Pharmacokinetics, Toxicities, and Efficacies of Sodium Stibogluconate Formulations after Intravenous Administration in Animals

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The pharmacokinetics and toxicities of free sodium stibogluconate (SSG) and two vesicular formulations of this drug (a nonionic surfactant vesicular formulation of SSG [SSG-NIV] and SSG-NIV-dextran) were determined after treatment with a single intravenous dose in healthy dogs and were related to their antileishmanial efficacies in mice. Analysis of the curves of the concentrations in plasma after intravenous administration of SSG and SSG-NIV in dogs showed that both formulations produced similar antimony (Sb) pharmacokinetics. In contrast, treatment with SSG-NIV-dextran significantly modified the pharmacokinetics of the drug. The elimination half-life was four times longer (280 min) than that observed after administration of SSG (71 min) (P = 0.01), and the volume of distribution at steady state (Vss) was also increased (Vss for SSG, 0.21 liters/kg; Vss for SSG-NIV-dextran, 0.34 liters/kg [P = 0.02]), thus indicating that drug encapsulation favors the distribution of Sb into organs and increases its residence time in tissues. This would explain the superior antileishmanial efficacy of this formulation compared to those of the free drug in mice. No signs of toxicity were found in dogs after SSG and SSG-NIV administration. However, SSG-NIV-dextran treatment was associated with short-term toxicity, demonstrated by the development of chills and diarrhea, which cleared by 24 h postdosing, and hepatic dysfunction at 24 h postdosing (P < 0.05). The levels of all the biochemical parameters had returned to normal at 1 month postdosing. No signs of toxicity were observed in mice treated with all three formulations.

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Dogs are the principal reservoir of Leishmania infantum, which is the etiologic agent of visceral leishmaniasis (VL) in Mediterranean countries (39). Therefore, one of the ways to prevent human VL would be to eradicate canine disease. Pentavalent antimonials, such as sodium stibogluconate (SSG) and meglumine antimoniate, are the first-line drugs used for the treatment of infections in humans (5) and dogs (2, 21). However, the multiple-dose treatment protocol used with dogs (twice-daily subcutaneous treatment with 75 mg/kg of body weight for 3 to 4 weeks) generally causes only a temporary remission of symptoms, and relapses are common. The pharmacokinetic behavior of antimony (Sb) after the administration of meglumine antimoniate to healthy dogs (4, 31, 33, 34) and dogs experimentally infected with L. infantum (35, 36) has been described previously. In contrast, the pharmacokinetic behavior of Sb in dogs after SSG administration has only been partially described (13). The lack of a correlation between administration protocols and the pharmacokinetic behavior of Sb could be an important parameter involved in therapeutic failures.

More recently, lipid-based amphotericin B formulations (e.g., Ambisome, Abelcet, and Amphocil), which were developed for the treatment of systemic fungal infections, are being used for the treatment of leishmaniasis (5). These formulations attain high levels of drug in the target organs infected with the parasite with reduced dosing schedules, thus providing more effective and safer therapies (17). However, no commercial lipid formulations of Sb are available, even though these types of formulations were shown to be 700 times more effective, based on the drug dose, than unencapsulated drug as early as the 1980s (9). A liposomal meglumine antimoniate formulation administered to dogs infected experimentally with L. infantum every 24 h allowed high and sustained plasma drug concentrations throughout the treatment period, and the dogs remained symptom free for at least 12 months posttreatment (36). Major barriers to the development of a phospholipid-based antimonial drug formulation for the treatment of canine leishmaniasis is the high commercial costs associated with the production of a new drug formulation and the emergence of Sb resistance within the Leishmania parasite populations in some parts of the world (20).

Previous studies have shown that the use of a nonionic surfactant vesicular formulation of SSG (SSG-NIV) can markedly improve the therapeutic efficacy of SSG (23, 37). In a murine model, SSG-NIV treatment was more effective or as effective as lipid-based amphotericin B drug formulations, which are now commercially available for the treatment of VL (23). In addition, recent studies have shown that an SSG-NIV formulation was more effective than free SSG against clinically derived Sb-resistant strains (8). Studies with an alternative
SSG-NIV formulation in mice showed that use of the carrier system increased the pentavalent Sb (SbV) concentrations in tissues infected with *Leishmania* (12). In addition, the pharmacokinetics of SSG were altered so that more of the drug dose was targeted to infected tissues, thus avoiding rapid renal excretion.

In this study the pharmacokinetics and toxicities of free SSG and two vesicular formulations of SSG were determined in healthy dogs, and these results were related to its antileishmanial efficacy in mice.

**MATERIALS AND METHODS**

**Materials.** SSG (31.1% [wt/wt] SbV) was provided by Glaxo Wellcome Ltd. (Ware, United Kingdom). The nonionic surfactant tetraethylene glycol mono-n-hexadecylether was purchased from Chesham Chemicals Ltd. (Harrow, United Kingdom). Diectyl phosphate and ash-free cholesterol were obtained from Sigma, and all other reagents were of analytical grade. Water for irrigation (lot 96/29B25, code F7114) was obtained from Baxter Healthcare (Northampton, United Kingdom), and dextran T10 was obtained from Pharmacia Biotech AB (Uppsala, Sweden). Pentostam (27 mg of SbV/ml; lot A5332A) was purchased from a commercial supplier (Unichem, Livingston, United Kingdom).

**Animals.** Eight healthy beagle dogs (four males and four females; age range, 2 to 3 years; weight range, 12 to 18 kg) were used in this study. The dogs were classified as healthy on the basis of a physical examination by a qualified veterinarian and hematological and biochemical profiles (19). Animals were housed indoors in separate rooms, given water ad libitum, and fed dry food once a day. The study was approved by the institutional animal use committee of the Instituto de Salud Carlos III.

Age-matched 8- to 10-week-old BALB/c mice (inbred male or female mice bred in-house) were used in this study. Golden Syrian hamsters (*Mesocricetus auratus*) bred in-house or obtained commercially (Harlan Olac, Bicester, Oxford, United Kingdom) were used for maintenance of *L. donovani* (strain MHHOM/ET/567/L.V82). The mice were infected by intravenous injection (in the tail vein without anesthesia) with 1 x 10^5 to 2 x 10^5 *L. donovani* amastigotes (7). The day of parasite administration to the mice was designated day 0 of the experiment.

**Drug formulations.** Four drug formulations, prepared under aseptic conditions at Strathclyde University, were used in this study: free SSG solution (27 mg of SbV/ml), an SSG and dextran solution (SSG-dextran; 27 mg of SbV/ml and 150 mg of dextran [10,680 Da] per ml), and two SSG-NIV formulations (SSG-NIV and SSG-NIV-dextran). A total of 740 ml of SSG-NIV (lipid concentration, 30 mM), consisting of a 3:3:1 molar ratio of tetraethylene glycol mono-n-hexadecylether, cholesterol, and dicetyl phosphate, was prepared as described previously (23). Briefly, the lipids were melted at 130°C for 5 min. The molten mixture was then cooled to 70°C, hydrated with 740 ml of preheated SSG solution (70°C, 27 mg of SbV/ml), and homogenized at 8,000 ± 100 rpm for 15 min at 70°C with an Ultra-turrax T25 homogenizer fitted with an S25N-25G dispersing tool (IKA-Werke GmbH, Staufen, Germany). A total of 700 ml of the resultant SSG-NIV formulation was then processed as follows to prepare the SSG-NIV-dextran formulation.

The SSG-NIV suspension was left for 45 min to cool to room temperature and then was subjected to ultrafiltration with a sterilized (by autoclaving at 121°C for 15 min) Spirasep 90 device (Intersep, Berkshire, United Kingdom) which incorporated a sterile 100,000 molecular-weight-cutoff polyethersulfone membrane. The vesicle suspension was passed through the device by using a Jencons peristaltic pump operated at the high speed setting. The ultrafiltration process used is described in Table 1. The SSG-NIV and SSG-NIV-dextran suspensions were sized by photon correlation spectroscopy with a Zetasizer 4 instrument (Malvern Instruments Ltd., Malvern, United Kingdom) and stored at room temperature until use.

**Treatment of dogs.** Four dogs (two males and two females; group A) were given a single intravenous injection of either SSG (40 mg of SbV/kg) or SSG-NIV (20 or 40 mg of SbV/kg). Four other dogs (two males and two females; group B) were given a single intravenous injection of SSG, SSG-dextran, or SSG-NIV-dextran with 10 mg of SbV/kg. Drug formulations were administered into the cephalic vein by slow (5 min) intravenous infusion, and a 4-week drug-free interval between trials was used. Blood samples were collected with disposable syringes that contained sodium heparin from a catheter placed contralateral to the hepatic cephalic vein. After each administration, blood sampling was carried out at 5, 10, 20, 30, 40, 60, 80, and 100 min and 2, 2.5, 3, 4, 5, 6, 8, and 24 h after administration. Blood samples (3 ml) were kept refrigerated until they were centrifuged (2,000 x g). The plasma was aspirated and stored at ~20°C until analysis, which was completed within 2 weeks after blood collection.

**Pharmacokinetic studies with dogs.** Detection and quantification of Sb were performed in a graphite furnace atomic absorption spectrometer (model Zee- man 3030 HGA 600; Perkin-Elmer) by using the optimized conditions described previously (11, 14). Before analysis and quantification, the plasma samples were diluted in ultrapure water (MiliQ system; Millipore). The values of the pharmacokinetic parameters for each animal were determined by means of the PCNONLIN (version 4.0) program. The weighted (1/x^2, where C is concentration) data for intravenous administration were fitted to different compartmental models by nonlinear least-squares regression analysis with first-order elimination from the central compartment. The criteria used for discrimination between models was residual sum of squares (2 Res^2) and Akaike’s information criterion (26).

**Treatment of mice.** Groups of infected mice (n = 4 or 5 mice per treatment) were treated intravenously on day 7 postinfection with one of the following: phosphate-buffered saline (controls), a single dose of SSG-NIV (222 mg of SbV/kg), SSG-NIV-dextran (33 mg of SbV/kg), or SSG solution (222 mg of SbV/kg). Animals were killed on day 14 postinfection, and the parasite burdens were determined by microscopic examination of Giemsa-stained tissue smears (7). Leishman-Donovan units (LDUs; where LDU is the number of amastigotes per 1,000 host cell nuclei × organ weight [in grams]) were calculated for the liver and spleen, and the numbers of parasites per 1,000 host cell nuclei were calculated for the bone marrow (6).

**Toxicity study with dogs.** A complete serum biochemical analysis was performed before dosing, at 24 h postdosing, and at 1 month postdosing by using an autoanalyzer (OPERA model; Bayer). The dogs were observed during the first 48 h after drug administration to evaluate possible clinical or behavioral changes. Food and water intake, temperature, and heart and respiratory rates were also observed.

**Statistical analysis.** Statistical analysis was performed by using the SPSS (version 10.0) software package (SAS Institute, Inc., Cary, N.C.). The data obtained for samples from dogs are presented as arithmetic means ± standard deviations (SDs). A Wilcoxon test and a Friedman nonparametric test were used to evaluate differences of paired comparisons in pharmacokinetic and biochemical parameters between the different formulations administered. For the studies with mice, the mean log ± standard error parasite burdens (LDUs per organ for the spleen and liver and the number of parasites/1,000 host cell nuclei for the bone marrow) are shown. Parasite burdens were analyzed by a Students’ unpaired t test with log_10-transformed parasite burden data. A P value <0.05 was considered significant.

**RESULTS**

**NIV formulations.** The SSG-NIV suspension used in this study had a mean ± SD hydrodynamic diameter of 526 ± 20 nm and a mean ± SD zeta potential of 3.8 ± 1.3 mV; this formulation had an entrapment efficiency of 6%. The SSG-NIV-dextran formulation was prepared from the SSG-NIV formulation used in this study, and the dextran concentration was selected to minimize any change in the osmotic pressure across the vesicular bilayer during processing, thereby minimizing the loss of entrapped drug. The SSG-NIV-dextran for-
mulation had a lipid concentration of 30 mM and an Sb\textsuperscript{V} concentration of 3.9 mg/ml (the concentrations were calculated by atomic absorption spectroscopy; data not shown). It was assumed that intravesicular drug would be responsible for the formulation having a higher Sb concentration per milliliter than that anticipated from the volume changes during processing (Table 1). Thus, it was calculated that 43\% of the Sb formulation was intravesicular (this value was obtained by subtracting the calculated expected antimony concentration [2.3 mg of Sb\textsuperscript{V}/ml; Table 1] from the measured antimony concentration [3.9 mg of Sb\textsuperscript{V}/ml]). Thus, the processing method had reduced the total antimony content to 15\% of the original starting concentration. Preparation of the SSG-NIV-dextran formulation from the SSG-NIV formulation was associated with significant physicochemical changes, including a reduction in the mean vesicle size (mean ± SD hydrodynamic diameter, 253 ± 13 nm) and the zeta potential (mean ± SD, 13.7 ± 1.8 mV). The changes in vesicle size and zeta potential may be related to the processing method (e.g., particle-particle attraction during filtration and the substitution of dextran for SSG) rather than to the presence of a specific physical change in the vesicle. The amount of Sb\textsuperscript{V} present in the SSG-NIV-dextran formulation (3.9 mg of Sb\textsuperscript{V}/ml) gave an entrapment efficiency of 43\%.

**Studies with mice.** Treatment with the vesicular formulation was more effective than treatment with free SSG (Table 2), since treatment with free SSG (222 mg of Sb\textsuperscript{V}/kg) caused a significant reduction in parasite burdens only in the liver, whereas treatment with the NIV formulations caused a significant reduction in parasite burdens in all three sites. Animals treated with SSG-NIV or SSG-NIV-dextran were treated with the same lipid dose. This equated to drug doses of 33 mg of Sb\textsuperscript{V}/kg for animals treated with SSG-NIV-dextran and 222 mg of Sb\textsuperscript{V}/kg for animals treated with SSG-NIV. Treatment with the SSG-NIV-dextran formulation was more effective than treatment with the SSG-NIV formulation since it caused a greater reduction in parasite burdens in the liver and bone marrow (P < 0.01). Thus, treatment efficacy is primarily dependent on the amount of entrapped drug present in the formulation. None of the treatments caused any signs of toxicity in dosed mice.

**Pharmacokinetic study with dogs.** The standard curve for determination of Sb concentrations was linear over the range of concentrations tested (0.1 to 100 µg/ml) in dog plasma diluted (1/10) in ultrapure water (r\textsuperscript{2} = 0.999). The intra- and interassay coefficients of variation were <6\%. The quantification limit of the analytical method was 9.2 ng of Sb/ml.

The concentrations of all four SSG formulations in plasma were best fitted by a bicompartimental open model with first-order elimination. The mean values for the pharmacokinetic parameters for each formulation derived for individual animals are shown in Tables 3 and 4. Sb\textsuperscript{V} levels dropped quickly in animals dosed with free SSG at 10 or 40 mg of Sb\textsuperscript{V}/kg. This is reflected by the very short elimination half-lives (t\textsubscript{1/2,el}) of 71 ± 18 and 88 ± 31 min, respectively. Administration of dextran along with SSG (SSG-dextran) did not have any significant effect on the pharmacokinetics of SSG, since the values for all the parameters determined were similar for the two formulations (Table 4). Treatment with SSG-NIV did not alter the pharmacokinetics of SSG, presumably since most of the SSG remains unentrapped (Table 3). In contrast, treatment with the SSG-NIV-dextran formulation, which contained a higher proportion of intravesicular SSG compared to the proportion in the SSG-NIV formulation, did have a significant effect on the pharmacokinetic behavior of SSG (Table 4). After SSG-NIV-dextran administration, the distribution half-life (t\textsubscript{1/2,d}) had significantly increased compared with that obtained after free drug administration (P = 0.01), the t\textsubscript{1/2,d} was four times longer than that observed after administration of SSG, and the mean residence time was two times longer (P = 0.02). These results indicate marked delays in the elimination of Sb. The volume of distribution at steady state (V\textsubscript{ss}) for the SSG-NIV-dextran formulation was also significantly increased (P = 0.02), but the clearance from plasma and the area under the plasma concentration-time curve were not modified (P > 0.05).

**Toxicity study.** No overt clinical signs of toxicity were detected in any dog given SSG (10 or 40 mg of Sb\textsuperscript{V}/kg) or SSG-dextran (10 mg of Sb\textsuperscript{V}/kg [Table 5]) and 40 mg of Sb\textsuperscript{V}/kg

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**TABLE 2. Effects of various SSG formulations on *L. donovani* parasite burdens in BALB/c mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spleen</th>
<th>Liver</th>
<th>Bone marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.49 ± 0.90</td>
<td>3.12 ± 2.24</td>
<td>2.07 ± 1.23</td>
</tr>
<tr>
<td>SSG, 222 of mg Sb\textsuperscript{V}/kg</td>
<td>1.78 ± 1.30</td>
<td>2.32 ± 2.05</td>
<td>2.14 ± 1.32</td>
</tr>
<tr>
<td>SSG-NIV, 222 of mg</td>
<td>0.85 ± 0.60</td>
<td>1.77 ± 1.36</td>
<td>1.04 ± 0.48</td>
</tr>
<tr>
<td>SSG-NIV-dextran, 33 of mg Sb\textsuperscript{V}/kg</td>
<td>0.48 ± 0.30</td>
<td>0.70 ± 0.30</td>
<td>ND*</td>
</tr>
</tbody>
</table>

a *L. donovani*-infected mice were treated on day 7 postinfection with a single dose of phosphate-buffered saline (controls), SSG, SSG-NIV, or SSG-NIV-dextran. Normalized parasite burdens were determined on day 14 postinfection.

b Parasite burdens are in mean log LDUs for the spleen and liver and are the mean log number of parasites per 1,000 host cell nuclei for the bone marrow.

d P < 0.0015 compared to the control treatment.

e ND, no parasite detected.

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**TABLE 3. Effects of dosing with SSG or SSG-NIV on various pharmacokinetic parameters in dogs**

| Treatment (Sb\textsuperscript{V} dose [mg/kg]) | k\textsubscript{12} (h\textsuperscript{-1} [10\textsuperscript{-3}]) | k\textsubscript{21} (h\textsuperscript{-1} [10\textsuperscript{-3}]) | t\textsubscript{1/2,el} (h) | t\textsubscript{1/2,d} (h) | AUC\textsubscript{0–∞} (mg·h/liter) | V\textsubscript{ss} (liters/kg) | V\textsubscript{c} (liters/kg) | CL (liters/kg·h) | MRT\textsubscript{0–∞} (h) |
|---------------------------------------------|-----------------|-----------------|----------------|----------------|----------------|--------------|-------------|-------------|---------------|---------------|
| SSG (40)                                    | 0.32 ± 0.26     | 0.32 ± 0.17     | 0.24 ± 0.08   | 1.46 ± 0.51   | 241.1 ± 60.2 | 0.11 ± 0.02  | 0.25 ± 0.08  | 1.71 ± 0.04  | 1.48 ± 0.56   |
| SSG-NIV (40)                                | 0.15 ± 0.25     | 0.21 ± 0.17     | 0.50 ± 0.42   | 1.97 ± 1.14   | 364.4 ± 121.1 | 0.22 ± 0.1   | 0.33 ± 0.08  | 1.70 ± 0.05  | 2.19 ± 1.25   |
| SSG-NIV (20)                                | 0.66 ± 0.71     | 0.56 ± 0.46     | 0.20 ± 0.17   | 1.42 ± 0.15   | 154.3 ± 16.9 | 0.10 ± 0.03  | 0.21 ± 0.05  | 1.15 ± 0.01  | 1.68 ± 0.39   |

a *Dogs (n = 4/group) were given a single intravenous injection of SSG (40 mg of Sb\textsuperscript{V}/kg) or SSG-NIV (20 or 40 mg of Sb\textsuperscript{V}/kg). The values are means ± SDs. Abbreviations: k\textsubscript{12} and k\textsubscript{21}, intercompartmental transfer rate constants; t\textsubscript{1/2,el}, distribution phase half-life; t\textsubscript{1/2,d}, elimination phase half-life; AUC\textsubscript{0–∞}, area under the plasma concentration-time curve projected to infinity; V\textsubscript{ss}, steady-state volume of distribution; CL, total body clearance; MRT\textsubscript{0–∞}, mean residence time projected to infinity.
[data not shown]). No serum biochemical alterations were observed in animals treated with 10 mg of SbV/kg as part of the SSG or the SSG-dextran formulation, but a significant increase in aspartate aminotransferase (AST) levels was obtained for animals given free SSG with 40 mg of SbV/kg at 24 h postdosing (AST level before dosing, 28 U/liter; AST level after dosing, 70 U/liter; AST level before dosing, 24 h after dosing, 33.0 U/liter; AST level after dosing, 37.0 U/liter). No serum biochemical alterations were observed in animals treated with 10 mg of SbV/kg as part of the SSG-NIV formulation with 40 mg of SbV/kg also produced a statistically significant increase in the levels of serum enzymes (alanine aminotransferase (ALT) and alkaline phosphatase) that determine hepatic functionality (Table 5) compared to the pretreatment levels. In addition, iron levels were lower at 24 h posttreatment (Table 5). There were no significant changes in any of the other parameters evaluated (data not shown). There were no differences in the values for any of the biochemical parameters assessed predosing and 1 month postdosing in any of the dogs (data not shown).

**DISCUSSION**

The results of this study confirm those of previous studies, i.e., that in the dog SSG is rapidly excreted from the body, has a rapid distribution phase (t1/2d), a rapid distribution phase (t1/2d), and has a low Vss (13). Similar results have been reported for dogs treated with meglumine antimoniate (4, 33) and human patients given antimonial drugs (10, 16, 25, 27). The short t1/2d obtained in this study for dogs given SSG solution (t1/2d 71.1 ± 18.4 min for 10 mg of SbV/kg and 88 ± 31 min for 40 mg of SbV/kg) are also indicative of rapid clearance of the drug. The main route of excretion for antimonial drugs is via the kidney (16, 27); therefore, the glomerular filtration rate influences how fast the drug can be cleared from the systemic circulation postdosing. In this study the calculated clearance rate for SSG solution is within the normal range for the glomerular filtration rate in the dog (24). Indeed, the clearance rates for all the SSG formulations were in this range. The results of this study are similar to those obtained by Belloli et al. (4) after treatment of dogs with meglumine antimoniate (t1/2d 85.6 min). A similar study by Collins et al. (13), in which dogs were treated with a single SSG dose equivalent to 45 mg of SbV/kg, gave a more rapid elimination phase (mean t1/2d 34.64 ± 5.08 min), which is considerably shorter than the t1/2d obtained in this study. However,

### TABLE 4. Pharmacokinetic parameters determined for dogs given different SSG formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>k12 (h⁻¹ [10⁻³])</th>
<th>k21 (h⁻¹ [10⁻³])</th>
<th>t1/2d (h)</th>
<th>t1/2e (h)</th>
<th>AUC0-∞ (mg·h/liter)</th>
<th>Vss (liters/kg)</th>
<th>CL (liters/kg·h)</th>
<th>MRT0–∞ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSG</td>
<td>0.69 ± 0.42</td>
<td>0.56 ± 0.22</td>
<td>0.14 ± 0.06</td>
<td>0.19 ± 0.31</td>
<td>60.7 ± 12.5</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.1</td>
<td>1.68 ± 0.04</td>
</tr>
<tr>
<td>SSG-dextran</td>
<td>0.38 ± 0.08</td>
<td>0.67 ± 0.07</td>
<td>0.13 ± 0.06</td>
<td>0.14 ± 0.14</td>
<td>58.9 ± 8.7</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>1.68 ± 0.02</td>
</tr>
<tr>
<td>SSG-NIV</td>
<td>0.11 ± 0.01</td>
<td>0.06 ± 0.07</td>
<td>0.12 ± 0.14</td>
<td>4.67 ± 0.88</td>
<td>67.1 ± 22.4</td>
<td>0.2 ± 0.1⁵</td>
<td>0.3 ± 0.1⁵</td>
<td>1.63 ± 0.07</td>
</tr>
</tbody>
</table>

* a. Dogs (n = 4/group) were given a single intravenous injection of SSG, SSG-NIV, or SSG-NIV-dextran with 10 mg of SbV/kg. k12 and k21, intercompartmental transfer rate constants; t1/2d, distribution phase half-life; t1/2e, elimination phase half-life; AUC0-∞, area under the plasma concentration-time curve projected to infinity; Vss, volume of distribution of the central compartment; CL, total body clearance; MRT0–∞, mean residence time projected to infinity.

**b**. P < 0.05 compared to values for animals given free SSG.

### TABLE 5. Effects of dosing with different SSG formulations on selected biochemical parameters before dosing and 24 h postdosing

<table>
<thead>
<tr>
<th>Formulation and time of evaluation</th>
<th>AST (U/liter)⁶</th>
<th>ALT (U/liter)⁶</th>
<th>Alkaline phosphatase (U/liter)⁶</th>
<th>Iron (μg/dl)⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal level</td>
<td>19–70</td>
<td>28–78</td>
<td>32–185</td>
<td>90–125</td>
</tr>
<tr>
<td>SSG</td>
<td>32.25 ± 16.74</td>
<td>56.75 ± 39.73</td>
<td>83.50 ± 24.28</td>
<td>126.00 ± 51.58</td>
</tr>
<tr>
<td>24 h after dosing</td>
<td>33.0 ± 11.05</td>
<td>54.5 ± 16.52</td>
<td>100.75 ± 32.2</td>
<td>141.25 ± 37.2</td>
</tr>
<tr>
<td>SSG-dextran</td>
<td>24.25 ± 5.91</td>
<td>32.00 ± 10.98</td>
<td>69.50 ± 28.01</td>
<td>139.00 ± 18.57</td>
</tr>
<tr>
<td>24 h after dosing</td>
<td>37.00 ± 12.11</td>
<td>54.50 ± 21.13</td>
<td>81.75 ± 35.27</td>
<td>128.00 ± 27.64</td>
</tr>
<tr>
<td>SSG-NIV-dextran</td>
<td>27.25 ± 10.05</td>
<td>40.00 ± 15.38</td>
<td>81.25 ± 32.75</td>
<td>158.75 ± 40.88</td>
</tr>
<tr>
<td>24 h after dosing</td>
<td>115.00 ± 90.18</td>
<td>97.50 ± 44.73</td>
<td>214.00 ± 150.90</td>
<td>39.25 ± 10.63</td>
</tr>
</tbody>
</table>

* a. Dogs (n = 4/group) were given a single intravenous injection of SSG, SSG-dextran, or SSG-NIV-dextran with 10 mg of SbV/kg. The normal physiological range for each parameter is shown. The values are means ± SDs.

**b**. Statistically significant differences between SSG-NIV formulation and the other two formulations.
differences in the dogs (beagles versus mongrels) or assay methods used may explain the discrepancies in the results. The \( t_{1/2}\) values for individual dogs in the study of Collins and coworkers (13) ranged from 15 to 77 min, so that for some of the dogs the \( t_{1/2}\) values were within the range found in this study.

Conjugation of drugs to dextrans can alter the in vivo pharmacokinetics of the drugs (22). However, in this study dual treatment with SSG and dextran did not alter the pharmacokinetics of SSG. This is perhaps not surprising since the two were not conjugated. In addition, this also indicates that the presence of dextran in the SSG-NIV-dextran formulation would not have a major impact on the pharmacokinetics of the SSG-NIV-dextran formulation.

Similar pharmacokinetic profiles were obtained for SSG and SSG-NIV. This result probably reflects the low proportion of drug entrapped in the SSG-NIV formulation relative to the total drug dose (entrapment efficiency, \(<7\%\)). In contrast, treatment with SSG-NIV-dextran (entrapment efficiency, 43\%), which was processed to remove unentrapped drug, had a profound effect on the in vivo pharmacokinetics of SSG. This is demonstrated by the significantly higher distribution, \( t_{1/2}\), area under the plasma concentration-time curve, volume of distribution in the central compartment, \( V_{SS}\) and mean residence time compared to those for free SSG. Only clearance from plasma was unaltered by use of a carrier system, indicating that the elimination of Sb was still reliant on the kidney glomerular filtration rate. Similar studies with a different SSG-NIV formulation, in which unentrapped drug was removed from the formulation by gel filtration, also showed that incorporation of the drug into an NIV system altered the drug’s pharmacokinetics and targeted more of the drug dose to tissues (13). However, the low blood \( Sb^V\) levels obtained postdosing in this study did not allow determination of the pharmacokinetic data for the SSG-NIV formulation (13). Treatment with SSG-NIV-dextran gave a \( V_{SS}\) value which was fourfold higher than that obtained after administration of the same dose of SGG, indicating that use of the carrier system favored tissue loading. It has been shown that retention of drugs in tissues is favored when lipid drug formulations are used (18). This supposition is supported by the higher \( t_{1/2}\) (four times that of SSG) and the longer mean residence time (twice that of SGG) of the SSG-NIV-dextran formulation compared to the values obtained with the same dose of free SSG. Data from murine in vivo studies ranked the antileishmanial efficacies of the three formulations as SSG-NIV-dextran > SSG-NIV > SSG. This would imply that it is the amount of entrapped drug present which controls in vivo efficacy.

There were interspecies differences in the toxicities of the formulations. The SSG-NIV-dextran formulation had no obvious adverse effects in mice given 33 mg of \( Sb^V\)/kg. In contrast, treatment with this formulation with 10 mg of \( Sb^V\)/kg caused acute toxic effects in dogs. The difference in toxicity could be explained by the differences in the half-lives between the mouse and the dog and, thereby, exposure versus time; but it is not possible to provide pharmacokinetic data for mice treated with the SSG formulations. Toxic effects in dogs included chills, salivation, respiratory distress, vomiting, and passing of mucous diarrhea, which turned hemorrhagic. In addition, serum hepatic enzyme levels increased and iron levels decreased considerably over the physiological range of values. The SSG-NIV formulation did cause a slight increase in AST levels, but treatment with the same dose of free drug caused a similar effect, suggesting that the carrier itself is not toxic. In addition, joint treatment with SSG and dextran (SSG with 10 mg of \( Sb^V\)/kg, 1,332 mg of dextran/kg) did not cause the same adverse effects, suggesting that the presence of dextran in the SSG-NIV-dextran formulation did not contribute to the toxicity of this formulation. Dosing of the dogs (SSG with 10 mg of \( Sb^V\)/kg) on the basis of the total \( Sb^V\) dose rather than the amount of entrapped \( Sb\) meant that dogs given SSG-NIV-dextran (2.5 ml/kg, equivalent to 76.2 \( \mu \)M lipid, 3.9 mg of \( Sb^V\)/ml and a 43\% entrapment efficiency) were given a larger amount of total lipid and entrapped drug than those given SSG-NIV (0.37 ml/kg, equivalent to 11.1 \( \mu \)M lipid, 27 mg of \( Sb^V\)/ml, and a 6\% entrapment efficiency). Either one or a combination of these could be responsible for the adverse effects observed, or more likely, the SSG-NIV-dextran formulation resulted in toxic \( Sb^V\) levels in tissues. The pharmacokinetic data allied with the high degree of efficacy of the SSG-NIV-dextran formulation against \( L. donovani\) in the mouse studies suggest that this formulation induces high tissue \( Sb^V\) levels. It is well known that \( Sb^V\) is toxic and can cause side effects such as nausea, vomiting, diarrhea, cardiotoxicity, hepatic damage, and respiratory problems (38). Treatment of rats with doses of SSG below those required to eradicate the organism caused a significant dose-related reduction in the hemoglobin concentration and the hematocrit count (1). Therefore, the reduction in iron levels observed in SSG-NIV-dextran-treated dogs is not surprising. It has previously been described that SSG treatment also cause significant dose-dependent rises in AST, ALT, alkaline phosphatase, and serum creatinine levels and can induce proteinuria (1), which would indicate liver and kidney damage. In this study all of these effects except significant changes in creatinine levels and the induction of proteinuria were observed, indicating that damage was restricted to the liver. This would not be surprising, since previous studies have shown that use of an NIV formulation directs a large proportion of the dose to tissues such as the liver (3, 13), and this fact could also be contributing to the hepatic toxicity of the NIV formulation in dogs. Thus, a potential problem with improved drug delivery is that it can lead to high concentrations in tissues and improved efficacy but increased toxicity if the drug has adverse side effects.

Previous studies have show that serum complement opsonizes particles for clearance by the reticuloendothelial system (15). Many cardiovascular and hematological changes observed following the administration of liposomes in vivo can be explained by complement C3a and C5a activation, and this phenomenon is called “complement activation-related pseudoallergy.” The adverse effects caused by this include a rise in pulmonary arterial pressure, a decline in cardiac output, increased heart rate, increased pulmonary vascular resistance, increased systemic vascular resistance, and up to a 100-fold increase in plasma thromboxane B2 levels. These may have caused some of the effects observed in the SSG-NIV-dextran-treated dogs, since they can occur rapidly postdosing (peak, 1 to 5 min after administration).


