High Frequency of Skin Reactions in Patients with Leishmaniasis Treated with Meglumine Antimoniate Contaminated with Heavy Metals. A Comparative Approach Using Historical Controls

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We analyzed data from historical controls treated with meglumine antimoniate to compare the frequency of adverse events observed in patients with cutaneous leishmaniasis treated with the same dose of meglumine antimoniate contaminated with heavy metals in an endemic area of the State of Bahia, Brazil. Group A patients were treated in 2000 with the drug produced by Eurofarma Laboratórios Ltda., São Paulo, Brazil (lot A) and group B patients were treated in 1996 with the reference drug produced by Rhodia Farma Ltda., São Paulo, Brazil (lot B). We observed an unusual higher frequency of skin reactions in group A patients. However, all type of adverse events observed in group A were also observed in group B. The physico-chemical analysis of these lots revealed that lot A had lower pH and higher concentration of total and trivalent antimony, lead, cadmium, and arsenic. Our findings suggest that the skin reactions could be attributed to heavy metal contamination of lot A.

Key words: pentavalent antimony - heavy metals - cutaneous leishmaniasis - lead - arsenic - toxicity

Pentavalent antimonials had been, for many years, the first-choice drugs for the treatment of leishmaniasis (Marsden 1985, Herwaldt & Berman 1992, Berman 1997). Two salts are commercially available, meglumine antimoniate and sodium stibogluconate. They are used by the IM or IV route. In Brazil, patients with cutaneous leishmaniasis are treated with meglumine antimoniate, 10-20 mg/kg/day IM or IV, for 20-30 days following the recommendations of the Brazilian Ministry of Health. The drug usually causes mild to moderate adverse events that rarely lead to treatment suspension. Myalgias, arthralgias, abdominal symptoms, headache, elevation of aminotransferases and amylase, and electrocardiographic changes affecting the ST segment and QTc are the most frequent events (Marsden 1985, Ballow et al. 1987, Franke et al. 1990, Gasser et al. 1994, Berman 1997). Rarely, severe toxicity is observed such as acute renal and hepatic failure (Kopke et al. 1993), thrombocytopenia (Hepburn 1993) and even sudden death, probably due to cardiac rhythm disturbances (Chulay et al. 1985). The presence of trivalent antimony contaminating pentavalent antimonials for clinical use was demonstrated and could explain the toxic events observed during treatment (Franco et al. 1995). Pharmacokinetics of both drugs is comparable and it is assumed that they produce similar therapeutic effects (Chulay et al. 1988). Toxicity caused by antimonials could be different for each drug and formal comparisons of sodium stibogluconate and meglumine antimoniate are scarce. We recently compared the efficacy, safety and toxicity of sodium stibogluconate produced in China (sodium stibogluconate BP88® Shandong, Xinhua, China) with meglumine antimoniate (Rhodia Farma, Ltda., São Paulo, Brazil) and our conclusions suggest that sodium stibogluconate was more toxic than the drug produced by Rhodia Farma Ltda. (Saldanha et al. 1999, 2000). There are no definite criteria to evaluate the quality of antimonials. High osmolarity could be a marker of higher toxicity as shown by one report from India (Sundar et al. 1998). We had measured the osmolarity and pH of different lots of meglumine antimoniate and that physico-chemical properties appear to be stable at least during a three-year period (Romero et al. 1996). The presence of metals such as lead has been reported as a cause of poisoning in patients treated with traditional Chinese remedies (Wu et al. 1996) but there is little information about this kind of contamination in pharmaceutical products (Popinska et al. 1999). Flores et al. (2000) drew attention to the fact that routine quality control of drugs with the appropriate methods to detect heavy metals has been traditionally neglected. Contamination of antimonials with heavy metals such as lead, arsenic and cadmium is not expected unless poor quality control of manufacturing process allows the use of impure salts. We use historical controls (patients with cutaneous leishmaniasis) treated with the unique lot of the reference drug, meglumine antimoniate (Rhodia Farma Ltda., São Paulo, Brazil) in 1996 to compare the frequency of unusual adverse events due to meglumine antimoniate (Eurofarma Laboratórios Ltda., São Paulo, Brazil) used in patients with similar conditions in 2000. We compared the physico-chemical properties of both drugs raising the hypothesis of toxicity attributed to heavy metal contamination.

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PATIENTS AND METHODS

Patients - Nineteen patients (Group A) with localized cutaneous leishmaniasis (LCL) were treated with meglumine antimoniate (Eurofarma Laboratorios Ltda., São Paulo, Brazil), 20 mg/kg/day I. V. for 20 days (Lot A). They were included in a therapeutic protocol aiming the identification of prognostic factors associated with failure to cure from August through December 2000. Lot A was produced for the Brazilian Ministry of Health and correspond to a unique lot No. 011/00 A, valid through May 2002. Patients were provided with disposable syringes and instructed to return every 10 days during treatment and monthly thereafter for a three months period to diagnose failure or cure. Data from group A was compared with data from 54 historical controls (Group B) treated and followed in a similar manner by the same researcher in 1996 with a unique lot of meglumine antimoniate, No. 0595 L061 (Rhodia Farma Ltda, São Paulo, Brazil) (Lot B) in a protocol designed to compare the characteristics of two species of the *Viannia* subgenus (Romero et al. 2001a, b). Lot B was valid through May 2000. Both groups attended the basic health unit of Corte de Pedra District, Presidente Tancredo Neves municipality, State of Bahia, Brazil, located in an endemic area of *Leishmania (Viannia) braziliensis* (França et al. 1991). All patients had positive Montenegro skin test performed as recommended by the World Health Organization (WHO 1984) using antigen prepared as described by Reed et al. (1986). All cases had confirmed parasitological diagnosis using the isolation and visualization methods previously described (Romero et al. 2001a). Both research protocols were done in agreement with the Helsinki Declaration and the Resolution 196/96 of the National Health Council of the Ministry of Health of Brazil, which regulates research in humans. The Ethics Research Committee of the University of Brasília approved the protocols. All patients signed informed consents to diagnostic tests and were treated in accordance to the recommendations of the Brazilian Ministry of Health. When the protocol was interrupted because the unusual frequency of adverse events all patients were informed of the potential causes of the phenomenon and instructed to stop medication and report any symptom related to the treatment.

Chemical analysis - All measurements were carried out at the same time in 2001 with an atomic absorption spectrometer Model 3030 B (Perkin Elmer, Germany) equipped with a continuum source background correction system. Equipment was coupled to an electrothermal atomizer (longitudinally heated graphite tube, Model HGA-400, Perkin Elmer) or to a batch hydride generation system (MHS-10, Perkin Elmer). Antimony determinations were performed after water dilution of samples using an air/acetylene flame (217.6 nm) and pneumatic nebulization. Determinations of arsenic (193.7 nm), cadmium (228.8 nm) and lead (283.3 nm) were performed by electrothermal atomization using a graphite tube with a specific heating programme (drying, pyrolysis and atomization) for each element. Argon (99.996%, White-Martins, Brazil) was used as protection gas. Integrated absorbance was used instead of peak height for measurements.

Determination of antimony on the trivalent state in the samples was performed by hydride generation atomic absorption spectrometry using a flame heated quartz cell as atomizer. Samples were diluted with water using 4% (m/v) tartaric acid solution as selective medium for trivalent antimony determination. Reductant was a daily prepared 1%, m/v, sodium tetrahydroborate solution. Reference trivalent antimony solutions were daily prepared and absorbance signals were completely recorded in 15 sec. Argon was used as purge gas and measurements were made in integrated absorbance mode.

All chemicals used were of analytical reagent grade from Merck (Darmstadt, Germany). Distilled and double-deionized water (maximum conductivity of 1.2 μS/cm) was used to prepare all solutions. All glass apparatus were soaked in 0.72 mol/l nitric acid, and thoroughly washed with water before use. Working reference solutions were prepared immediately before use by serial dilution from a stock solution containing 1,000 mg/l for each element. All determinations were made in triplicate from five test samples for each individual lot. pH was measured with a digital apparatus (Model 10, CELM, Brazil) in 10 ampoules, one measure for each ampoule. The final mean for each lot corresponds to the mean of 10 ampoules.

Osmolarity - This property was measured with a digital Fiske Osmometer (Fiske Associates, Norwood, MA, USA) in 10 ampoules of each lot, obtaining three independent measures for each ampoule for a mean estimate. The mean for each lot was obtained using the mean values of the ten ampoules.

Statistical analysis - The comparisons of the frequency of the observed adverse events were performed with the chi-square test or Fisher exact test and the medians were compared with the Mann-Whitney-U test using the Statistical Package for Social Science, version 9.0, 95% confidence intervals were calculated using the EPI Info, version 6.04.

RESULTS

Both groups were comparable by age, sex and total body weight. Most patients were agricultural workers. Table I shows the characteristics of the disease and the results of the Montenegro skin test observed in both groups. Table II shows the frequency of adverse events observed in each group. Although the absolute proportion of patients who experiences at least one adverse event was similar between groups, the quality of the observed events was different mainly due to the unusual higher frequency of skin reactions in group A patients. Arthralgias were more frequently observed in group B. The episodes of skin reactions were characterized by the onset of erithematous and itching plaques at the site of the intravenous injections in the forearms and the appearance of generalized morbiliform rash during a secondary stage. Symptoms disappeared 3 to 14 days after the interruption of the medication. Some patients needed antihistaminic medication to improve itching. All patients with skin reactions in group A were instructed to stop treatment. The patient in group B who experienced a skin reaction had a generalized rash without any plaques on the 19th day of treatment and consulted on the 20th day

after the administration of the last prescribed dose and her symptoms relief during the next five days. Table III shows the physico-chemical characteristics in the samples of the lots used by each group. Lot A was characterized by lower pH and osmolarity and higher concentrations of total and trivalent antimony, lead, cadmium and arsenic. The total concentration of antimony was 22% higher than the expected concentration of 85mg/ml indicated by the manufacturer.

TABLE I

Clinical findings observed at entry time in two groups of patients with cutaneous leishmaniasis treated with different lots of meglumine antimoniate in Brazil [a]

Clinical finding	Group A [b] n=19	Group B [c] n=54	Statistical significance [d]
Age-years (median)	21.5 (14.0-32)	22.0 (17.0-31.0)	0.505
Sex (male)	10/19 (52.6)	40/54 (74.1)	0.084
Number of lesions (median)	1 (1-2)	1 (1-2)	0.840
Total body weight-kg (median)	50.0 (45.0-59.0)	52.0 (42.8-60.3)	0.816
Total ulcerated area-cm^2 (median)	2.33 (1.22-5.42)	1.37 (1.02-2.86)	0.152
Disease duration-weeks (median)	4.0 (3.0-6.0)	4.0 (2.0-12.0)	0.458
Montenegro skin test-mm (median)	16.0 (14.0-20.2)	18.0 (14.0-2.0)	0.549

a: numbers in parenthesis are 25-75 quartiles for medians and % for proportions; *b*: group A received meglumine antimoniate, lot 011/00 A (Eurofarma Laboratórios, Ltda., São Paulo, Brazil); *c*: group B received meglumine antimoniate, lot 0595L061, (Rhodia Farma, São Paulo, Brazil); *d*: statistical significance corresponds to Mann-Whitney U test except for the proportion of male sex comparison that was performed using Chi square test

TABLE II

Comparison of adverse events observed in two groups of patients with cutaneous leishmaniasis treated with different lots of meglumine antimoniate in Brazil

Adverse event	Group A [a] n = 19 (%)	Group B [b] n = 54 (%)	Statistical significance [c]
Mylagias	5 (26.3)	13 (24.1)	1.000
Arthralgias	2 (10.5)	19 (35.2)	0.045
Anorexia	2 (10.5)	14 (25.9)	0.210
Headache	2 (10.5)	11 (20.4)	0.492
Fever	3 (15.8)	16 (29.6)	0.363
Rash	7 (36.8)	1 (1.9)	< 0.001
Fatigue	2 (10.5)	2 (3.7)	0.276
Stop work [d]	2 (10.5)	4 (7.4)	0.647
Any adverse event	14 (73.7)	36 (66.7)	0.571

a: group A received meglumine antimoniate, lot 011/00 A (Eurofarma Laboratórios, Ltda., São Paulo, Brazil); *b*: group B received meglumine antimoniate, lot 0595L061 (Rhodia Farma, São Paulo, Brazil); *c*: statistical significance corresponds to Fisher exact test except for comparisons of the categories: arthralgias and any adverse event that were performed with the Chi square test; *d*: proportion of individuals who stopped normal activities

TABLE III

Physico-chemical characteristics of two lots of meglumine antimoniate used for treatment of patients with cutaneous leishmaniasis in Brazil [a]

Characteristic	Lot A [b]	Lot B [c]
PH	4.5 (0.05) [4.48-4.56]	6.7 (0.05) [6.78-6.83]
Osmolarity (mosm/l)	708.9 (5.86) [704.7-713.1]	959.3 (22.44) [952.5-965.7]
Total antimony (Sb^{III} + Sb^{V}) (mg/ml)	103.9 (1.4)	88.1 (1.2)
Trivalent antimony (mg/ml)	3.45 (0.31)	1.82 (0.25)
Heavy metals		
Lead (mg/l)	52.71 (1.29)	< 0.20
Arsenic (mg/l)	35.79 (1.54)	< 0.90
Cadmium (mg/l)	0.132 (0.014)	< 0.04

a: numbers in parenthesis correspond to SD. Numbers in brackets correspond to 95% CI; *b*: meglumine antimoniate, lot 011/00 A (Eurofarma Laboratórios, Ltda., São Paulo, Brazil); *c*: meglumine antimoniate, lot 0595L061 (Rhodia Farma, Ltda., São Paulo, Brazil)

DISCUSSION

Our data showed that the frequency of skin reaction, an unusual adverse event, was higher in patients treated with the drug containing a high concentration of lead, cadmium and arsenic. Although historical controls had limitations for comparisons in our case the therapeutic protocols were identical and the same researcher was responsible for the adverse events monitoring and follow-up. Furthermore the chemical analysis was performed at the same time to avoid variations in the assay sensitivity. We storage samples of the lot B used to treat the historical controls avoiding exposure to light at room temperatures. We do not perform biochemical and electrocardiographic tests to identify other kind of effects such as cardiac, hepatic and pancreatic toxicity but it would be expected a higher level of cardiac abnormalities since patients received also a higher dose of total and trivalent antimony that have shown a dose dependent toxicity on the heart (Chulay et al. 1985). The type of the skin reactions observed in group A patients could be attributed initially to local sensitization with contaminant heavy metals with posterior generalized rash. Arsenic, lead and cadmium have been described as causes of contact allergy (Cavelier & Foussereau 1995). Pentavalent antimony could cause generalized rash but the phenomenon is rare even with prolonged courses of the maximum recommended dose (Franke et al. 1994). In our case it appears to be more plausible the hypothesis of reactions due to arsenic, lead or cadmium. The concentration of total and trivalent antimony was higher in lot A. The trivalent species is considered more toxic and more active than the pentavalent form but patients usually tolerate high antimony doses and rarely develop skin reactions.

Mean osmolarity levels in both lots was in the expected range of values based in our experience measur-

ing osmolarity of at least ten different lots of meglumine antimoniate. Lower pH could explain in part the local skin reactions at the injection site but do not explain the systemic toxicity manifested as generalized rash.

Our results together with the reported analysis of the reactions observed with other two lots of the drug produced by Eurofarma Laboratórios Ltda., São Paulo, Brazil confirming heavy metal contamination (Brazilian Ministry of Health 2001), indicates that the unusual frequency of cutaneous reactions observed in group A patients could be attributed to heavy metal contamination.

The use of drug salts from dubious origin could explain the contamination with heavy metals despite good manufacturing practices during the dilution process. Our data raise the urgent need to establish stringent criteria for quality control of pentavalent antimonials including the inspection of sources of chemical supplies. Recent report of successful treatment of visceral leishmaniasis in Kenya using a cheaper generic sodium stibogluconate produced following good manufacturing practices shows that the production of generic drugs of adequate quality could be an achievable goal (Moore et al. 2001). However the unexpected high frequency of rash episodes observed in both intervention groups in that research deserves more attention to identify the causes of that phenomenon. Pentavalent antimonial characteristics are the antithesis of the ideal drug profile for the treatment of any disease, the situation is the worst possible if we consider that leishmaniasis affects mainly poor people living in less developed countries where health services usually show precarious conditions. Until the development of cheaper oral medications with minor toxicity, the important next step should be the construction of a surveillance system for adverse events observed with pentavalent antimonials aiming the early detection of the more dangerous forms of toxicity to avoid potentially lethal outcomes.

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