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Article

Influence of the Nucleobase on the Physicochemical Characteristics and Biological Activities of Sb^{V}-Ribonucleoside Complexes

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A influência da nucleobase (uracila, U; citosina, C; adenina, A; guanina, G) nas propriedades físico-químicas e atividades biológicas *in vitro* dos complexos Sb^{V}-ribonucleosídeo foi investigada. Os complexos 1:1 Sb-U e Sb-C foram obtidos no estado sólido e caracterizados por RMN, ESI-MS e análise elementar. As constantes de estabilidade e as constantes aparentes de velocidade de formação e dissociação dos complexos 1:1 Sb-U, Sb-C e Sb-A foram determinadas. Embora o Sb^{V} se ligue através dos mesmos átomos de oxigênio das posições 2' e 3' nos diferentes nucleosídeos, as mudanças conformacionais na ribose e as propriedades físico-químicas do complexo dependem da nucleobase. A nucleobase influenciou fortemente as atividades citotóxica e leishmanicida dos complexos Sb^{V}-ribonucleosídeo. Os complexos Sb^{V}-nucleosídeos purínicos foram mais citotóxicos e eficazes contra a *Leishmania chagasi* do que os pirimidínicos, o que reforça o modelo de que a interação do Sb^{V} com os nucleosídeos purínicos pode mediar a atividade dos medicamentos antimoniais.

The influence of the nucleobase (uracyl, U; cytosine, C; adenine, A; guanine, G) on the physicochemical characteristics and *in vitro* biological activities of Sb^{V}-ribonucleoside complexes has been investigated. The 1:1 Sb-U and Sb-C complexes were characterized by NMR and ESI-MS spectroscopies and elemental analysis. The stability constant and the apparent association and dissociation rate constants of 1:1 Sb^{V}-U, Sb^{V}-C and Sb^{V}-A complexes were determined. Although Sb^{V} most probably binds *via* oxygen atoms to the same 2' and 3' positions in the different nucleosides, the ribose conformational changes and the physicochemical characteristics of the complex depend on the nucleobase. The nucleobase had a strong influence on the cytotoxicity against macrophages and the antileishmanial activity of the Sb^{V}-ribonucleoside complexes. The Sb^{V}-purine complexes were more cytotoxic and more effective against *Leishmania chagasi* than the Sb^{V}-pyrimidine complexes, supporting the model that the interaction of Sb^{V} with purine nucleosides may mediate the antileishmanial activity of pentavalent antimonial drugs.

Keywords: antimony, kinetics, nucleosides, cytotoxicity, leishmaniasis

Introduction

Two pentavalent organoantimonial complexes, meglumine antimoniate (MA) and sodium stibogluconate, are the first line drugs for the treatment of all forms of leishmaniasis. Despite their clinical use for more than half a century, the mode of action of these drugs remains poorly understood.[1] It is still not clear whether the final active form of pentavalent antimonials is Sb^{V} or Sb^{III}. According to a first model, Sb^{V} would behave as a prodrug, being reduced within the organism into more toxic and active Sb^{III}.[2]

The formation of 1:1 and 1:2 Sb^{V}-ribonucleoside complexes was evidenced by electrospray ionization mass spectrometry (ESI-MS), nuclear magnetic resonance (NMR) and circular dichroism,[3-6] suggesting the involvement of these complexes in the mode of action of pentavalent antimonials. The two different 1:1 and 1:2 Sb^{V}-adenosine complexes were obtained individually and characterized in the solid state.[5] Moreover, Sb^{V} was found to react with guanosine forming a hydrogel containing a mixture of 1:1 and 1:2 Sb^{V}-guanosine

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complexes. The failure to detect any complex formed between Sb^V and 2'-deoxyadenosine, adenosine-3,5-cyclic monophosphate and 2'-deoxyribose using HPLC-ES-MS (high performance liquid chromatography - electrospray mass spectrometry) and HPLC-ICP-MS (high performance liquid chromatography - inductively coupled plasma - mass spectrometry) analyses supported the specific binding of Sb^V to vicinal 2'- and 3'-hydroxyl groups.[3,5]

Kinetic and thermodynamic characterization of the reaction of Sb^V with 5'-GMP (guanosine monophosphate) showed that complexation was faster at acidic pH,[4] indicating that it would be kinetically favored in the acidic biological compartments in which *Leishmania* parasite resides. Moreover, the value of the stability constant determined for the 1:1 Sb^V-5'-GMP complex[4] is consistent with the formation of such complex in the vertebrate host following treatment with pentavalent antimonial drugs, especially if one considers the high accumulation and prolonged retention of antimony in macrophages[7] which harbor *Leishmania* parasites. Another remarkable property of Sb^V-5'-GMP complexes is their low dissociation rate constant in aqueous solutions at neutral pH.[4]

A model has been suggested for the possible involvement of Sb^V-nucleoside complexes in the leishmanicidal action of pentavalent antimonials. Since *Leishmania* is a true auxothroph for purine, it has been proposed that Sb^V-purine nucleoside derivatives may inhibit *Leishmania* purine transporters or act as potent enzyme inhibitors of the purine salvage pathway, as already reported in the case of other purine analogs.[8]

Although much progress has been achieved regarding the physicochemical characterization of Sb^V-ribonucleoside complexes, their pharmacological relevance and the possible influence of the nucleobase on their physicochemical and biological actions still have to be investigated. Accordingly, this paper reports the influence of the nucleobase on the physicochemical characteristics, as well as the cytotoxicity and *in vitro* antileishmanial activity of Sb^V-ribonucleside complexes.

Experimental

Materials

Cytidine (C), uridine (U), adenosine (A) and guanosine (G) were obtained from Sigma Chemical Co. (St. Louis, MO). Potassium antimoniate ($[KSb(OH)_6]$) was obtained from Fluka Chemie GmbH (> 99% purity). $SbCl_5$ (99%) and *N*-methyl-*D*-glucamine (99%) were obtained from Aldrich Chemical Co. (Milwaukee, Wis). All other reagents were of at least reagent grade. Double-distilled-deionized water was used throughout the experiments.

General experimental techniques

C, H and N analyses were carried out using a Perkin-Elmer 240 Elemental Analyzer. Antimony content was determined by atomic absorption using a HITACHI Z 8200 spectrophotometer. Sb and K were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer plasma emission spectrometer, model Optima 3000. The content of crystallization water molecules of the synthetic products was determined by thermogravimetric analysis (TGA) using a Shimadzu TGA-50 instrument operating under a nitrogen atmosphere.

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX400-AVANCE (^1H: 400 MHz; ^{13}C: 100 MHz) spectrometer using D_2O as solvent and tms (3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid, sodium salt) as an internal reference. Heteronuclear multiple quantum correlation (HMQC) and heteronuclear multiple bond coherence (HMBC) spectra for C, U and their complexes with Sb^V were obtained in D_2O. Standard pulse sequences were used for homo- and heteronuclear correlation experiments. The proportions of each species (free ribonucleoside (Rb) and 1:1 Sb-Rb complex) were calculated by integration of the ^1H NMR signals corresponding to H-6. The chemical shift for the H-6 of U was assigned according to a previous reference.[9]

Electrospray mass spectrometry (ESI-MS) analyses were performed on a 2000 QTrap Applied Biosystem equipment. ESI-MS was acquired in the negative mode using H_2O/CH_3OH (1:1, v/v) solutions of each compound and spraying the solution mixture through the ESI source at 25 °C. Values of *m/z* are reported for the principal ion, that is, for the most abundant isotopomer of the cluster ion.

Preparation of the Sb^V-C and Sb^V-U complexes in the solid state

About 0.9 mmol of ribonucleoside was dissolved in 15 mL of water and 0.45 mmol of $[KSb(OH)_6]$ was added to this solution. The pH of the mixture was adjusted to 7.0 and the solution was kept under stirring at 25 °C. After 24 h, a white precipitate was formed. This precipitate was washed with acetone and dried over $CaCl_2$. A reaction yield of 30-40% was determined.

Sb^V-cytidine complex: Elemental analysis, found: C, 20.69; H, 3.84; N, 8.02; Sb, 23.50; K, 7.86%. Calc. for $C_9H_{15}KN_3O_9Sb$ ($C_9H_{15}KN_3O_9Sb$ + 3 H_2O): C, 20.61; H, 4.00; N, 8.01; Sb, 23.22; K, 7.50%. mp 225 °C (water).

Sb^V-uridine complex: Elemental analysis, found: C, 21.60; H, 3.39; N, 5.40; Sb, 24,04; K, 7.67%. Calc. for $C_9H_{14}KN_2O_{10}Sb$ ($C_9H_{14}KN_2O_{10}Sb$ + 1.7 H_2O): C, 21.53; H, 3.48; N, 5.58; Sb, 24.18; K, 7.76%. mp 225 °C (water).

Preparation of meglumine antimoniate, Sb^V-adenosine (Sb^V-A) complex and Sb^V-guanosine (Sb^V-G) hydrogel

Meglumine antimoniate was synthesized, according to Demicheli *et al.*[10], from an equimolar mixture in water of *N*-methyl-*D*-glucamine and freshly precipitated, hydrated antimony pentoxide obtained from $SbCl_5$ previously hydrolyzed in water. After precipitation with acetone, the resulting product was dried. The meglumine antimoniate product contained 29% of Sb by weight, which corresponds to the expected theoretical value for 1:1 Sb-*N*-methyl-*D*-glucamine complex [$C_7H_{17}NO_5 \cdot HSbO_3 \cdot 3H_2O$].

The 1:1 Sb^V-A complex was obtained in the solid state, as described previously,[5] with a reaction yield of 20-25%.

The Sb^V-G hydrogel was prepared as reported previously.[5] Briefly, G and [$KSb(OH)_6$] were co-dissolved in H_2O at 1:0.75 molar ratio, the pH was adjusted to 5 and the resulting mixture was kept under stirring at 60 °C for 1.5 h. After cooling at 25 °C, a translucent hydrogel was obtained.

Formation rate constants of 1:1 Sb^V-ribonucleoside complexes

The initial rates of complex formation (V_i) were calculated from the concentration of complex formed after 5 min of reaction between potassium antimoniate and the ribonucleoside in D_2O. The complex concentration was determined through integration of ^{1}H NMR signals corresponding to H-6. In all aqueous solutions (D_2O), which contained 0.1 mol L^{-1} KCl, the pD was adjusted to 6.5 using DCl or NaOD and the reaction was run at 37 °C. The value of pD was obtained from pH measurements and calculated as: pD = pH + 0.4.[11]

The concentrations of nucleoside and antimony were typically either 10 or 20 mmol L^{-1}. In these initial conditions of reaction, the amount of complex formed did not exceed 15% of the total complex amount at equilibrium. Data were determined as the mean values from three independent experiments.

In the case of cytidine, the reaction partial orders, n and m, with respect to nucleoside (Rb) and Sb, respectively, were determined according to the following equations:

$$V_i = k_f[Rb]^n[Sb]^m \quad (1)$$

$$\ln V_i = n \ln[Rb] + m \ln[Sb] + \ln k_f \quad (2)$$

where [Rb] = initial concentration of nucleoside, expressed in mol L^{-1}, [Sb] = initial concentration of Sb (mol L^{-1}) and k_f = apparent rate constant for complex formation.

The reaction of cytidine with potassium antimoniate was run in three different conditions:

experiment 1: [Rb] = 5 mmol L^{-1} and [Sb] = 10 mmol L^{-1}
experiment 2: [Rb] = 20 mmol L^{-1} and [Sb] = 20 mmol L^{-1}
experiment 3: [Rb] = 20 mmol L^{-1} and [Sb] = 10 mmol L^{-1}.

From the results of experiments 1 and 3 and equation 2, the reaction partial order n was determined as n = 1.0 ± 0.2. From the results of experiments 2 and 3 and equation 2, the reaction partial order m was determined as m = 0.9 ± 0.2.

According to these results, k_f could be determined from equation 3:

$$V_i = k_f[Rb][Sb] \quad (3)$$

k_f was determined for each nucleoside (C, U and A) as the mean value obtained from three independent experiments.

Dissociation rate constants for 1:1 Sb^V-ribonucleoside complexes

First, 1:1 Sb^V-nucleoside complexes (Sb^V-C, Sb^V-U, Sb^V-A or Sb^V-G) were formed through incubation of 20 mmol L^{-1} nucleoside with 20 mmol L^{-1} potassium antimoniate for 2 h at 60 °C at pD 6.5. Dissociation was induced by diluting the mixture from 20 to 1 mmol nucleoside L^{-1} in D_2O containing 0.1 mol L^{-1} KCl. The decrease of the complex concentration was then followed as a function of time (0, 2, 4, 24 h) at 37 °C and pD 6.5. The apparent rate constant of complex dissociation (k_d) was determined from the following equation:

$$\ln[SbL] = k_d t + \ln[SbL]_0 \quad (4)$$

where [SbL] and $[SbL]_0$ are the molar concentrations of the 1:1 complex at time t and time zero, respectively. [SbL] was calculated through integration of ^{1}H NMR signals corresponding to H-6. k_d was determined, for each nucleoside, as the mean value obtained from three independent experiments.

Stability constants for 1:1 Sb^V-ribonucleoside complexes

Potassium antimoniate and nucleoside (C, U or A) were incubated in D_2O containing 0.1 mol L^{-1} KCl at 37 °C and pD 6.5, and the reaction was run until equilibrium as evidenced

by ^{1}H NMR. The nucleoside and antimony concentrations were typically in the range of 1 to 2 mmol L^{-1}. The stability constant (*K*) was determined from the following equation:

$$K = [SbL]/[Sb][L] \quad (5)$$

where [Sb] and [L] are the concentrations, expressed in mol L^{-1}, of free (non-complexed) antimoniate and nucleoside. [SbL] and [L] were calculated through integration of ^{1}H NMR signals corresponding to H-6. *K* was determined, for each nucleoside, as the mean value obtained from three independent experiments.

Biological assays of 1:1 Sb^V-ribonucleoside complexes

Parasites and animals

The *Leishmania (leishmania) chagasi* (MCAN/BR/2002/BH400) strain was maintained in hamsters (*Mesocricetus auratus)* experimentally infected and cryopreserved in the bank of strains (*Leishmania* Biology Laboratory / UFMG-Brazil). The *Leishmania (leishmania) chagasi* (MCAN/BR/2002/BH400) amastigotes used in the present study were obtained from the spleen of hamsters experimentally infected. The spleen was ground in RPMI-1640 (Cultilab, Brazil) with the aid of a tissue grinder and filtered through a thin nylon. The material was centrifuged at 3000 rpm for 10 min (Centrifuge Hermle, 2323 K) and then subjected to lysis solution for 10 min at 37 ºC. The resulting suspension was centrifuged and the amastigotes were suspended in RPMI-1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 IU mL^{-1} penicillin and 100 µg mL^{-1} streptomycin.

Balb/c mice (male, 6-8 weeks) were obtained from Cebio (Centro de Bioterismo do Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais). Free access was allowed to standard diet and tap water was supplied *ad libidum.*

Cytotoxicity assay (MTT assay)

The MTT assay is a semi-automated assay based on the ability of viable cells to reduce the tetrazolium salt MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] into a colored product, formazan, by the action of the mitochondrial enzyme succinate dehydrogenase. The original protocol was described by Mosmann, 1983.[12]

Thioglycollate (Gibco®) elicited peritoneal macrophages of Balb/c mice were harvested in RPMI 1640 medium. Cells were plated in 96-well plates (TPP, Switzerland®) in RPMI medium supplemented with 10% FBS, and allowed to adhere for 4h at 37 ºC in 5% CO_2. The non-adherent cells were then removed by washing with RPMI medium, and the macrophages which adhered to the plates were then treated with Sb^V-ribonucleosides complexes, meglumine antimoniate or antimoniate at Sb concentrations varying from 10 to 100 µg mL^{-1} for 72 h at 37 ºC under 5% CO_2. At the end of the period of incubation, 10 µL of MTT (Sigma®) was added to each well and plates were incubated for 4 h at 37 ºC in 5% CO_2. Enzyme reaction was then stopped by the addition of 100 µL of dimethylsulfoxide (dmso) solvent. The relative optical density (OD) was then measured at a wavelength of 570 nm using a spectrophotometer (ELISA reader).[13,14]

Anti-amastigote activity

Resident peritoneal cells from Balb/c mice were plated onto 13 mm^2 coverslips inside 24-well plates (TPP, Switzerland®) and allowed to adhere for 4 h at 37 ºC in 5% CO_2. The macrophages adhered to the coverslips were infected with *Leishmania chagasi* as described previously.[15] Amastigotes obtained from the spleen of experimentally infected hamsters were incubated with adhered macrophages for 2 h at 37 ºC, 5% CO_2, at a parasite/macrophage ratio of 10:1.[15] After the incubation period, non-adherent cells and free parasites were removed by washing with RPMI medium, and the culture was treated with Sb^V-ribonucleosides complexes, meglumine antimoniate or $[KSb(OH)_6]$, at 10 µg mL^{-1} or 30 µg mL^{-1} of Sb for 72 h at 37 ºC in 5% CO_2. Subsequently, the coverslips were removed from the wells, stained with Laborclin® and fixed to a slide with Canada Balsam (Vetec®). The number of amastigotes/macrophages and infected cells was determined by counting at least 300 macrophages in duplicate cultures, and the results were expressed as percentage of survival in comparison to that of the controls.

Results and Discussion

Physicochemical characterization of Sb^V-ribonucleoside complexes

The reaction of $[KSb(OH)_6]$ with cytidine (C) and uridine (U) led to the formation of 1:1 Sb^V-ribonucleoside complexes that were isolated in the solid state. ESI-MS analyses of these complexes in the negative mode revealed major ions at *m/z* 430 and 431, which can be assigned to $[SbU(OH)_4]^-$ and $[SbC(OH)_4]^-$, respectively (Supplementary Information).

The two new complexes (Figure 1) were further characterized by ^{1}H and ^{13}C NMR spectroscopy analysis. Tables 1 and 2 display the attribution of ^{1}H and ^{13}C NMR resonances for the Sb^V-C and Sb^V-U complexes. The assignment of the NMR resonances was achieved using

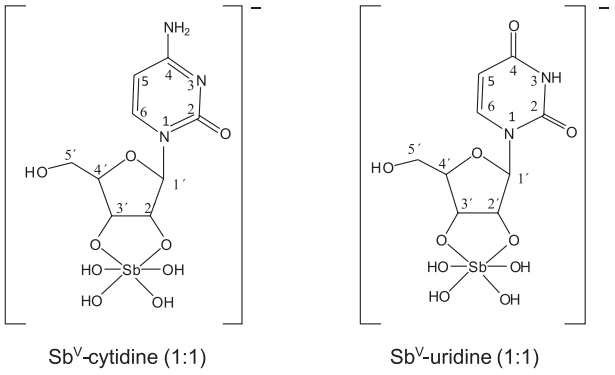

Figure 1. Structures proposed for the 1:1 Sb-C and Sb-U complexes.

HMBC and HMQC experiments, as illustrated for the Sb^{V}-U complex in Figures 2 A and B.

The most important changes in NMR parameters resulting from the formation of the complexes were observed for H-4', H-6 (shifted to lower frequencies), H2', H-3' (shifted to higher frequencies), C-1', C-4', C-5' and C-6 (high frequency shift). These can be attributed to a change in the sugar conformation upon binding of Sb^{V}. Interestingly, the profile of hydrogen resonance changes differed between the Sb^{V}-C and Sb^{V}-U complexes, whereas very similar profiles of carbon resonances changes were observed. Furthermore, these hydrogen resonances

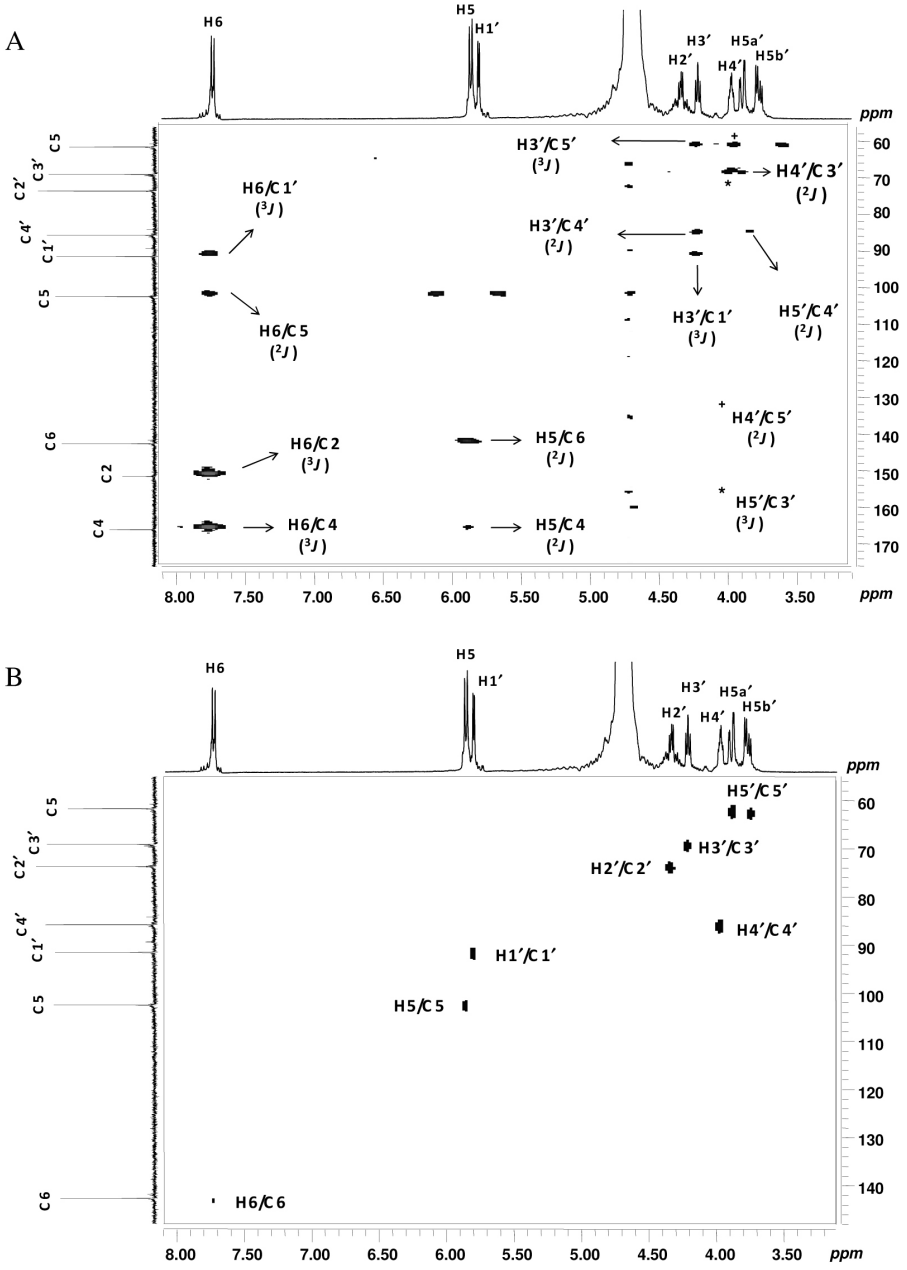

Figure 2. HMBC (A) and HMQC (B) spectra obtained for the Sb^{V}-U complex.

changes differ from those reported previously for the Sb^V-A complex,[5] which showed greater changes for H-1'and H-2'.

To further investigate the influence of the nucleobase on the binding of Sb^V to the ribose moiety, kinetic and thermodynamic parameters were determined for the formation of 1:1 Sb^V complexes with C, U, A and G ribonucleosides. Table 3 reports the stability constants (*K*) and apparent association and dissociation rate constants determined for the different Sb^V-ribonucleoside complexes at 37 ºC and pD 6.5. Strikingly, Sb^V-U showed a significantly higher value of stability constant when compared to Sb^V-C. Since these complexes exhibited comparable values of association rate constants but different dissociation rate constants, the dependence of *K* upon the nature of the pyrimidine nucleobase could be attributed to differences in the dissociation rate constants of the complexes. Other interesting features of the present data are the lower values of the association and dissociation rate constants of the Sb^V-A complex when compared to the other Sb^V-ribonucleosides complexes.

These data indicate that, although Sb^V most probably binds to the same donor atoms in the different nucleosides, the ribose conformational changes induced by its binding and the physicochemical characteristics of the resulting complex depend on the nucleobase.

Table 1. ^{1}H NMR parameters (400 MHz) for cytidine (C) and uridine (U) and their Sb^V derivatives (Sb^V-C and Sb^V-U)

Hydrogen	C δ / ppm	Sb^V-C complex δ / ppm	$\Delta\delta$	U δ / ppm	Sb^V-U complex δ / ppm	$\Delta\delta$
1'	5.76 (d)	5.77 (d)	0.01	*5.90 (d)	5.86 (d)	–0.04
2'	4.17 (t)	4.27 (t)	0.10	4.36 (t)	4.39 (q)	0.03
3'	4.07 (t)	4.18 (t)	0.11	4.23 (t)	4.27 (t)	0.04
4'	3.99 (m)	3.92 (m)	–0.07	4.14 (m)	4.04-3.99 (m)	–0.13
5a'	3.81 (dd)	3.84 (d)	0.03	3.92 (dd)	3.94 (dd)	0.03
5b'	3.68 (dd)	3.74 (dd)	0.06	3.81 (dd)	3.82 (dd)	0.02
5	5.90 (d)	5.89 (d)	–0.01	*5.89 (d)	5.92 (d)	0.03
6	7.70 (d)	7.66 (d)	–0.04	7.88 (d)	7.79 (d)	–0.09

*data from reference 9.

Table 2. ^{13}C NMR parameters (100 MHz) for cytidine (C) and uridine (U) and their Sb^V derivatives (Sb^V-C and Sb^V-U)

Carbon	C δ / ppm	Sb^V-C δ / ppm	$\Delta\delta$	U δ / ppm	Sb^V-U δ / ppm	$\Delta\delta$
2	157.69	157.71	0.02	151.67	151.70	0.03
4	166.23	166.24	0.01	166.21	166.28	0.07
5	96.22	96.59	0.37	102.26	102.55	0.29
6	141.69	142.68	0.99	141.85	142.75	0.90
1'	90.38	92.13	1.75	89.41	91.57	2.16
2'	73.98	73.95	-0.03	73.67	73.61	-0.06
3'	69.34	69.21	-0.13	69.44	69.12	-0.32
4'	83.80	85.67	1.87	84.41	85.81	1.40
5'	60.79	61.76	0.97	60.77	61.67	0.90

Table 3. Stability constants (*K*) and apparent association and dissociation rate constants (k_f and k_d, respectively) for different Sb^V-ribonucleoside complexes at 37 ºC in D_2O containing 0.1 mol L^{-1} KCl at pD 6.5 (mean ± standard deviation, n = 3)

	Sb^V-U	Sb^V-C	Sb^V-A	Sb^V-G
K (L mol^{-1})	1756 ± 58	1227 ± 86	1594 ± 91	-
k_f (L mol^{-1} h^{-1})	158 ± 8	142 ± 6	82 ± 15	-
k_d (h^{-1})	0.0155 ± 0.0022	0.0363 ± 0.0020	0.0081 ± 0.0018	0.036 ± 0.011

Biological activities of the Sb^V-ribonucleoside complexes

The different Sb^V-ribonucleoside complexes were evaluated for their cytotoxicity towards murine peritoneal macrophages, as well as for their *in vitro* activity against *L. chagasi* parasite in the *Leishmania*-infected macrophage system. Figure 3 shows the cytotoxicity of different Sb^V complexes towards macrophages at 10 and 100 μg mL^{-1} of Sb. Antimoniate and meglumine antimoniate were also evaluated in the same assay. Although none of the antimonial compounds was found to be toxic at 10 μg mL^{-1}, the following complexes showed cytotoxicity at 100 μg mL^{-1} according to the order: Sb-G > $[KSb(OH)_6]$ > Sb-A > Sb-C > Sb-U.

Interestingly, the higher toxicity of antimoniate when compared to most Sb^V-complexes suggests that the complexation of Sb^V reduces its cytotoxicity, presumably by promoting a slow release system for Sb^V. This result is consistent with the higher cytotoxic activity of antimoniate against a tumor cell line, when compared to meglumine antimoniate.[16] In the case of Sb^V-C, Sb^V-U and meglumine antimoniate, an inverse relationship was observed between the stability constant of the complex and its cytotoxicity. Thus, no significant cytotoxicity of the highly stable complex meglumine antimoniate[4] was observed, even at 100 μg mL^{-1} of Sb.

Figure 4A displays the *in vitro* antileishmanial activity of the different Sb^V-ribonucleoside complexes at 10 μg mL^{-1} of Sb. A significant reduction of the fraction of infected macrophages was observed only in the case of Sb-A and Sb-G complexes. Interestingly, these complexes were found to be more effective than the first-line antileishmanial drug meglumine antimoniate. Figure 4B illustrates that meglumine antimoniate, although inactive at 10 μg mL^{-1} of Sb, promoted a significant reduction of the fraction of infected macrophage at 30 μg mL^{-1} of Sb.

The high cytotoxicity towards host cells of Sb^V-purine nucleoside complexes suggests that the formation of these complexes in the vertebrate host may contribute to the

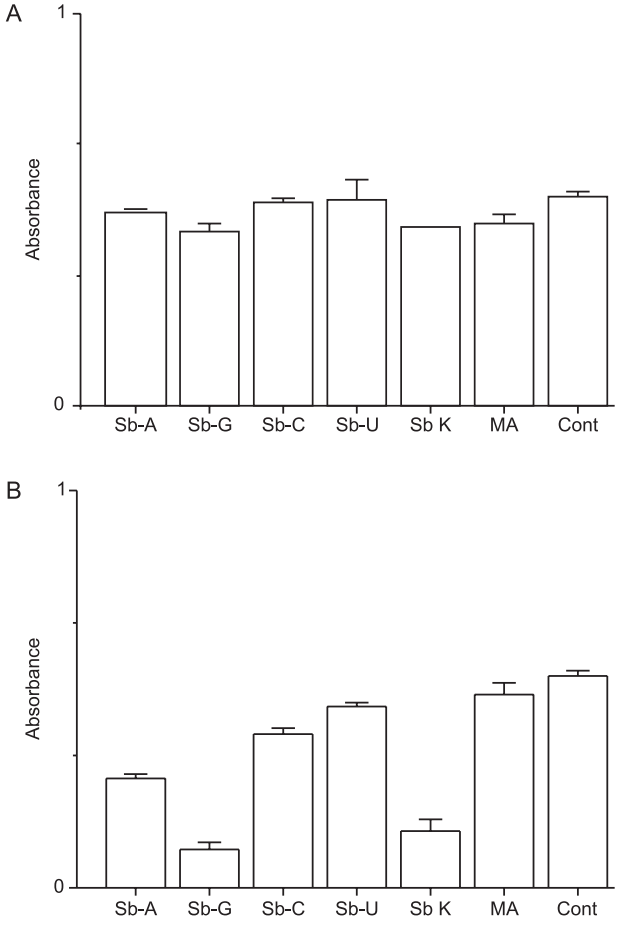

Figure 3. Cytotoxicity of different Sb^V complexes against murine macrophage at 10 μg mL^{-1} of Sb (A) and 100 μg mL^{-1} of Sb (B), as determined by the MTT assay. "Sb K" and MA represent $[KSb(OH)_6]$ and meglumine antimoniate, respectively. "Cont" represents untreated control. Data are shown as means of absorbance ± standard error (n = 3).

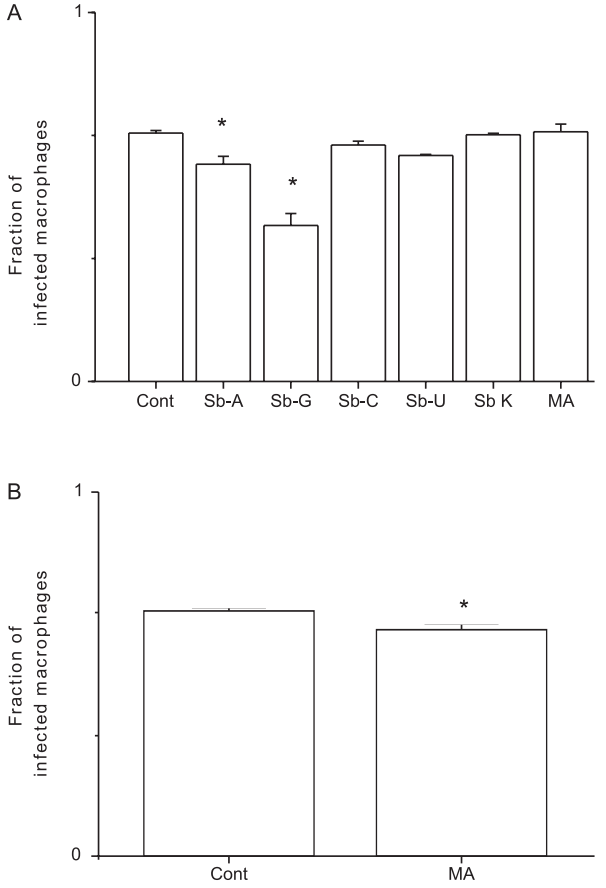

Figure 4. *In vitro* antileishmanial activity of different Sb^V complexes at 10 μg mL^{-1} of Sb (A) and 30 μg mL^{-1} of Sb (B) in the *Leishmania chagasi*-infected macrophage model. "Sb K" and MA represent $[KSb(OH)_6]$ and meglumine antimoniate, respectively. "Cont" represents untreated control. Data are shown as means of fraction of infected macrophage ± standard error (n = 2). *$P < 0.05$ according to One-way ANOVA, followed by Newman Keuls multiple comparison Post-test.

side effects of pentavalent antimonial drugs.[1] Furthermore, the higher antileishmanial activities of Sb-A and Sb-G complexes also support the model that the interaction of Sb^V with purine nucleosides may mediate the antileishmanial action of pentavalent antimonial drugs. According to this model, following treatment with an antimonial drug such as meglumine antimoniate, the drug would reach macrophage phagolysosomes in which the *Leishmania* parasite resides, either by simple diffusion or following binding to the cell surface and subsequent endocytosis. Sb^V-purine nucleoside complexes would then be formed within the phagolysosomes. The formation of these complexes is supported by both biological and physicochemical features. Since *Leishmania* is a true auxothroph for purine, its survival depends on the capture of purine ribonucleosides from the host.[8] Therefore, purine ribonucleosides are expected to be present in significant amount within phagolysosomes. Furthermore, the stability constants determined for Sb^V-A and Sb^V-GMP[4] are consistent with the *in vivo* formation of these complexes. In addition, the pH dependence of the rate of formation of Sb-ribonucleosides[4] indicates that this reaction would be kinetically favored in the acidic environment of phagolysosomes. Ultimately, the Sb^V-purine nucleoside complexes may kill the parasites, through inhibition of the *Leishmania* purine transporters or interference with the purine salvage pathway.[8]

Conclusions

The present work indicates for the first time that, although Sb^V most probably binds *via* oxygen atoms to the same 2' and 3' positions in the different nucleosides, the ribose conformational changes induced by its binding and the physicochemical characteristics of the resulting complexes depend on the nucleobase. It is also established that the nucleobase has a strong influence on the cytotoxicity and antileishmanial activity of the Sb^V-ribonucleoside complex, the Sb^V-purine nucleoside complexes being more cytotoxic towards macrophages and more effective against *Leishmania chagasi* than their Sb^V-pyrimidine nucleoside analogues. The higher cytotoxicities of Sb-A and Sb-G towards both *Leishmania* and host cells also support the model that the interaction of Sb^V with purine nucleosides may mediate the toxicity and antileishmanial activity of pentavalent antimonial drugs.

Supplementary Information

Supplementary information related to the ESI-MS analysis of the Sb^V-C and Sb^V-U complexes is available free of charge at http://jbcs.org.br, as a PDF file.

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Influence of the Nucleobase on the Physicochemical Characteristics and Biological Activities of Sb^{V}-Ribonucleoside Complexes

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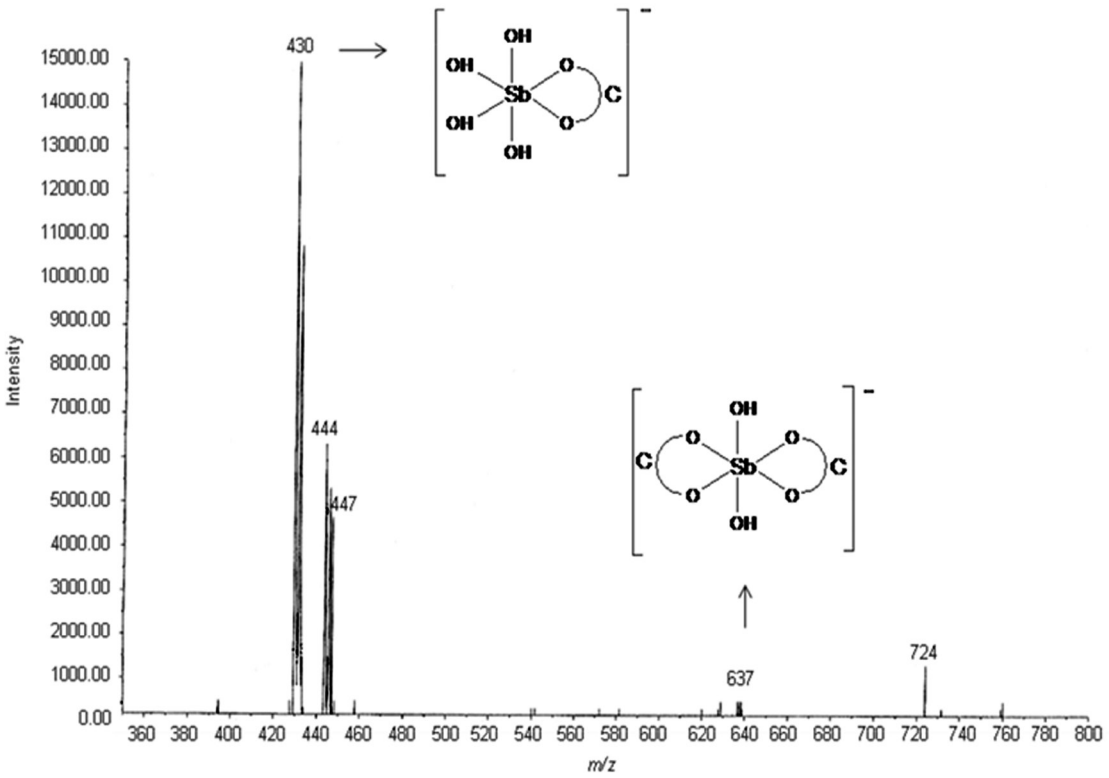

Figure S1. Negative ESI-MS spectrum of Sb^{V}-C.

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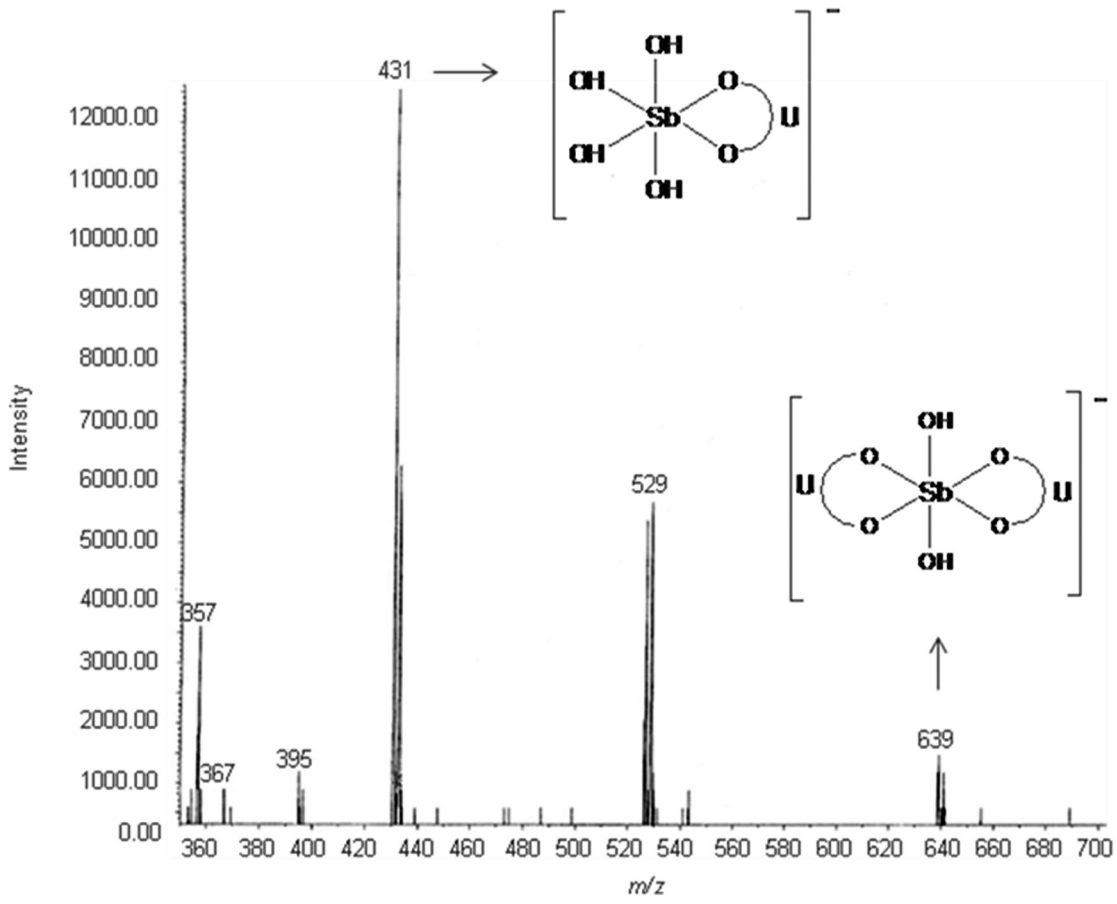

Figure S2. Negative ESI-MS spectrum of Sb^{V}-U.