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Antimony in plasma and skin of patients with cutaneous leishmaniasis – relationship with side effects after treatment with meglumine antimoniate

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Summary

OBJECTIVE To evaluate the levels of antimony in plasma and skin of patients being treated with pentavalent antimonials (Glucantime®) and their relationship with side effects.

METHODS We evaluated 19 patients treated endovenously at the conventional dose (20 mg Sb^v/kg/day), two at a smaller dose (5 mg Sb^v/kg/day) and three treated intralesionally (up to 4.0 ml/week). During treatment, patients underwent periodic blood exams and were interviewed weekly about the incidence of adverse symptoms. The levels of antimony in plasma and skin samples were determined by Inductively Coupled Plasma with Mass Spectrometry (ICP-MS).

RESULTS The patients under conventional treatment presented a mean initial antimony plasma concentration of 3.39 μg/l; at the end of treatment, these levels were 0.21 before Glucantime® application and 125.8 mg after Glucantime® application. The mean antimony level in their skin at the end of the treatment was 9.24 μg/g. The main adverse symptoms were arthralgia and myalgia; laboratory results showed mainly lymphocytosis and eosinophilia.

CONCLUSIONS We found some significant correlations between antimony concentrations, adverse symptoms and laboratory alterations, strengthening the hypothesis of a dose–dependent relationship between antimony concentration in plasma and skin and side effects.

keywords cutaneous leishmaniasis, side effects, antimony, inductively coupled plasma with mass spectrometry

Introduction

Leishmaniasis is a major public health issue, threatening around 350 million people in 88 countries (Reguera *et al.* 1998). The disease is caused by many species of the *Leishmania* parasite, and presents in cutaneous, mucosal or visceral forms. The seriousness of the disease can range from a single self-healing lesion to the most dangerous visceral form, also known as kala-azar, which has a mortality rate of up to 100% in developing countries, if left untreated (World Health Organization 2008). In Brazil, the incidence of the disease from 1990 to 2005 has been around 19.0 cases/100 thousand individuals, with about 90% of them of the cutaneous type (RIPSA 2009).

Although there are alternative treatments, pentavalent antimonials, introduced about 50 years ago, remain the drug of first choice for all forms of Leishmaniasis (Arana *et al.* 2001; Croft & Coombs 2003). The wide variety of side effects attributed to this drug are well described in the literature, ranging from simple adverse symptoms, such as headache and myalgia, to serious effects such as hepatitis, pancreatitis, renal failure and cardiopathy (Arana *et al.* 2001; Oliveira *et al.* 2005; Lawn *et al.* 2006; Mlika *et al.* 2008). Some studies have indicated that there is a relationship between the length of the treatment, the dose used and the incidence of side effects (Grevelink & Lerner 1996; Name *et al.* 2005). However, a statistical relationship between these parameters still needs to be shown.

In this work, we determined the levels of antimony in plasma and skin of patients being treated with Glucantime®, the pentavalent antimonial commercially available in Brazil, and evaluated the adverse symptoms and laboratory values alterations as a result of the treatment. The objective was to search for a possible dose–dependent relationship between the antimony concentrations in the body and side effects.

Materials and methods

Study population and treatments

Patients with confirmed diagnostics of cutaneous leishmaniasis, who were initiating the treatment with

Glucantime® in the University Hospital of Brasilia (UHB), in the Central West region of Brazil, were invited to participate in this study. They answered questions about their smoking habits, occupation, number and place of lesions and the existence of a previous treatment with pentavalent antimonials. UHB patients with cutaneous leishmaniasis routinely receive the conventional treatment (CT), which consists of daily endovenous injections of Glucantime®, at 20 mg Sb^v/kg bw/day, for 20 days. For this study, patients who were not eligible for the CT, such as those with Chagas disease, were included on low dose (LD) (5 mg Sb^v/kg bw/day, for 20 days) or intralesional treatments (IL) (weekly intralesional injections, at 1.0 ml/cm^2 of lesion, at a maximum of 4.0 ml, for 4 weeks). To enhance the chances of therapeutic success, patients were only included in these two last treatments when they had only one lesion measuring no more than 4 cm^2. Patients included in the IL treatment should not have the lesion in the head, as infections due to this treatment are more frequent in this area (Masmoudi *et al.* 2006). Twenty-six patients participated at the beginning.

Side effects — adverse symptoms and alteration in laboratory exams

Patients were interviewed weekly about the occurrence of adverse symptoms. Results of laboratory blood exams, performed weekly by the patients during the treatment, were obtained from patients' medical records. Not all patients underwent all exams every week. The laboratory parameters evaluated were related to liver function – aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (Alk-Ph), to pancreas function – amylase, to cardiotoxicity – QT interval (Qtc), and to hematological features – number of lymphocytes, eosinophiles and neutrophiles, hematocrit (Htc) and hemoglobin (Hgb). Measured values were considered altered when AST, ALT, Alk-Ph, amylase, number of lymphocytes, eosinophiles and neutrophiles were above the upper limit of normal (ULN); when Htc and Hgb were below the lower limit of normal; and when Qtc: increased by 50 ms from baseline or rose to an absolute value ≥450 ms.

Biological samples

Blood samples were collected by venipuncture before the beginning of the treatment, on day 8 and on the last day. It was previously defined that the blood samples would be collected 5 min after the injection, so that we could also evaluate the day's dose. However, in some cases the collection was made before the injection, due to the unavailability of the patient to do it afterwards. The last day's blood sample was collected within 5 min to 48 h of the last injection. For the CT and LD treatments, a biopsy was taken from healthy skin within 1 cm of the lesion site up to 48 h after the last dose. For the IL patients, the initial protocol defined the skin biopsy occurring immediately before the last application of the drug. However, this procedure produced a severe skin irritation on the first patient, and for the other patients of this treatment the biopsies were performed 1 week after the last application. Blood samples were centrifuged and plasma and skin biopsies were frozen at –20 °C, until they were analysed.

Antimony analysis

All volumetric flasks used in the analysis were polypropylene flasks decontaminated with 10% nitric acid for 12 h, followed by a triple rinse with deionised water. All reagents were at least of analytical grade. Multi-element calibration standard 4 (Perkin Elmer Pure Plus) and indium standard (Perkin Elmer Pure) were used. The reference materials used to validate the analytical method were Human Serum, from the Centre de Toxicologie — INSPQ — Canada, Control ID # QMEQAS07S-04, and the Reference Material 8414 — Bovine Muscle Powder, from the National Institute of Standards & Technology.

Plasma samples were diluted with a solution of 0.5% nitric acid and 0.01% Triton X-100. Skin samples were digested in a Start D Microwave Digestion System (Milestone), using 10% nitric acid and hydrogen peroxide. In both cases, a solution of indium was added as internal standard. For the plasma analysis, a matrix-matching calibration curve with ovine plasma was used. Antimony was quantified by Inductively Coupled Plasma Mass Spectrometry – ICP-MS (Perkin–Elmer ELAN DRC II, with a Meinhard nebulizer and a cyclonic spray chamber). The analytical curves used for plasma and skin analysis had coefficients of correlation >0.999. The limit of detection (LOD) of the method was 0.020 μg/l for plasma and 0.008 μg/l for skin. The levels of antimony found in the reference materials using the procedure applied to the samples were within the reported range.

Data analysis

The SPSS software version 13.0 (Statistical Package for Social Sciences) was used to perform chi-square, Pearson's correlation and *t*-tests; *P* values ≤0.05 were considered as significant. In cases where multiple statistical

comparisons were made, the Bonferroni correction was used to ensure the significance of the relationships. In this case, significance was found when $P \leq 0.05/N$, where N is the number of comparisons.

This study was reviewed and approved by the Research Ethics Committee of the Health Sciences Faculty of the University of Brasilia.

Results

The study began with 26 patients with cutaneous leishmaniasis under treatment with Glucantime® at the UHB; three patients were later excluded for not tolerating the treatment since the first day or not returning to hospital after the first week. Twenty-three patients finished the study: 18 from the CT treatment, 2 from the LD treatment and 3 from the IL treatment. On average, patients were 38 years old and had one to six lesions characteristic of the disease; most of them were men (74%) and 35% were smokers.

The mean antimony concentration in the plasma samples taken from all patients before the beginning of the treatment (basal level) was 3.93 ± 3.42 μg/l. For the CT patients only, this level was 3.39 ± 3.12 μg/l, ranging from <0.02 to 9.7 μg/l (Table 1). The mean plasma concentrations just before and after the eighth dose for the CT patients were 0.114 and 106.1 mg/l, respectively. The levels found after dosing were over two times higher than those found for the two LD patients (mean of 43.6 mg/l).

At the 20th day of treatment, the mean plasma concentrations were 0.210 and 125.8 mg/l when the sample was collected before (C_{20}pre) and after the last dose (C_{20}post), respectively. The C_{20}pre/C_8pre and C_{20}post/C_8post ratios show that plasma Sb concentration increased during the treatment for at least half the patients who had both blood samples collected before or after dosing (Table 1). For the IL patients, the plasma sample taken after the second dose (C_8post) had levels lower than those found for both intravenous groups (CT and LD). While the plasma

Table 1 Antimony levels in the plasma and skin samples of patients from the three treatment groups

Patient	C_0 (μg/l)	C_8pre (mg/l)	C_8post (mg/l)	C_{20}pre (mg/l)	C_{20}post (mg/l)	C_{20}pre/ C_8 pre	C_{20}post/ C_8 post	Cskin (μg/g)
CT1	1.76	–	92.76	–	–	–	–	7.61
CT2	<0.02†	–	135.2	–	187.5	–	1.39	–
CT3	3.27	–	95.38	–	74.44	–	0.78	33.4
CT4	3.94	0.208	–	0.159	–	0.76	–	14.25
CT5	3.79	–	101.2	0.061	–	–	–	6.57
CT6	<0.02	0.079	–	0.452	–	5.72	–	9.32
CT7	2.94	–	108.5	–	–	–	–	–
CT8	<0.02	–	94.72	0.639	–	–	–	7.29
CT9	1.92	0.129	–	0.077	–	0.60	–	8.95
CT10	6.20	0.061	–	0.186	–	3.05	–	3.43
CT11	2.03	–	71.10	0.104	–	–	–	2.01
CT12	<0.02	–	115.0	0.080	–	–	–	3.94
CT13	6.44	–	86.95	0.076	–	–	–	0.83
CT14	3.25	–	53.98	–	125.9	–	2.33	6.17
CT15	9.19	–	173.7	–	115.4	–	0.66	6.06
CT16	6.56	–	67.31	0.452	–	–	–	2.47
CT17	9.71	0.089	–	0.131	–	1.47	–	6.01
CT18	<0.02	–	183.8	0.101	–	–	–	1.12
Mean ± SD	3.39 ± 3.12	0.114 ± 0.06	106.1 ± 38.51	0.210 ± 0.19	125.8 ± 46.74	–	–	7.46 ± 7.7
LD1	3.40		45.68	0.037	–	–	–	27.84
LD2	5.40	–	41.46	0.066	–	–	–	9.49
IL1‡	0.60	–	7.20	0.001	–	–	–	9.06
IL2	10.3	–	2.527	–	–	–	–	–
IL3	9.64	–	2.525	1.002	–	–	–	94.75

CT = conventional treatment; LD = low dose treatment; IL = intralesional treatment; C_0 = Initial concentration, before starting the treatment; C_8pre = concentration at the eighth day of treatment, before the application of the drug; C_8post = concentration at the eighth day of treatment, after the application of the drug; C_{20}pre = Concentration at the 20th day of treatment, before the application of the drug; C_{20}post = Concentration at the 20th day the treatment, after the application of the drug; Cskin = Concentration on the skin at the end of the treatment.

† Samples <0.02 μg/l (LOD) were considered to be at 0.02 μg/l level.

‡ For the IL patients, C_8 corresponds to the second intralesional dose and C_{20} to the fourth dose.

antimony level of IL1 patient before the fourth dose (C_{20}pre) represented <0.02% of the level found after the second dose (C_8post), for other patient of this group (IL3), it corresponded to 40% (Table 1).

The mean antimony concentration in the skin biopsies was 7.46 μg/g ± 7.7 for the CT patients (n = 16), ranging from 0.83 to 33.3 μg/g. The levels found in skin of the other patients ranged from 9.06 to 94.75 μg/g (Table 1).

Symptomatic side effects and alterations in values of laboratory exams

CT patients reported, in average, two adverse symptoms during the course of the treatment, ranging from 0 to 8. Arthralgia and myalgia were the most frequently reported (by 50% of the patients), followed by headache (17%), fever and itching (13% each). Other adverse symptoms were nausea and lack of appetite. These patients showed a mean of four laboratory alterations, ranging from 1 to 7. The incidence of these alterations is shown in Figure 1. Eosinophilia and lymphocytosis were the most frequent alterations, followed by increase in ALT. Prolongation of the QTc interval, one of the most critical adverse effects caused by pentavalent antimonials, was presented by about one third of the CT patients.

Adverse symptoms and alterations in laboratory exams shown by the patients from the LD and IL are summarized in Table 2. These patients reported up to two symptomatic side effects and showed two to five laboratory alterations.

In most cases, the laboratory alterations had no clinical significance. However, three patients showed AST values of more than two times the ULN, two others ALT values of more than five times the ULN, and one had Alk-Ph of more than two times the ULN. Two of these patients (CT17 and CT4) had to temporarily suspend treatment, in addition to CT9, whose treatment was suspended due to alteration in amylase levels. Alterations in the electrocardiogram necessitated temporary suspension of treatment of CT10 and complete suspension of treatment for CT7 and IL2.

Evaluation of significant correlations in the CT patients

Searching for possible relationships between the amount of adverse symptoms and alterations in laboratory values and characteristics of the CT patients, only one relationship was found – number of lesions and

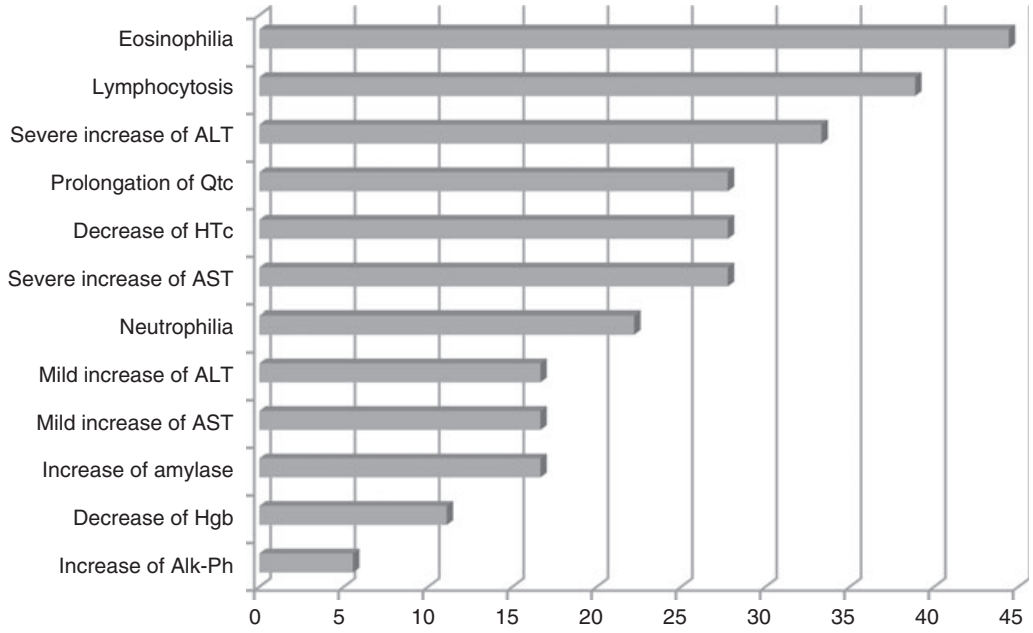

Figure 1 Incidence of the most frequent laboratory alterations shown by the CT patients. ALT = alanine aminotransferase; AST = aspartate aminotransferase; Htc = hematocrit; Hgb = hemoglobin; Alk-Ph = alkaline phosphatase. Mild increase of AST indicate up to 150% upper limit of normal (ULN); severe increase of AST and ALT indicate more than 150% ULN; lymphocytosis, neutrophilia and increase of amylase and Alk-ph indicate more than 110% ULN; eosinophilia indicates more than 150% ULN; decrease of Htc and Hgb indicate less than 95% of the lower limit of normal.

amount of adverse symptoms ($n = 18$, $P < 0.0001$, Pearson's correlation = 0.710). When we evaluated the correlations between antimony levels in plasma and skin biopsies, we also found only one significant relationship ($n = 5$, $P = 0.021$, Pearson's correlation = 0.893), between C_8pre (plasma) and Cskin.

The difference between the levels of antimony in plasma of CT patients at the end of the treatment and with 1 week of treatment (Table 1) is a dichotomic variable, defined as Df–1. This variable had a significant correlation with the amount of alterations in laboratory values, as shown in Table 3, which also shows the other significant correlations between the antimony levels in plasma and skin biopsies and the number of adverse symptoms and alterations in laboratory values.

We tested the significance of the laboratory alterations of the CT patients that occurred during the course of the treatment ($N = 70$). With the exception of AST values, 23 significant correlations were found ($P \leq 0.05$). Once we applied the Bonferroni correction, the only significance found was the decreasing of the Hgb levels from the first to the last week of treatment ($n = 11$; $P < 0.0007$; Pearson's correlation = 0.885).

We tested 150 relationships between the antimony levels (Table 1) and values of laboratory exams, from which 18 were found significant ($P \leq 0.05$). When the Bonferroni correction was applied, the only significant relationship was between the number of lymphocytes and the antimony levels in the skin at the end of the treatment ($n = 13$; $P < 0{,}0005$, Pearson's correlation = 0.914).

Finally, no significant correlation was found between the levels of antimony in plasma or skin and the suspension/interruption of the treatment.

Table 2 Symptomatic side effects and laboratory alterations in patients from the LD and IL

Patient	Side effects	Laboratory alterations
LD1	None	Neutrophilia, eosinophilia
LD2	Arthralgia and myalgia	Increase of AST and ALT, lymphocytosis. Decrease of Htc, anaemia
IL1	Itching at the place of injection and arthralgia	Lymphocytosis, eosinophilia
IL2	Pain in the place of injection	Neutrophilia, increase of ALT and Alk-Ph. Prolongation Qtc (treatment interrupted)
IL3	Itching at the place of injection	Neutrophilia. Increase of Alk-Ph and amylase

Discussion

The basal plasma antimony concentration in the patients participating in this study was higher than the values reported in the literature. In a study in southeast region of Brazil, the mean baseline plasma antimony level found in 10 patients with Leishmaniasis was 0.69 μg/l (Miekeley *et al.* 2002). Bazzi *et al.* (2005) reported mean levels of antimony in blood of South African children at 0.85 ± 0.9 μg/l and of North American adults at 2.51 ± 1.1 μg/l. Goullé *et al.* (2005) found a mean plasma and blood antimony level of 100 heath individuals in France of 0.11 μg/l and 0.08 μg/l, respectively. In our study, the only patient reported having previously been treated with antimonials (CT14) had a plasma antimony concentration close to the mean for all patients.

Jaser *et al.* (1995a) evaluated the pharmacokinetics of antimony in the blood of Saudi Arabian patients treated with daily intramuscular doses of 600 mg Sb^v, for 10 days. The time for the antimony to reach its maximum blood concentration of 8.77 mg/l was estimated in 1.34 h. A maximum skin concentration of 5.0 μg/g was reached after 2.1 h (Jaser *et al.* 1995b). The authors found two different profiles of patients, those whose antimony concentration augmented during treatment, which they called the 'slow eliminators' and those where the concentration diminishes in the end, the 'fast eliminators'. We also found these two patient profiles in our study.

The effect of other drugs on the antimonial metabolism cannot be discarded. In our study, the patient using

Table 3 Significant correlations found between the amount of side effects and antimony levels

Variables	*P*	Pearson's correlation value
Number of laboratory alterations × Df–1 ($n = 9$)	0.001	0.867
Number of laboratory alterations × C_8 pre ($n = 5$)	<0.0001	0.992
Number of symptomatic effects × C_8 pre ($n = 5$)	0.037	0.841
Number of symptomatic effects × C skin ($n = 16$)	<0.0001	0.781

C_8 pre = concentration at the eighth day of treatment, before the application of the drug; Cskin = Concentration of antimony in the skin at the end of the treatment; Df–1 = Difference between the concentration of antimony at the end of the treatment and after its first week.
1w = first week; 2w = second week; f = final.

captopril (CT11) presented plasma and skin antimony concentrations smaller than the means found for the group. Apparently, the use of fluoxetine, atenolol, bromazepam and omeprazole by one patient from the LD treatment (LD2) did not affect the antimony metabolism compared with the other individual from the same group.

The concentrations of antimony found in the skin biopsies of the CT patients in the present study were lower than the values reported by Dorea *et al.* (1990) in seven Brazilian patients treated with endovenous injections of pentavalent antimonials (10 or 20 mg Sb^v/kg/day, for 10 or 20 days). The biopsies were collected in the last day of treatment and antimony analysed by neutronic activation. In our study, both LD patients had skin antimony concentrations within the range of those found in the CT patients. Unlikely of what would be expected, the skin levels were higher for only one IL patient.

The main adverse symptoms and alterations in laboratory exams found were similar to those described in the literature for leishmaniasis patients (Mattos *et al.* 2000; Saldanha *et al.* 2000; Nogueira & Sampaio 2001; Paula *et al.* 2003; Lawn *et al.* 2006; Kashani *et al.* 2007). In a previous study conducted in the UHB, patients treated with meglumine antimoniate at the conventional dose also showed arthralgia and myalgia as the main adverse symptoms (Paula *et al.* 2003). Laboratory alterations such as elevation in seric creatinine, leukopenia, eosinophilia and elevation in transaminases were observed in 42.2% of the patients. In a study conducted in the southeast region of Brazil, patients treated either with meglumine antimoniate or sodium stibogluconate reported mainly inappetence, headache, arthralgia, fever and muscular weakness (Deps *et al.* 2000). In Iran, Kashani *et al.* (2007) found altered AST, ALT and Alk-Ph levels in patients treated by intramuscular injections at 20 mg Sb^v/kg/day for 15 days, but the authors did not consider these alterations clinically relevant. We found no study reporting neutrophilia induced by antimonials in the literature, whereas a reduction of Htc was also mentioned by Kashani *et al.* (2007).

The exact mechanism of the leishmanicidal action of pentavalent antimonials remainsl unknown (Croft & Yardley 2002; Rath *et al.* 2003; Singh 2006), however, it was never considered that its action was due only to a direct cytotoxic action. Many studies demonstrated that, despite its direct leishmanicidal action *in vitro* and in animal models, full recovery demands an intact T-cell population (Croft & Yardley 2002; Singh 2006). Thus lymphocytosis, which was one of the most frequent laboratory alterations in this study, can be attributed to the fact that the drug induces the proliferation of lymphocytes, as a part of its antiparasitic action, by the activation of the host defense cells (Lindoso *et al.* 1996).

Alterations in the electrocardiogram, as the Qtc prolongation, an indicator of possible arrhythmias, were also observed by other authors (Deps *et al.* 2000; Paula *et al.* 2003; Lawn *et al.* 2006). In our study, they were responsible for the temporary suspension of the treatment of one patient and ending treatment for two patients (8.7% of all patients). Lawn *et al.* (2006) found that 6% of 65 patients with cutaneous or mucosal leishmaniasis had to interrupt treatment due to cardiac toxicity, generalized erythroderma, fever and/or severe musculoskeletal pain.

Few studies describe the occurrence of side effects in patients receiving antimonial doses different than the conventional protocol. In a study with 40 patients receiving intramuscularly one ampoule of Glucantime® (405 mg of antimony)/day on alternate days, up to clinical cure, only six reported side effects, mainly pain at the injection site, slight arthralgia and nausea (Oliveira-Neto & Mattos 2006). Oliveira-Neto *et al.* (1997b) observed adverse symptoms in only 6% of 159 patients receiving 5 mg Sb^v/kg/day, for 30 days, endovenously. In the present study, the two LD patients had Chagas disease, but the one who had also hypertension and cardiopathy presented more side effects. Disconfort at the injection site, reported by all three IL patients, has also been reported previously by patients using IL treatment (Oliveira-Neto *et al.* 1997; Mutjaba & Khalid 1999).

It was thought that a great advantage of the intralesional treatment would be to avoid the systemic side effects of the drug (Faghihi & Tavakoli-Kia 2003). However, as clearly demonstrated in this study, systemic absorption of the drug occurs, and in our case it lead to affection of hepatic and renal function, Qtc prolongation and changes in white cells count.

In this study, we found that the number of lesions was significantly correlated to the incidence of adverse symptoms. The antimony concentration in plasma of CT patients after 1 week of treatment (C_8pre) was correlated both with the number of adverse symptoms and laboratory alterations, which were also correlated to the concentration of antimony in the skin at the end of the treatment. Higher antimony levels appears to mean a higher concentration of AST, ALT and Alk-Ph, a higher count of lymphocytes, neutrophiles and eosinophiles, a higher Qtc value and a smaller concentration of Hgb and Htc, although only one of these relationships was significant using the Bonferroni correction The group of results obtained indicates that there is indeed a relationship between the administration of pentavalent antimonials and the incidence of side effects.

The present work had two major limitations. During the course of the study, not all patients were available to donate biological material as previously planned, which limited the number of data for all patients in all dosing days. Additionally, the small number of individuals in the LD and IL groups did not allow a statistical analysis of the data from these groups.

Conclusion

This study supports the conclusion that LD and intralesional patients had both lower blood antimony levels and fewer adverse symptoms than CT patients. The concentration of antimony in the body of patients under Conventional Treatment was significantly correlated to the incidence of adverse symptoms and alteration in laboratory exams, indicating a possible dose-dependent relationship between these parameters. In a posterior phase, this relationship could be explored further using animal models, where the levels of antimony in target organs would be compared with the clinical and laboratorial manifestations.

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