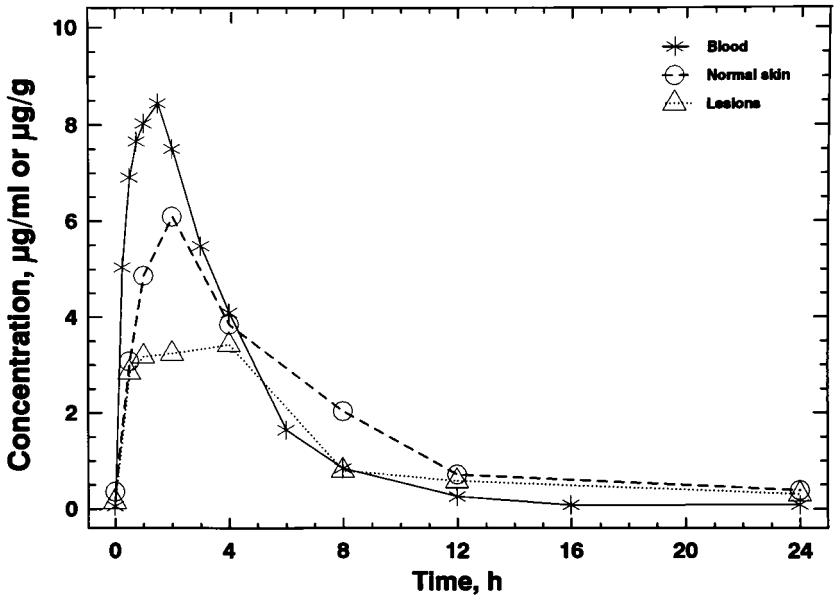

FIG. 1. Profiles of the mean antimony concentrations in leishmania lesions, normal skin, and whole blood as functions of time in nine patients following intramuscular injection of a dose of sodium stibogluconate equivalent to 600 mg of antimony.

TABLE 2. Pharmacokinetic parameters of antimony in skin leishmaniasis lesions of patients treated intramuscularly with sodium stibogluconate[a]

Patient	T_{max} (h) [2.1 ± 0.4]	C_{max} (μg/g) [5.02 ± 1.44]	$t_{1/2}$ (h) [6.88 ± 0.54]	AUC (μg · h/g) [32.8 ± 6.1]	MRT (h) [10.4 ± 1.2]	CL[b] (liters/h) [16.4 ± 1.2]	V[c] (liters) [73.4 ± 8.1]
1	2.0	7.13	6.68	47.5	9.70	13.5	48.6
2	1.0	2.62	6.46	19.5	9.53	12.2	51.2
3	1.0	3.06	5.72	26.7	8.29	17.7	81.2
4	2.0	10.30	6.92	58.3	8.92	14.8	55.9
5	4.0	0.37	8.67	2.7	12.87	22.9	103.3
6	0.5	2.49	8.34	26.2	11.65	13.7	46.7
7	8.0	3.85	6.12	41.1	8.45	20.5	102.9
8	2.0	2.08	9.10	19.7	18.16	16.6	101.7
9	4.0	13.30	3.93	53.1	5.56	16.1	69.5

[a] Means ± SEMs are given in brackets.
[b] CL, clearance from blood.
[c] V, volume of distribution in blood.

these parameters between lesions and normal skin according to matched-pair t-test statistics or Wilcox sign or rank tests.

The $t_{1/2}$ (3.04 h [SEM, 0.19]) of disposition of antimony from blood was significantly shorter than that of disposition from the lesions ($P \leq 0.000091$) or from normal skin ($P \leq 0.0368$). Similarly, MRT in blood (4.08 h [SEM, 0.37]) was significantly smaller than those in affected skin and normal skin ($P \leq 0.00022$ and ≤ 0.0317, respectively). However, the difference in C_{max} was significant ($P \leq 0.0356$) only when that in blood (8.81 μg/ml [SEM, 0.77]) was compared with that in affected skin. The penetration of antimony into the skin was evaluated from the AUC_{skin}/AUC_{blood} ratio for each patient; there was no significant difference in this ratio between the affected lesions (mean, 0.86 [SEM, 0.16]) and normal skin (mean, 1.13 [SEM, 0.39]) according to the matched-pair t test or Wilcox sign or rank test.

DISCUSSION

Because of infiltration of the skin by the leishmania parasites and the resulting damage to both tissue and blood vessels, it was of importance to examine the impact of this infection on the pharmacokinetics of antimony in skin. The lack of significant difference in any of the pharmacokinetic parameters examined, including the AUC_{skin}/AUC_{blood} ratio between the affected skin and normal skin, suggests the absence of such an effect on either the pharmacokinetics or the penetration of Sb into the skin. This is in agreement with a previous report (2) indicating that there was no significant difference in liver antimony concentration between infected and noninfected hamsters following the administration of 10 mg of Sb/kg of body weight intramuscularly.

TABLE 3. Pharmacokinetic parameters of antimony in normal skin of patients treated intramuscularly with sodium stibogluconate for cutaneous leishmaniasis

Patient	T_{max} (h) [2.6 ± 0.8]	C_{max} (μg/g) [6.56 ± 2.01]	$t_{1/2}$ (h) [5.44 ± 0.83]	AUC (μg · h/g) [44.0 ± 15.8]	MRT (h) [8.08 ± 1.34]
1	2.0	6.60	7.08	37.5	9.82
2	2.0	6.41	5.00	40.0	6.62
3	8.0	1.40	9.92	18.5	16.27
4	2.0	16.28	6.22	145.3	9.00
5	2.0	0.92	2.39	3.9	4.16
6	2.0	2.00	4.81	14.0	6.76
7	1.0	5.17	4.90	30.3	7.14
8	2.0	13.66	3.18	62.2	4.83

[a] Means ± SEMs are given in brackets.

Several studies have examined the uptake and distribution of antimony in various body tissues (e.g., liver, spleen, bone marrow, etc.) following the administration of a pentavalent antimonial drug such as SSG or meglumine antimonate in animal models (2, 4, 6, 9, 17), but only one looked at the concentrations of this metal in skin in addition to other tissues and serum (2). Thus, our study is the first to examine the pharmacokinetics of Sb in skin biopsies of patients treated with SSG for cutaneous leishmaniasis. The penetration of antimony into the skin as measured by the AUC_{skin}/AUC_{blood} ratio for both affected lesions (mean, 0.86 [SEM, 0.16]) and normal skin (mean, 1.13 [SEM, 0.39]) appears to be complete and rapid (short T_{max}), though less rapid than that in blood. Also, the data indicate that antimony is retained by the skin longer than in the blood (significantly longer $t_{1/2}$ and MRT). Burguera et al. (3) and Dorea et al. (7) also reported long retention of this metal by the skin, although their finding was based on a single biopsy concentration after treatment of patients with meglumine antimonate for cutaneous leishmaniasis.

The interpatient variability (SEM) of the data obtained from skin is much larger than the variability of the data generated from the blood; however, if one is to exclude the data generated from patient 5 (Table 2), for whom the concentrations in the affected lesions for unknown reasons were exceedingly small, the values of SEM become reasonable. Nevertheless, the variability observed in this study is by far smaller than those observed by Burguera et al. (3) and Dorea et al. (7). This may be attributed to the narrower ranges of age (25 to 42 years) and weight (60 to 75 kg) of our patients (all male) than those included in the previous studies (three males and two females 9 to 25 years old [3] and 10 to 71 years old, weighing 21 to 70 kg [7]).

As mentioned earlier, the mean concentration of Sb in skin was smaller than that in blood for the first 4 h following SSG administration but became larger thereafter. This is in perfect concurrence with the profiles reported by Berman et al. (2) for Sb in skin following the administration of SSG in hamsters. Unfortunately, these animal data were not subjected to a further analysis, and no pharmacokinetic parameters were determined. Thus, our values for disposition $t_{1/2}$, MRT, and AUC, etc., in skin are the first to be reported for humans or animal models.

In conclusion, we present in this report much needed data on the pharmacokinetics of antimony in the skin of patients with cutaneous leishmaniasis. The penetration of this metal

into the skin, either affected or normal, appears to be complete and quite rapid, but the disposition is slow compared with that from the blood. The leishmania infection apparently has no impact on the pharmacokinetics or penetration of antimony into skin.

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