PHARMACOKINETICS AND TISSUE DISTRIBUTION OF ANTIMONY$^{(V)}$ AFTER MULTIPLE INTRAMUSCULAR ADMINISTRATIONS IN THE HAMSTER

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إن مصير عنصر الأنتيمون خماسي التكافؤ في الأنسجة المختلفة للجسم بعد التعاطي العضلي له أهمية خاصة وذلك للدراسة المستقبلية للعلاج بالأدوية المحتوية على عنصر الأنتيمون خماسي التكافؤ في المنظومات المختلفة. ولقد تم دراسة حركية الدواء وتوزيع عنصر الأنتيمون خماسي التكافؤ في فئران هامستر بعد جرعة يومية من مادة ستيبوجلوكونات الصوديوم مكافئة لـ 120 مغ/كغ من عنصر الأنتيمون خماسي التكافؤ يتم إعطاؤها عضلياً لمدة أسبوعين. وتم عزل كل من الكبد والطحال والقلب والكليتين وأنسجة الجلد بعد جمع الدم عند أوقات محددة ثم تم قياس تركيز عنصرالأنتيمون خماسي التكافؤ في هذه الأنسجة، وذلك بعد المعالجة المناسبة وتحويلها إلى رماد، بواسطة مطياف الامتصاص الذري معدوم اللهب. وأظهر رسم تراكيز عنصر الأنتيمون خماسي التكافؤ في الدم بدالة الزمن تناقصاً خطياً سريعاً بعمر نصف حيوي مقداره 1.7 ساعة. وقد تناقص تركيز الدواء (مكغ/غ) بطريقة ثنائية الطور من جميع الأنسجة تقريباً. ومع ذلك فإن تراكيز عنصر الأنتيمون خماسي التكافؤ قد تناقصت من أنسجة فئران هامستر بطريقة أبطأ من التناقص من دمائها. وكان أقصى تركيز لعنصر الأنتيمون خماسي التكافؤ في أنسجة الكلية هو (3416 ± 631 مكغ/غ) بينما كان أدنى تركيز في الطحال هو (209 ± 187 مكغ/غ). وقد بلغ أقصى تركيز لعنصر الأنتيمون خماسي التكافؤ في الكلية (مكغ/غ) أكثر من 25 ضعفاً أكثر مما تم قياسه في الدم (مكغ/مل). وكان ترتيب المساحة تحت المنحنى لعنصر الأنتيمون خماسي التكافؤ في الأنسجة على النحو التالي: الكليتين > الكبد > الجلد > الطحال > القلب > الدم. وقد كان عمر النصف الحيوي لعنصر الأنتيمون خماسي التكافؤ في القلب والطحال والكبد متساوياً (5.2 – 6.2 ساعة) بينما بلغ عمر النصف الحيوي في الكليتين والجلد 3 ساعات. وهكذا فإن حركية الدواء لعنصر الأنتيمون خماسي التكافؤ يمكن وصفها بنموذج متعدد الحجرات ، قد وجد أعلى تركيز للدواء المحتوي على عنصر الانتيمون خماسي التكافؤ كان في الكليتين وهذا قد يؤدي إلى حدوث تسمم في الكلية بعد العلاج طويل المدى بهذا النوع من الدواء.

The fate of pentavalent antimony (Sb^V) in different tissues in the body after intramuscular administration is of great interest for the future study of Sb^V therapy in different sitting. Pharmacokinetics and tissue distribution of antimony (Sb^V) were studied in the hamster after daily dose of sodium stibogluconate equivalent to 120 mg $kg^{-1}$of Sb^V, administered intramuscularly for two weeks. Liver, spleen, heart, kidney and skin tissues were isolated after blood collection at the specified time. Antimony was measured in these tissues after suitable treatment, ashing and processing, by flameless atomic absorption spectrophotometry. The concentrations of Sb^V time profile in blood showed a linear rapid decline with elimination half life ($t_{1/2}$) of 1.7 h. The concentration of drug (µg/gm) declined in a biphasic manner from almost all tissues. However, the concentrations of Sb^V were declined in slower fashion from the hamster tissues than from the blood. The maximum concentration of Sb^V was determined in the kidney tissues (3416 ± 631 µg/gm) while the lowest concentration was in the spleen (209 ± 187 µg/gm). The maximum concentration of Sb^V in the kidney (µg/gm) was more than 25 fold higher than that measured from

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blood (µg/ml). The AUC of Sb^V in the studied tissues was in this rank: kidney > liver > skin > spleen > heart > blood. Surprisingly, the heart, spleen and liver showed a similar $t_{1/2}$ of 5.2-6.2 h while the kidney and skin had a $t_{1/2}$ of about 3 h. Therefore, disposition of Sb^V seems to kinetically follow multicompartmental compartmental model. The kidneys got the highest concentration of drug which may lead to nephrotoxicity on long term therapy.
Keywords: Antimony, pharmacokinetics, tissue distribution, atomic absorption, hamsters

Introduction

Leishmaniasis is a major tropical and subtropical parasitic disease. The yearly prevalence is estimated at 12 million people worldwide and 200–350 million people are at risk. In the Mediterranean region, leishmaniasis caused by *Leishmania infantum* has emerged as one of the important opportunistic infections of human immunodeficiency virus (HIV)-positive individuals (1,2). Moreover, the prevalence of canine leishmaniasis in this region may be as high as 42%. Dogs and wild canids are important reservoirs and are mainly responsible for the persistence of the disease in this region (3, 4). Pentavalent antimonial agents (Sb^V) in the form of sodium stibogluconate (Pentostam^R) or N-methyl-D-glucamine antimoniate (Glucantime^R) are the first line drugs for the treatment of visceral leishmaniasis (VL) and the New World Cutaneous leishmaniasis (CL) (1,5). Despite their clinical use for over half a century, the mode of action of pentavalent antimonials remains poorly understood. The two pentavalent antimony compounds are thought to be of comparable efficacy and safety (6, 7). Following an intramuscular injection in the dog, peak plasma levels are reached within 2 hours. The drug distributes throughout the extracellular body space with a volume of distribution of 0.22 L/kg (8). Pentavalent antimonials are probably not metabolized in the body. Elimination is characterized by two phases: an initial phase with a plasma elimination half-life ($t_{1/2}$) of around 2 hours, followed by a slow elimination phase with a $t_{1/2}$ of between 33 and 76 hours (8). More than 80% of Sb^V was excreted in urine in the first nine hours after IV administration of 100 mg/kg of N-methylglucamine antimoniate containing 27.2% Sb^V (9). It was reported (10) that increasing Sb^V dose from 20 to 40 mg/kg/day resulted in sever nephrotoxic effect and an increase in the number of leucocytes at urinary sediment examination. The fate of Sb^V in the body after intramuscular administration (IM) is of great interest for the future study of Sb^V therapy in different sitting. However, because of the rapid blood clearance of these drugs (11-13), an increase in dose and dosing frequency has been Recommended by the World Health Organization (14). The amount of exposure of the infectious parasite to Sb^V is believed to be an important factor in eradicating the cutaneous leishmania disease. 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 Sb^V. 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.

In Saudi Arabia, VL as well as CL is endemic, but CL is more prevalent throughout the country with annual reported cases of more than 4,400 cases per year (Saudi Ministry of Health, 2002). There are several studies of Sb^V disposition after IM administrations in human and animals (5,12,13,15-19). However, the majority of these studies has placed more emphasis on the biodistribution of Sb^V in blood (12,13,15,17-19), skin (16) or some organ tissues (liver, spleen and skin) (5). Although blood kinetic data are clinically relevant to the extent that they reflect those of the site of action, the site of *leishmania* infection is generally not the blood but certain tissues, such as liver organ or skin tissue. Therefore, for infections, tissue concentrations rather than blood concentrations determine the clinical outcome and may be expected to allow a better prediction of the therapeutic effect than blood concentrations. To our knowledge no such study has yet been reported the tissue disposition of antinomy in different organ and its relation to blood pharmacokinetics. Therefore, this investigation undertook to examine the pharmacokinetics of Sb^V in blood and its distribution in different organ tissues after daily administrations of IM sodium stibogluconate in the hamster.

Materials and methods

Materials:

Pentostam® is a commercial product of Burroughs Wellcome, Research Triangle Park, NC, USA. All other reagents and chemicals were analytical grade, and were used as received.

Hamsters' dosing scheme:

Thirty Six male Syrian hamsters (132 ± 15 g) were randomly divided into 6 groups for different sampling time. The hamsters were given IM a daily dose Sb^V (120 mg/kg) for two weeks. Each group (n = 6) was housed in one cage and was used for each data point. At the specified time blood samples (0.5 ml) were collected, from the orbital venous plexus from one group at 30 min, 1, 2, 4, 6 and 12 h following each administration at the end of the two weeks. Therefore, each data point is the mean of 6 replicates. Hamsters were lightly anesthetized with halothane during blood sampling. Then the animals were excised and the liver, spleen, heart, kidney and skin tissues were isolated. Every tissue was pressed between two filter papers, rinsed with normal saline and pressed between two filter papers again to remove any blood. Each of the collected tissues was accurately weighed before storing in liquid nitrogen at -70^{o}C till assayed for Sb^V content while the wholel blood was maintained at 4^{o}C till assayed as described below.

Determination of antimony in whole blood Antimony was measured in whole blood by flameless atomic absorption spectrophotometer (15). Briefly, a solution of Triton X-100 (2% v/v) was used for dilution of blood samples prior to injection into the furnace. Depending upon the expected concentration of $Sb^{(V)}$, the dilution ratio was either 1:19 (i.e. 0.05 ml blood diluted to1 ml with Triton solution, for samples collected within the first two hours of drug administration) or 1:4 (i.e. 0.2 ml blood diluted with 0.8 ml Triton solution, for the remaining samples). After the mixture was vortex-mixed for 30 s. and centrifuged at 1000g for 5 min, 0.5 ml of the clear solution was transferred into an atomic absorption plastic sample cup. The instrument (AA-680 Shumatzu, Japan) was fully automated and the parameters used were those recommended by the manufacturer. The autosampler was programmed to inject 20 µL of the diluted sample (n=3).

Determination of antimony in the tissues:

The tissues were thawed and suitable weight of the collected tissues were recorded and digested by heating for 3 h in a crucible with 2 ml of a mixture of concentrated nitric and sulphuric acids (1:1, v/v). The residue was ashed in an ashing oven for 6 h. The white ash was dissolved in 0.5 ml of 1% HCl solution by sonication for 2 min. The solution was then transferred to small plastic cups, and Sb^V was measured by electrothermal atomic absorption spectrophotometry in the same manner applied for blood. Normal tissue samples, free from Sb^V, were used as blank or spiked with Sb^V for the standard curve calculation, and were treated as mentioned before. Antimony tissue concentrations were converted to µg/g for comparisons to blood concentrations. Tissue to blood partition coefficients (K_p) were calculated according to the following equation (20).

$$K_p = \frac{C_T}{C_b(1-E)}$$

Where C_T (µg/g) and C_b (µg/mL) are the tissue and blood maximum concentrations at steady state, respectively, and E stands for the tissue extraction ratio. Since Sb^V is mainly excreted in the urine (11), the E value was set equal to zero for all tissues.

Evaluation of kidney function:

To evaluate renal function, the serum of the collected blood samples, at the end of the two weeks study, was separated by centrifugation at 4000 rpm for 15 min. The serum concentration of creatinine (Sr_{Cr}) and blood urea nitrogen (BUN) in each of these samples were then determined colorimetri-cally, using commercial kits from BioSystems (Barcelona, Spain) and Pierce Chemical (Rockford, IL, USA), respectively.

Data analysis:

All calculations were performed on Microsoft Office Excel 2003 and the results were expressed as mean ± SD. Pharmacokinetic parameters were estimated using model-independent methods (21). The data points were arbitrary divided into 6 groups but all data belong to each animal blood and tissues got the same number for ratios calculations. The terminal elimination rate constant (λ_n) was estimated by linear regression analysis of the terminal portion of the log-linear blood concentration-time profile of a drug. The mean peak drug concentration (C_{max}) and the time to reach C_{max} (T_{max}) were derived directly from the individual blood levels. The area under each drug concentration time curve (AUC, µg ml^{-1} h) to the last data point were calculated by the linear trapezoidal rule and extrapolated to time infinity by the addition of C_{Last}/λ_n where, C_{Last} is concentration of the last measured blood sample. The

area under the first moment (AUMC) was determined using the same rules followed for AUC calculation. The mean residence time (MRT) was estimated from MRT = AUMC/AUC and terminal elimination half-life ($t_{1/2}$) was calculated from the terminal elimination rate constant using the formula $t_{1/2} = 0.693 / \lambda_n$. The apparent total clearance (Cl/F = (Dose/ AUC)) and apparent volume of distribution at steady state (V_{SS}/F = (Cl * MRT)) were also calculated. All data were compared using the *t*-test, ANOVA, and Tukey's tests. Statistical significant differences were assumed when $p < 0.05$.

Results and Discussion

The mean Sb^V blood and different tissues concentration-time profiles obtained at steady state after IM dosing of 120 mg kg^{-1} to hamsters are depicted in Figure 1 and 2. The mean pharmacokinetic parameters derived from a noncompartmental analysis of Sb^V in blood and different tissues are presented in Table 1. The absorption of Sb^V from the intramuscular tissue was rapid with C_{max} values from 128.4 to 167.2 (151 ± 13) µg mL^{-1} in blood appeared 0.5 to 1 h after administrations. After reaching C_{max}, the blood drug level decreased monoexponentially, Figure 1, with $t_{½}$ of 1.7 ± 0.12 h (r = 0.97). It is also shown that, Sb^V was extensively distributed into many organs reaching higher tissue concentrations than blood at most of the time post dosing. The concentration of drug (µg/gm) declined in a biphasic manner from almost all tissues. However, the concentrations of Sb^V were declined in slower fashion from the hamster tissues than from the blood.

The mean of the terminal logarithmic concentrations time profile of Sb^V declined from tissues with r ranging from 0.90 to 0.99. However, the disposition half-life of Sb^V in tissues was different than that of blood. Liver, spleen and heart showed $t_{½}$ in the range of 5.4 to 6.3 h, while the kidney and skin had the same $t_{½}$ value of 2.95 h. Therefore, a multicompartmental model for Sb^V disposition could be the explanation of the difference in $t_{½}$ among tissues. Since the number of data points was limited, authentication of this postulation was not there. The apparent volume of distribution of Sb^V at steady state was 0.72 L/kg. These results are similar to that obtained with lithium (22-25).

Lithium, a small cation unlinked to proteins, diffuses in the entire organism: the volume of distribution varies from 0.7 to 1.0 L/kg according to the species (22). Studies carried out on tissue distribution of lithium in rats and in chickens are in agreement with the use of a three-compartment open model, demonstrating that three groups of tissue compartments can be observed following intravenous injection. In some tissues (e.g. the kidney), lithium displays similar kinetics to plasma (central compartment). In the majority of other tissues, the maximum concentration is reached approximately 1 h after the intravenous injection (shallow peripheral compartment), with the exception of erythrocytes, bones and the brain where lithium penetrates and is eliminated slowly (deep peripheral compartment) (22-25).

The apparent blood clearance (Cl/F) is 0.29 L/h/kg and it is approximately the summation of all the apparent organs' clearances of Sb^V for all the studied tissues (0.301 L/h/kg). Interestingly, the lowest Cl/F of Sb^V was from the spleen (0.009 L/h/kg) while the highest Cl/F was from the kidneys (0.10 L/h/kg), the eliminating organ of Sb^V among the examined tissues.

Table 1. Mean Pharmacokinetic parameters of Sb^V (± SD) at steady - state after multiple IM administrations of 120 mg kg^{-1} in blood and different tissues in hamsters (n = 6).

Parameters	Blood	Liver	Kidney	Spleen	Heart	Skin
C_{max}, µg/ml or µg/g	151± 13	290±31	3478±573*	235±160	261.5±59	1077±324*
T_{max}, h	0.83±0.26	1.67±2.2	0.67±0.26	1.17±0.68	0.5±0	0.5±0
$AUC_{0-\infty}$, µg ml^{-1} h	413	2264	13516	1163	1332	1712
$t_{1/2}$, h	1.7	5.7	2.95	5.4	6.3	2.95
MRT, h	2.1	8.2	4.0	5.7	4.6	2.4
Cl/F, L/h/kg	0.29	0.05	0.009	0.10	0.076	0.066
V_{ss}/F, L/kg	0.72					

* Statistically significant at $p<0.001$

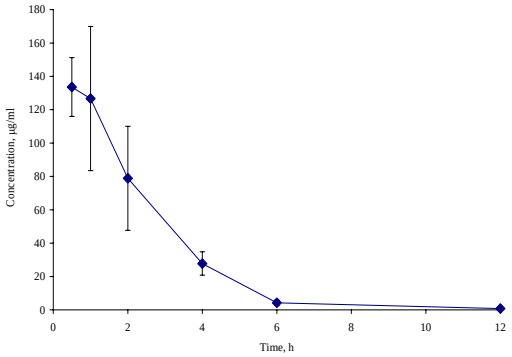

Figure 1. Mean (± SD) blood concentration (µg/ml) - time profiles after multiple IM administrations of 120 mg kg^{-1} of Sb in the hamster (n = 6).

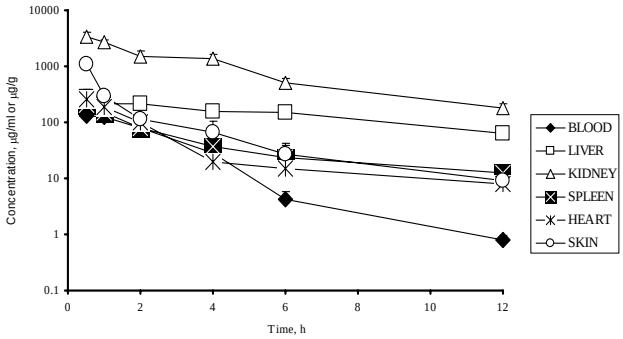

Figure 2. Mean (± SD) blood or tissue concentration (as µg/ml or µg/gm) - time profiles after multiple IM administrations of 120 mg kg^{-1} of Sb in the hamster (n = 6).

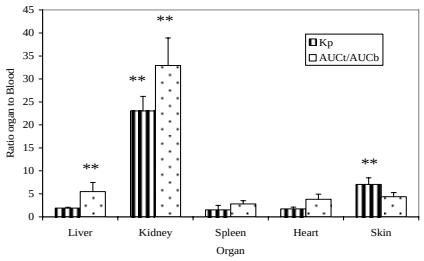

Figure 3. Ratios of tissue–to–blood levels (Mean ± SD) K_p achieved at C_{max} and AUC_T/ AUC_b following multiple IM administrations of 120 mg kg^{-1} of Sb in the hamster (n = 6).
** $p<0.001$

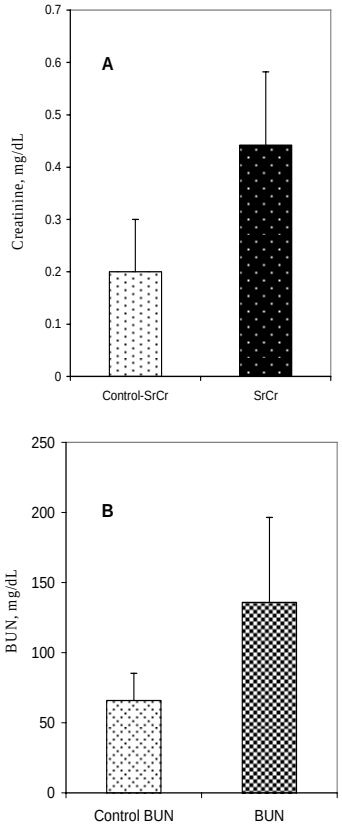

Figure 4. The effect of antimony administration on the mean (± SD) creatinine concentration (panel A) and BUN (panel B) after multiple administrations.(n = 6).

Berman and his group (5) reported that tissue disposition of Sb^V up to 48 h in liver, spleen and skin and compared that with serum after 150, 300 or 600 mg/kg (n = 3 for each time point). Analyzing their reported data for the 300 mg dose (150 mg dose was not tabulated) showed that Sb^V in the liver and skin declined identically with time but different from serum and spleen, which had the lowest Sb^V concentrations. The present study in agreement with that the decline was different among tissues and spleen had the lowest concentrations. Although from their tabulated data the $t_{½}$ of Sb^V in the liver or the skin was about 14 h, and it was 1.1 and 4.4 h in the serum and spleen, respectively.

Al Jaser et al. (16) reported the pharmacokinetics of Sb^V in the affected skin and normal skin

of patients infected with cutaneous leishmaniasis and compared the results with those for the blood after 600 mg IM dosage. The mean concentration of Sb^V in skin was smaller than that in blood for the first 4 h following drug administration but became larger thereafter. Although the C_{max} of Sb^V in blood was significantly higher than that in normal skin, their areas under the curves were comparable. Therefore, their finding, in patients was not in agreement with the present investigation in hamsters.

In order to normalize the results, an assessment of tissue levels near peak blood concentration after multiple dosing over two weeks was performed. It revealed that the highest Sb^V concentrations were in this rank: kidney> liver > skin > heart > spleen. The tissue-to-blood level ratios (K_p) were calculated as shown in Figure 3. Similar drug tissue affinities were found in liver, heart and spleen where there was no significant difference ($p>0.05$) in their K_p values. On the other hand a significant difference ($p<0.001$) in the K_p values corresponding to the kidney and skin was detected compared to the other tissues. The average Sb^V levels achieved in the kidneys and the skin were 23 (± 3) and 7 (± 1.4)-fold above blood levels, respectively while the spleen showed the lowest K_p values (1.5 ± 0.98) among all tissues. These results were not in agreement with the Berman and co workers (5) discussed earlier in that the K_p was in the order skin > spleen > liver. It should be mentioned that the discrepancy in results among researchers could be attributed to the difference in subjects used, dosage regimen or animal handling besides the animals in the present study were not infected.

The tissue /blood AUC ratios (AUC_T/AUC_b) for each tissue was also calculated at steady state to measure the extent of drug distribution in tissues compared to blood at all times. The same trend was observed with the kidneys showed the highest ratios (about 33-fold higher, $p<0.001$) while the spleen was the lowest (about 3-fold higher) but the liver ($p<0.001$) area ratio was higher than that of the skin. This indicates that although the skin showed a higher C_{max} than the liver, the extent of drug distribution was higher in the liver than that in the skin. Therefore, Sb^V distribution was in this order kidneys > liver > skin > heart > spleen.

To evaluate Sb^V toxicity to the kidney, creatinine concentration and blood urea nitrogen (BUN) were measured. Figure 4 shows the changes in creatinine or BUN after two weeks of Sb^V although the means were higher than the controls, both were not statistically significant. Therefore, Sb^V toxicity to the kidney exited but would take longer time to show its evidence.

In conclusion, this investigation have shown that antimony is declined rapidly from blood and distributed quickly to tissues with the highest concentrations in the kidneys where elimination takes place. Antimony is also distributed to the skin where CL is localized. Even though numerous publications are continuously added to the literature about antimony, it needs further investigations about its disposition in different organs for longer duration with different doses for better understanding of this drug.

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