Tropical Medicine and International Health doi:10.1111/tmi.13130

VOLUME 23 NO 10 PP 1110–1117 OCTOBER 2018

Plasma antimony determination during cutaneous leishmaniasis treatment with intralesional infiltration of meglumine antimoniate

Marta Gontijo de Aguiar[1], José Eduardo Gonçalves[1], Mirna d'Auriol Souza[2], Rosiana Estefáne de Silva[3], Josianne Nicacio Silveira[2] and Gláucia Cota[3]

1 *Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
2 *Departamento de Análises Clínicas e Toxicológicas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
3 *Fundação Oswaldo Cruz-Fiocruz, Instituto René Rachou, Centro de Referência em Leishmanioses, Pesquisa Clínica e Políticas Públicas em Doenças Infecto-Parasitárias, Belo Horizonte, MG, Brazil*

Abstract

OBJECTIVES To evaluate the antimony (Sb) in plasma of patients who underwent a standardised meglumine antimoniate (MA) intralesional infiltration protocol for cutaneous leishmaniasis treatment.
METHODS The level of Sb in plasma was determined by atomic absorption spectroscopy, before and 1, 2, 4 and 6 hours after the first intralesional infiltration of MA to determine the parameters peak concentrations ($C_{1\ h}$), area under curve of drug concentration in plasma from zero to 6 h ($AUC_{0\text{-}6\ h}$) and elimination half-life ($t½$) of Sb. Blood samples were also collected weekly during the treatment period, always before infiltration.
RESULTS Fourteen patients underwent MA intralesional infiltration with doses ranging from 0.8 to 9 mg Sb/kg at the first infiltration. The $C_{1\ h}$ ranged from 3850 to 47 095 mg × h/L and was the highest concentration obtained for 11 of 14 patients after the first intralesional infiltration of MA. A rapid initial phase of distribution lasting up to 4 h (2.6 ± 0.34 h) was followed by a slower elimination phase. Total skin lesion area, $C_{1\ h}$ and $AUC_{(0\text{-}6\ h)}$ were related to the dose of Sb infiltered ($P < 0.05$). Plasma Sb in samples collected weekly before the infiltration revealed antimony concentrations below the quantification limit (15.0 μg Sb/l) during the treatment period.
CONCLUSIONS Sb is quickly absorbed and eliminated after intralesional administration of MA, in a pattern similar to that reported with the Sb systemic administration. Using a therapeutic schedule limited to weekly intralesional infiltration of doses <10 mg Sb/kg does not result in plasma Sb accumulation.

keywords cutaneous leishmaniasis, intralesional therapy, meglumine antimoniate and antinomy

Introduction

Leishmaniasis is a neglected tropical disease caused by the intracellular *Leishmania* parasite highly prevalent and distributed throughout the world, either in its systemic, visceral leishmaniasis (VL), or cutaneous form, cutaneous leishmaniasis (CL) [1, 2]. The clinical manifestation depends on both the *Leishmania* species involved and the immune response of the host. The form with cutaneous involvement comprises 2/3 of cases. According to WHO and based on Global Health Observatory (GHO) data from September 2017, the majority of cases occur in Afghanistan, Algeria, Brazil, Colombia, the Islamic Republic of Iran, Pakistan, Peru, Saudi Arabia and the Syrian Arab Republic. Almost 90% of mucocutaneous leishmaniasis cases occur in Bolivia, Brazil and Peru [3].

Pentavalent antimonial compounds administered parenterally remain the most used therapy for CL globally despite their recognised toxicity. The most commonly reported adverse events are myalgia, arthralgia, nausea, headache, anorexia and fatigue. Serious clinical complications have been reported, such as hepatitis, pancreatitis and arrhythmias, the latter being the most lethal. Due to these serious adverse events, systemic therapy with antimony requires clinical and laboratory monitoring, which is difficult in remote areas with minimal laboratory infrastructure [4]. Alternatives to antimony are few, surrounded by uncertainties about their efficacy and safety.

Local therapies remain under researched, especially in the New World, where the risk of developing mucosal leishmaniasis, a feared complication due to *Leishmania braziliensis,* is the main reason for the historic use of systemic treatments. It is a paradigm that has been recently challenged as this metastatic complication has never been systematically studied, and there are no reports of mucosal complications developing in CL patients who were treated with topical therapies [5]. In 2010, the WHO Expert Committee on Leishmaniasis recommended the inclusion of the antimony intralesional infiltration in its guidelines as an acceptable therapeutic alternative for New World leishmaniasis [6]. In 2013, the Pan American Health Organization Expert Committee on leishmaniasis also included this approach in regional guidelines restricted to reference centres and to single lesions not involving the face or joints [7]. Recently, a systematic review confirmed a global efficacy of 75% for pentavalent antimony intralesional therapy, a similar cure rate to that described in the New World for parenteral antimony treatment [8]. It is important to note that this is an off-label route of a drug that has been used for decades, but was only just incorporated into the Brazil's public health system officially, with expanding large-scale use.

The potential advantages of the intralesional infiltration of meglumine antimoniate (MA) are the use of lower total doses of antimony (Sb) and thus fewer toxic effects and a more flexible schedule without the requirement of daily drug administration. In addition, it is a therapeutic modality exempting investment in equipment, which makes it feasible to implement in the short term. Information on the pharmacokinetics of Sb after intralesional infiltration of MA is scarce [9]. Large-scale incorporation of the intralesional approach requires knowledge of the magnitude of systemic antimony absorption and accumulation in the body, which is directly related to its safety profile. Thus the aim of this study was to determine the Sb in plasma of patients who underwent a standardised MA intralesional infiltration protocol for CL treatment.

Materials and Methods

Study subjects and design

The study was designed to determine the concentrations of Sb in plasma of patients undergoing MA intralesional infiltration as therapy for localised CL in a single-arm phase II clinical trial. The patients were treated in an outpatient setting, the Leishmaniasis Referral Centre of Instituto René Rachou (IRR), Fundação Oswaldo Cruz (FIOCRUZ), Belo Horizonte, Minas Gerais, Brazil. The study protocol was approved by the Ethical Review Board of IRR-FIOCRUZ (approval number 1.136.132) and registered on the Brazilian Clinical Studies Registry Database (REBEC) under number RBR-44KG5X.

Only patients over 14 years old presenting fewer than four lesions, with a total lesion area up to 9.0 cm^2, were included. Their *Leishmania* infection was confirmed by direct smear or culture or k-DNA based polymerase chain reaction (PCR) test. In this study, the usual contraindications for systemic use of antimony were exclusion criteria, namely history of allergy to MA; presence of congestive heart failure or cardiac arrhythmia; renal or hepatic impairment; use of medications with the potential to cause cardiac arrhythmia such as amiodarone, beta blockers, tricyclic antidepressants; pregnancy or lactation. The lesion area was calculated according to current recommendations for clinical trials on CL [10] as the product of the two longest ulcer diameters. All patients included in this study will be clinically evaluated annually for 5 years after cure achievement.

Drug administration

The patients were treated with MA – Glucantime® – ampoules of 81 mg/ml, using the same lot control number (528240), produced by Sanofi-Aventis Pharma and assigned by the Brazilian Ministry of Health. Patients were submitted to weekly intralesional infiltration using a standardised and previously validated technique [8]. According to the study protocol, a maximum of eight infiltrations was performed deeply in the dermis and in the adjunctive subcutaneous tissue aiming to achieve saturation of the lesion, defined as swelling of the entire lesion accompanied or not by pallor. A smaller number of infiltrations could be performed in patients who evolved with lesion cure before the eight programmed treatment sessions. Based on the adopted protocol, the amount of the MA infiltrated was not previously established but a variable volume defined according to saturation state of each lesion during the infiltration, respecting the maximum limit of 15 ml or three ampoules of Glucantime®, the total accepted as the upper limit for the parenteral use, in a conservative approach. The total volume infiltrated was registered at the end of the procedure.

Blood samples

On the first day of treatment, EDTA-anticoagulated blood specimens were obtained before and 1, 2, 4 and 6 h after the MA intralesional infiltration. In the

subsequent weeks, blood samples were also collected from patients during all the treatment phase, always immediately before the intralesional infiltration. The samples were centrifuged and the obtained plasma stored at −80 °C until analysis of the Sb content.

Antimony analysis in the plasma

The plasma antimony concentration was determined by atomic absorption spectrometry, a method adapted from Cruz and colleagues (2007) [11] and validated [12] for our analysis conditions. The analytical curves used for analysis had coefficients of correlation >0.99. The precision had a variation coefficient <15% and the standard error of the median was <15%.

Integrated absorbances were obtained using an atomic absorption spectrometer SpectrAA Zeeman-220 equipped with a graphite furnace, an autosampler (PSD-31-972) and a polarised Zeeman background correction (Varian®, Sidney, Australia). Hollow cathode lamps (HCL) for Sb (Agilent Technologies®, Mississauga, Canada), were used as a light source. Antimony was determined at 10.0 mA with a spectral band-pass of 1.0 nm at 231.2 nm. Argon, 99.999% (Air Products®, São Paulo, Brazil), was used as a purge gas at 3 l/min. Pyrolytic graphite-coated tubes without L'Vov platform (Agilent Technologies®, Mississauga, Canada) were used. The autosampler injected 10 µl of sample into a graphite tube. These methods determine total antimony and do not distinguish between its different chemical forms. Plasma samples were diluted according to the total volume of MA infiltered into the lesion and collection time. The dilutions of plasma samples were made with 65% nitric acid solution (Merck®, Darmstadt, Germany) and hydrochloric acid (Merck®, Darmstadt, Germany), Zirconium 5–10 µl/ml (Merck®, Darmstadt, Germany) was used as chemical modifier, and 0.4% Triton X-100 (Merck®, Darmstadt, Germany). Two calibration curves for Sb in the plasma were used. The final concentration in the optimal assay working range was given between 15.0–120.0 µg/l. The temperature and time conditions (ramp and hold) by the graphite furnace were 100°C (10.0 s, 20.0 s), 140°C (30.0 s, 30.0 s), 200°C (10.0 s, 30.0 s), 350°C (35.0 s), 1300°C (10.0 s, 16.0 s), 2,400°C (0.5 s, 2.0 s) and 2600°C (0.5 s, 3.0 s). The calibrator was analysed at the beginning and at the end of each set of specimens, and the calibration curve was prepared by the mean of three replicates signals of each calibrator, as well as the samples analysed. The sample concentrations were calculated by the equation of line obtained by the ordinary least squares method. The dose of Sb (mg/kg) administered was calculated by the ratio of the Sb infiltered and the patient's weight at the first day of treatment.

Statistical analysis and Pharmacokinetics of Sb

Based on the measured Sb concentration, the following parameters were calculated: peak plasma concentration at 1 h ($C_{1\ h}$), area under the concentration-time curve from time 0–6 h ($AUC_{0\text{-}6\ h}$) and the elimination half-life ($t½$). The non-compartmental pharmacokinetic parameters described above were estimated using GraphPad Prism 7 and the summary data presented as mean and standard error (SE). Values for $AUC_{0\text{-}6\ h}$ were determined by trapezoidal method. Eliminations rates constants (k) were determined from the linear regression of the two last points of the plasma log concentration-time curve, and elimination $t½$ was calculated using the equation 0.693/k. Clinical data were were summarised using descriptive statistics and expressed in median and interquartile range of 25–75% (IQR) after analysis of the presence of outliers.

The regression model and Pearson's correlation were used to determine the correlation between the total lesion area (cm^2), $C_{1\ h}$ and $AUC_{(0\text{-}6\ h)}$ and the individual dose (mg/kg of Sb) administered to each patient on the first day of the treatment. For all analysis, differences were considered significant when P values were <0.05.

Results

A total of 14 patients presenting a total of 17 lesions were enrolled in this study and their main characteristics on the day of recruitment are presented in Table 1. The patients' median age was 38 years with an IQR of 18–56 years. 64% were male, matching the sex proportion of CL observed in the Brazilian population. Most lesions were ulcered and the median area of the lesions was 4.6 (IQR 2.4–8.2) cm^2. Lesions mainly occurred in exposed areas such as legs 43%, head 21% and arms/hands 21%. The median duration of lesions prior to treatment was 12 (IQR 2–21) weeks. Patients achieved cure in a median time of 7 (IQR 5–13) weeks. In total, the median volume of Glucantime® infiltrated throughout the treatment was 21.8 ml, with the median volume per cm^2 of lesion in the first day of treatment of 0.8 (IQR 0.6–1.0) ml and treatment duration of 46 (IQR 28–56) days.

The individual plasma concentration-time profile of Sb after the first intralesional infiltration of MA is illustrated in Figure 1. As expected, the plasma Sb concentration before the first MA infiltration, in all patients, was lower than the quantification limit of the method of analysis (15.0 µg Sb/l). The concentration-time curve, built for all the studied patients, revealed that for 79% (11/14) the $C_{1\ h}$ was the maximal value observed; for the other

Table 1 Baseline demographic and clinical characteristics of fourteen patients with CL treated with meglumine antimoniate (MA) intralesional infiltration

Number Patient	Age (years)	Weight (kg)	Gender	Number of lesions	Lesion site	Lesion morphology	Total lesion area (cm^2)	Number of infiltrations performed	Total Volume Glucantime® infiltrated (ml)
1	30	52.0	F	1	H	NU	1.2	6	2.18
2	59	72.6	F	1	H	NU	1.7	5	2.86
3	68	55.7	F	1	C/H	NU	3.1	3	10.80
4	16	96.0	F	1	C/H	U	4.5	4	16.00
5	39	58.0	M	2	A/H	U	3.8	3	12.20
6	37	66.9	M	1	A/H	U	4.6	8	23.20
7	47	65.3	F	1	H	U	1.6	8	15.30
8	20	65.0	M	3	L	U	5.6	8	69.30
9	15	50.0	M	1	L	U	7.6	7	23.40
10	53	81.5	M	1	L	U	9.0	8	62.00
11	14	70.5	M	1	A/H	U	8.8	4	20.30
12	70	73.0	M	1	L	U	4.1	6	45.30
13	25	67.0	M	1	L	U	6.7	7	59.00
14	39	63.0	M	1	L	U	8.8	7	41.00
Median	38	66.1	–	–	–	–	4.6	6.5	21.75
IQR25%	18	56.8	–	–	–	–	2.4	4.9	11.50
IQR75%	56	72.8	–	–	–	–	8.2	8.0	52.15

A/H, Arms/hands; C/H, chest/back; F, Female; H, head; IQR, interquartile range (percentile 25 and 75); L, Leg; M, male; NU; non ulcer; U, ulcer.

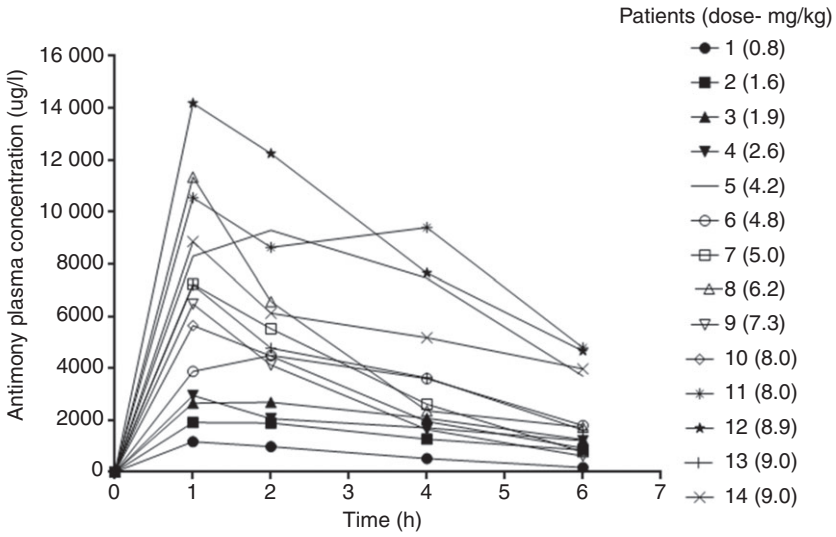

Figure 1 Plasma profile of Sb in patients after the first meglumine antimoniate (MA) intralesional infiltration.

21%, the mean peak plasma concentration was achieved in 2 h (3/14). A rapid initial phase of decreasing Sb concentration lasting up to 4 h and, after that, a slow downward curve, were observed. Sb determinations performed weekly alongside treatment, immediately before the MA infiltration, failed to identify the presence of antimony in all patients that is Sb was below the quantification limit of the analytical method for Sb determination (15.0 μg/l).

Table 2 shows the pharmacokinetic parameters $C_{1\ h}$ (μg/l), $AUC_{0\text{-}6\ h}$ (μg/l × h) and elimination $t½$ (h) obtained for each patient after the first MA intralesional infiltration (the first treatment day). The dose of Sb (mg/kg) infiltrated

Table 2 Pharmacokinetic parameters of Sb after the first intralesional infiltration of meglumine antimoniate (MA) to patients

Patient	Total lesion area (cm^2)	Dose Sb mg/Kg	$C_{1\ h}$, µg/l Sb	$AUC_{0\text{-}6\ h}$, µg h/l Sb	$t_{1/2}$,h
1	1.2	0.8	1165	3850	1.3
2	1.7	1.6	1912	8126	2.7
3	3.1	1.9	2686	12 081	3.3
4	4.5	2.6	2947	10 636	4.1
5	3.8	4.2	9292	40 848	2.0
6	4.6	4.8	4491	19 608	2.0
7	1.6	5.0	7222	21 511	1.1
8	5.6	6.2	11 342	27 622	4.4
9	7.6	7.3	6470	16 559	1.4
10	9.0	8.0	5642	17 159	1.8
11	8.8	8.0	10 544	47 095	2.1
12	4.1	8.9	14 187	52 522	2.8
13	6.7	9.0	7201	23 238	1.7
14	8.8	9.0	8855	32 347	5.2

was related to the total lesion area and to the $C_{1\ h}$ and $AUC_{0\text{-}6\ h}$ (Figure 2). There were no outliers in the analysed data.

Discussion

While the mechanism of action of Sb is not completely understood, the pharmacokinetics of pentavalent antimonial derivatives after both intramuscular and intravenous injection are known and seem to be similar, with a short half-life ($t½$) of approximately 1–2 h and a terminal elimination phase of 1–3 days [11, 13, 14]. Miekeley and colleagues (2002), using a more sensitive plasma mass spectrometry for Sb analysis, reported an even slower terminal ($t½$) of 50 days, hypothesised to be the intracellular conversion of SbV to SbIII, and subsequent slow release [15]. The drug-related toxicity after systemic administration includes both common and non-serious symptoms (myalgia, arthralgia, gastrointestinal disturbances and headache) and clinically significant ones (cardiac arrhythmia, hepatitis and pancreatitis) [4]. In contrast, only a few anecdotal reports on the Sb pharmacokinetics and toxicity profile of antimony intralesional infiltration are available [9]. This is the first study designed to evaluate the Sb in plasma after MA intralesional infiltration for treatment of localised CL. We confirm that the Sb pharmacokinetics profile after MA intralesional infiltration is similar to that after pentavalent antimonial administration by intramuscular route: a fast absorption phase, followed by rapid elimination. Besides the efficient Sb systemic absorption promoted by the intralesional infiltration, we confirmed a lack of Sb accumulation in plasma with the protocol adopted here, which was based on weekly infiltration sessions with daily doses not exceeding 10 mg/kg.

The extrapolation and comparison of our results is limited by two factors: First, an indirect comparison, as the data about the Sb concentrations observed with antimony parenteral use were obtained from the literature and not determined by us, using a standardised protocol, as we did with the intralesional infiltration approach; this analysis was possible from the understanding that the pattern of plasma Sb after antimony administered parenterally is well studied and established. Second, most of the available pharmacokinetic studies with antimony derivatives in parenteral administration were performed with sodium stibogluconate and not with MA. Due to structural differences in these compounds, differences in pharmacokinetics could be expected although they have not been confirmed so far. Chulay and colleagues (1988) found a similar pharmacokinetics profile among patients with visceral leishmaniasis treated with sodium stibogluconate intramuscularly (2 patients) and MA endovenously (3 patients). For both drugs, the data were best described by a two-compartment model. In turn, Cruz and colleagues (2007) compared the pharmacokinetic parameters of the intramuscular MA administration in children and adults using a non-compartment model. According to these two studies, in adults, the peak concentration was achieved approximately 1–2 h after the initial dose, followed by a short $t½$ of elimination of approximately 2 h and a terminal phase of 1–3 days [11, 16]. In another study, Zaghaloul and colleagues (2010), evaluating patients with CL treated with intramuscular administration of sodium stibogluconate (600 mg Sb daily), also demonstrated a two-compartmental open model of Sb plasma concentration characterised by a rapid distribution phase and a slower elimination half-life of about 10 h [13]. Similarly, our findings after MA intralesional infiltration confirmed an initial fast phase in the Sb concentration curve (up to 4 h), similar to that described in the studies with antimonial intramuscular administration as the representation of the distribution phase, followed by a slow downward curve, the representation of the elimination process [11, 16].

The elimination $t½$ of 2.6 ± 0.34 h (mean ± SE) observed here is similar to the values reported by other authors after the intramuscular administration of Sb at a dose of 20 mg/kg and 10 mg/kg (1.99 ± 0.08 and 2.02 ± 0,11 h, respectively) [11, 16]. This observation suggests that the distribution phase of MA infiltered intralesionaly is not different from that described after the intramuscular administration of pentavalent antimonial derivatives.

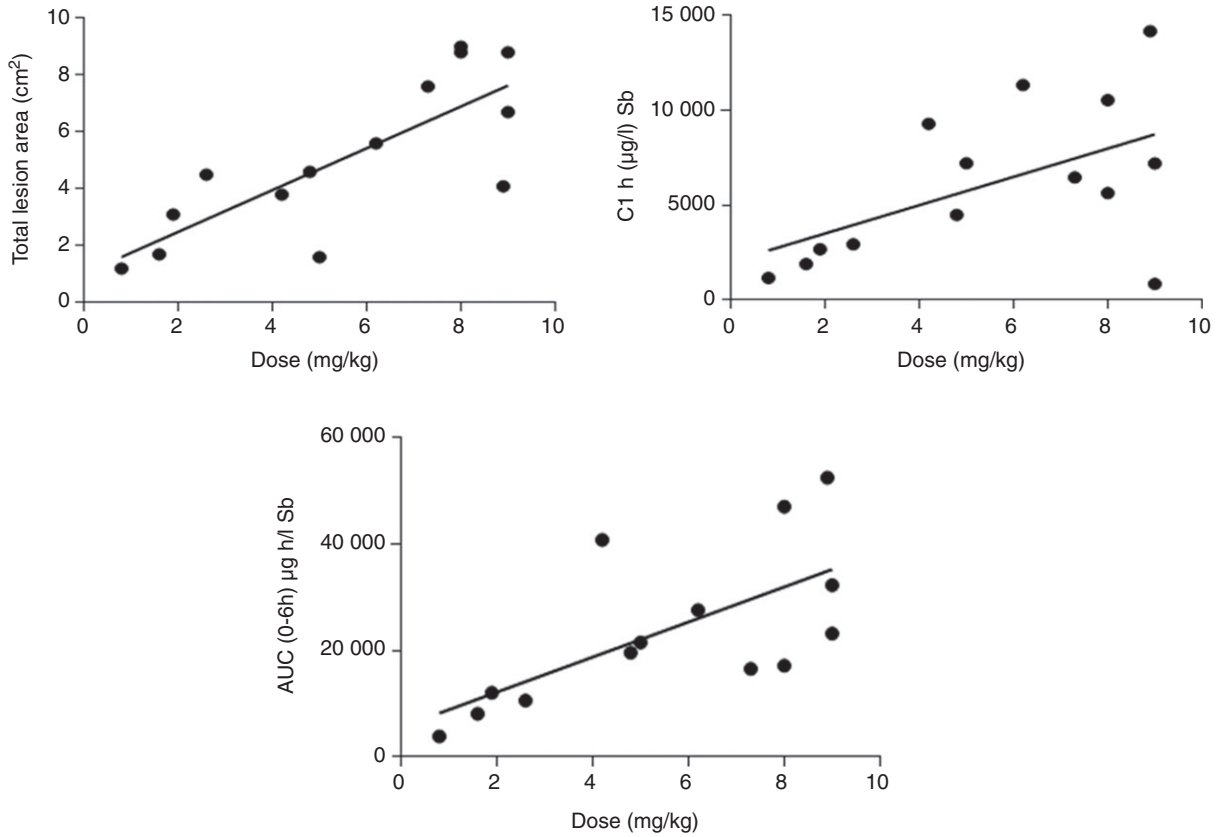

Figure 2 Correction between the total lesion area (cm^2) (a) $C_{1\ h}$ (b) and $AUC_{(0–6\ h)}$, (c) with the dose (mg/Kg) of Sb intralesional administered to patients. (a) Pearson (r) = 0.7906, confidence interval (CI) (95%) = 0.4479–0.9308, P value = 0.0008. (b) r = 0.583, CI (95%) = 0.3809–0.9191, P value = 0.0017. (c) r = 0.6615, CI (95%) = 0.20 l6–0.8824, P value = 0.001.

The values of $C_{1\ h}$ and $AUC_{0\text{-}6\ h}$ observed after MA intralesional infiltration are lower than those reported in the literature for antimony use by intramuscular route [11]. Moreover, these pharmacokinetic parameters are proportional to the total daily dose of Sb administered, independent of the route of administration. As an example, the highest value found for $C_{1\ h}$ after intralesional administration was 14.2 mg/l, obtained after an infiltered dose of 8.9 mg/kg. In the study performed by Cruz and colleagues, adults receiving the standard dose of 20 mg Sb/kg of MA (about 2.2 times greater) showed $C_{máx(1\ h)}$ of 38.8 mg/l, a concentration about 2.7 times higher [11]. These numbers suggest an equivalence in the absorption between the intralesional and the intramuscular routes. Thus it can be hypothesised that the difference between systemic and intralesional infiltration Sb administration is due to the smaller drug volumes used in infiltration rather than less efficient absorption capacity.

Another observation of interest is the lack of Sb plasma accumulation over the treatment period. Zaghloul and colleagues (2010) also demonstrated no significant differences in pharmacokinetic parameters between a single and multiple pentavalent antimony doses administered intramuscularly [13]. This observation suggests that the regimen based on multiple MA infiltrations adopted in this study has no effect on the Sb disposition, at least in the bloodstream. However, a progressive accumulation has been observed in the tissues [17]. The highest accumulation of Sb in human volunteers, after administration of radioactively labelled (Sb^{124}) sodium antimony mercapto succinate, was recorded in the liver, followed by the thyroid and heart [18]. Studies evaluating Brazilian CL patients reported higher skin concentrations with high variability after treatment with 10–20 mg Sb/kg/day for 20 days (8.32–70.68 ng/g) [19] and 20 mg/kg/day (7.46 ± 7.7 µg/g) [9]. This wide range of Sb tissue concentration could possibly influence Sb efficacy and

toxicity related to the CL treatment. In the present study, we did not determine the Sb concentration in the skin or another tissue, which would require an invasive procedure without direct benefit to the patient. Another limitation is the non-discrimination of the different Sb chemical forms. Given the tenfold higher toxicity of SbIII species, the evaluation of SbIII pharmacokinetics could play an important part in evaluating adverse effects and therapeutic action.

Other questions remain unanswered and will require more studies, with different designs, such as the association between effectiveness/safety profile and plasma/tissue Sb concentrations, and whether therapeutic regimens based on lower doses of antimony could lead to an increase in the rate of the *Leishmania* resistance.

Conclusion

Our observations demonstrate that Sb absorption provided by MA intralesional infiltration is as efficient as that observed after the pentavalent antimonial intramuscular administration. The potential risk of toxicity remains with the intralesional therapeutic approach if high Sb loading is administered, which may occur with infiltration of large volumes of the drug. This study provides novel and useful information that can support a safe implementation of a new therapeutic approach for CL insofar as it confirms the effectiveness of the absorption by intralesional administration route and alerts to the need to control the daily doses of Sb used.

Acknowledgements

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico for funding approved through the Universal Project (Process number 405407/2016-5).

References

1. Guerrero ET, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R. Leishmaniasis: a review. *F1000 Res* 2017: **6**: 750.
2. Kip AE, Schellens JHM, Beijnen JH, Dorlo TPC. Clinical pharmacokinetics of systemically administered antileishmanial drugs. *Clin Pharmacokinet* 2018: **57**: 151–176.
3. WHO. World Health Organization Map Production: Control of Neglected Tropical Diseases (NTD) World Health Organization. (Available from: http://www.who.int/lesihmaniasis/burden/en/.) Assessed at 22 Jun2 2018.
4. Oliveira LF, Schubach AO, Martins MM *et al.* Systematic review of the adverse effects of cutaneous leishmaniasis treatment in the New World. *Acta Trop* 2011: **2**: 87–96.
5. Blum J, Lockwood DN, Visser L *et al.* Local or systemic treatment for New World cutaneous leishmaniasis? Re-evaluating the evidence for the risk of mucosal leishmaniasis. *Int Health* 2012: **4**: 153–163.
6. WHO. Control of the leishmaniases: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases: World Health Organization; 2010.
7. PAHO - Organization Panamericana de La Salud. Leishmaniasis em lãs Americas: recomendaciones para El tratamiento. Washington (DC); 2013. Available from: http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&gid=22226&Itemid=270&lang=en
8. Brito NC, Rabello A, Cota GF. Efficacy of pentavalent antimoniate intralesional infiltration therapy for cutaneous leishmaniasis: A systematic review. *PLoS ONE* 2017: **9**: e0184777.
9. Neves DB, Caldas ED, Sampaio RNR. Antimony in plasma and skin of patients with cutaneous leishmaniasis—relationship with side effects after treatment with meglumine antimoniate. *Trop Med Int Health* 2009: **14**: 1515–1522.
10. Olliaro P, Vaillant M, Arana B *et al.* Methodology of clinical trials aimed at assessing interventions for cutaneous leishmaniasis. *PLoS Negl Trop Dis* 2013: **7**: e2130.
11. Cruz A, Rainey PM, Herwaldt BL *et al.* Pharmacokinetics of antimony in children treated for leishmaniasis with meglumine antimoniate. *J Infect Dis* 2007: **195**: 602–608.
12. Brasil, Agência Nacional de Vigilância Sanitária. Resolução RDC 27, Dispõe sobre os requisitos mínimos para validação de métodos bioanalíticos em estudos com fins de registro e pós-registro de medicamentos; Ministério da Saúde. Brasília – DF, 2012.
13. Zaghloul IY, Radwan MA, Al Jaser MH, Al Issa R. Clinical efficacy and pharmacokinetics of antimony in cutaneous leishmaniasis patients treated with sodium stibogluconate. *J Clin Pharmacol* 2010: **50**: 1230–1237.
14. Jaser MAL, El-Yazigi A, Croft SL. Pharmacokinetics of antimony in patients treated with sodium stibogluconate for cutaneous leishmaniasis. *Pharm Res* 1995: **12**: 113–116.
15. Miekeley N, Mortari SR, Schubach AO. Monitoring of total antimony and its species by ICP-MS and on-line ion chromatography in biological samples from patients treated for leishmaniasis. *Anal Bioanal Chem* 2002: **372**: 495–502.
16. Chulay JD, Fleckenstein L, Smith DH. Pharmacokinetics of antimony during treatment of visceral leishmaniasis with sodium stibogluconate or meglumine antimoniate. *Trans R Soc Trop Med Hyg* 1988: **82**: 69–72.
17. Coelho DR, Miranda ES, Saint'Pierre TD, Paumgartten FJ. Tissue distribution of residual antimony in rats treated with multiple doses of meglumine antimoniate. *Mem Inst Oswaldo Cruz* 2014: **109**: 420–427.
18. Abdallah A, Saif M. Trace studies with antimony 124 in man. In: Wolstenholme GEW, O'Connor M (eds). *Bilharziasis*. Churchill: London, 1962, 287–309.

19. Dorea JG, Merchan-Hamann E, Ryan DE, Holzbecher J. Retention of antimony in skin biopsies of Leishmaniasis patients after treatment with N-ethylglucamine antimoniate. *Clin Chem* 1990: **36**: 680–682.

Corresponding Author **Marta Gontijo de Aguiar**, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Departamento de Produtos Farmacêuticos. Avenida Presidente Antônio Carlos, no 6627, Campus Pampulha, CEP: 31270-901, Belo Horizonte, MG, Brazil. E-mail: martagontijo@ufmg.br