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*Brief Original Article*

# Tolerability and pharmacokinetics of two antimony products after subcutaneous administration in dogs

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## Abstract

Introduction: Pentavalent antimony is the first choice drug for leishmaniasis in dog. Leishmaniasis has a complex pathogenesis and it manifests various clinical signs, some of which are often similar to those associated with the toxicity induced by antimonial treatment. Among the reasons for this toxicity, also a general problem of drug's quality has been reported.

Methodology: The general and local tolerability of two commercially available meglumine antimoniate based veterinary products was evaluated in 12 healthy dogs, 6 receiving Antimania (Fatro, Italy) and 6 receiving Glucantime (Merial, Spain), following repeated subcutaneous administrations of therapeutic doses for 14 days.

Results: Individual and mean values of haematological and biochemical parameters in both groups remained in the physiological range, with no considerable differences within the two groups. The general tolerability of the drugs was also supported by clinical observations and physical examination of the dogs throughout the whole study period. Only slight local reactions at the injection sites, that spontaneously disappeared, were observed for both products starting from 12-84 hours after the administration. The pharmacokinetic parameters indicated no antimony accumulation.

Conclusions: These results suggest that meglumine antimoniate administered at the recommended dosage regimen is well tolerated by healthy dogs, and that there is no significant difference between the two tested products.

**Key words:** antimony; dog; leishmaniasis; meglumine antimoniate; tolerability.

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## Introduction

Leishmaniasis is a parasitic disease caused by more than 20 different species of the protozoan Leishmania that are transmitted by the bite of infected phlebotomine sandflies [1]. Leishmaniasis is endemic in all countries of the Mediterranean basin and affects both humans and dogs [2]. Dog is a reservoir host for leishmaniasis, which represents a serious veterinary and public health problem, also because the few available drugs cannot completely eliminate the infection in this species, and remission of clinical signs is only temporary. Although many drugs have been tested experimentally for this disease, pentavalent antimony products (SbV) remain the first choice since their introduction to the market for human use in the mid 1940's and later authorization for veterinary use in 1996 [3-4]. In Europe, the most used pentavalent antimony for dog treatment is N-methyl-D-glucamine antimoniate [5]. The mechanism of action of SbV is unknown, but it is presumed that the drug selectively inhibits leishmanial glycolysis and fatty acid oxidation, thus reducing the energy available for the survival of the parasite [6]. Furthermore, due to its complex pathogenesis, leishmaniasis may manifest various and controversial clinical signs [6], leading to late diagnosis and treatment [7]. The therapy outcome is influenced also by the individual immune response and by the susceptibility of the parasite strain to the drug [8-10]. Moreover, a general problem of quality and batch-to-batch variability of both branded and generic antimony products has been reported, which may affect their efficacy and sometimes lead to serious toxicity [11]. It is known that cardiotoxicity and pancreatitis are associated with antimonial therapy in humans [12]. In dogs, common adverse effects include apathy, anorexia, vomiting, diarrhea and pain at the site of injection [13-14]; pancreatitis has also been associated with N-methyl glucamine treatment [15]. Yet, the frequency and severity of these adverse effects need to be furtherly investigated, and it is often difficult to assess if they are related to the infection itself or to the therapeutic agent.

In order to highlight any potential adverse effects related to antimony administration, the aim of the

present study was to evaluate in healthy dogs the general and local tolerability of the two meglumine antimoniate based veterinary products available across Europe, after daily subcutaneous administration of drugs during 14 consecutive days.

## Methodology

The trial was approved by the Institutional Animal Care and Use Committee and the Committee for Animal Protection of the Ministry of Health of the Czech Republic (32/2016), and conducted in compliance with Good Laboratory Practice requirements. Twelve clinically healthy Beagle dogs of 3-5 years of age and 9.5-13.8 kg of bodyweight were randomly allocated in two experimental groups (A and B) of 6 animals each (3 males and 3 females) using an Excel randomisation file. Group A was treated with Antimania 300 mg/mL (FATRO S.p.A., Ozzano dell'Emilia, Bologna, Italy); group B was treated with Glucantime 300 mg/mL (Merial Laboratorios s.a., Barcelona, Spain). Both products were analysed to assess the antimony content, which was 97 and 96 mg/mL, for Antimania and Glucantime, respectively. The two groups of animals were treated in parallel, and each subject received a total daily dose of 100 mg of meglumine antimoniate/kg b.w., divided in two subcutaneous administrations (50 mg every 12 hours). Meglumine antimoniate was administrated from day 0 to day +13 (27 total injections).

General tolerability was evaluated by clinical observation of the animal health status, physical examination, urine analysis and abdominal ultrasonography (focusing on the kidney) throughout the study. Special attention was paid to episodes of vomiting, prostration, myalgia and arthralgia, being potential consequences of the treatment. In addition,

**Table 1.** Hematological and biochemical parameters.

| | | Group A | | | Group B | | |
|---|---|---|---|---|---|---|---|
| | | **D0** | **D7** | **D13** | **D0** | **D7** | **D13** |
| ***Hematological*** | | | | | | | |
| WBC | $10^9$/L | 10.3 ± 2.8 | 11.5 ± 2.3 | 13.4 ± 2.5 | 9.5 ± 2.3 | 11.7 ± 3.3 | 11.3 ± 3.2 |
| LYM | $10^9$/L | 1.89 ± 0.60 | 2.24 ± 1.91 | 1.44 ± 0.55 | 1.76 ± 0.47 | 1.90 ± 0.59 | 1.82 ± 0.57 |
| MON | $10^9$/L | 0.59 ± 0.39 | 0.63 ± 0.35 | 0.81 ± 0.56 | 0.49 ± 0.16 | 0.71 ± 0.27 | 0.68 ± 0.26 |
| NEU | $10^9$/L | 6.63 ± 1.58 | 8.23 ± 1.76 | 10.40 ± 2.43 | 5.72 ± 2.04 | 8.27 ± 3.28 | 6.86 ± 2.15 |
| EOS | $10^9$/L | 1.17 ± 0.77 | 0.41 ± 0.30 | 1.77 ± 0.60 | 1.49 ± 1.27 | 0.77 ± 0.76 | 1.91 ± 1.22 |
| BAS | $10^9$/L | 0.04 ± 0.03 | 0.03 ± 0.02 | 0.03 ± 0.02 | 0.04 ± 0.03 | 0.03 ± 0.02 | 0.03 ± 0.02 |
| LIC | $10^9$/L | 0.42 ± 0.26 | 0.29 ± 0.22 | 0.79 ± 0.27 | 0.38 ± 0.18 | 0.32 ± 0.21 | 0.59 ± 0.32 |
| RBC | $10^{12}$/L | 7.01 ± 0.49 | 6.14 ± 0.59 | 5.76 ± 0.55 | 7.17 ± 0.26 | 6.17 ± 0.30 | 6.41 ± 0.48 |
| HGB | g/L | 171 ± 13 | 147 ± 13 | 136 ± 13 | 172 ± 5 | 146 ± 6 | 153 ± 9 |
| HCT | L/L | 0.49 ± 0.04 | 0.42 ± 0.04 | 0.39 ± 0.04 | 0.50 ± 0.02 | 0.42 ± 0.02 | 0.44 ± 0.03 |
| MCV | fL | 69.8 ± 2.0 | 68.5 ± 1.9 | 68.2 ± 1.9 | 69.3 ± 2.3 | 68.3 ± 2.3 | 68.3 ± 2.3 |
| MCH | pg | 24.3 ± 0.7 | 23.9 ± 0.5 | 23.7 ± 0.7 | 24.1 ± 0.8 | 23.7 ± 0.8 | 23.9 ± 0.8 |
| MCHC | g/L | 348 ± 3 | 349 ± 4 | 348 ± 5 | 347 ± 3 | 347 ± 2 | 350 ± 5 |
| PLT | $10^9$/L | 347 ± 50 | 309 ± 44 | 373 ± 34 | 327 ± 68 | 332 ± 72 | 393 ± 91 |
| ***Biochemical*** | | | | | | | |
| Glu | mmol/L | 4.56 ± 0.18 | 4.73 ± 0.49 | 4.63 ± 0.31 | 5.16 ± 0.30 | 4.71 ± 0.31 | 4.94 ± 0.30 |
| Cl | mmol/L | 109 ± 3 | 112 ± 5 | 110 ± 2 | 111 ± 3 | 109 ± 3 | 112 ± 2 |
| Ca | mmol/L | 2.29 ± 0.08 | 2.32 ± 0.15 | 2.28 ± 0.10 | 2.32 ± 0.06 | 2.30 ± 0.11 | 2.35 ± 0.11 |
| Mg | mmol/L | 0.76 ± 0.04 | 0.77 ± 0.07 | 0.73 ± 0.08 | 0.78 ± 0.02 | 0.76 ± 0.04 | 0.79 ± 0.07 |
| P | mmol/L | 1.19 ± 0.24 | 1.40 ± 0.19 | 1.57 ± 0.36 | 1.22 ± 0.14 | 1.43 ± 0.21 | 1.42 ± 0.24 |
| Urea | mmol/L | 3.85 ± 1.11 | 3.60 ± 0.69 | 3.48 ± 0.62 | 4.07 ± 1.22 | 3.88 ± 0.90 | 3.92 ± 0.77 |
| Crea | µmol/L | 47.7 ± 7.2 | 44.2 ± 4.1 | 40.5 ± 3.8 | 54.0 ± 10.2 | 46.2 ± 10.0 | 48.2 ± 9.8 |
| LDH | µkat/L | 1.27 ± 0.36 | 1.11 ± 0.46 | 1.39 ± 0.51 | 0.94 ± 0.45 | 1.04 ± 0.25 | 0.76 ± 0.33 |
| ALT | µkat/L | 0.53 ± 0.13 | 0.45 ± 0.13 | 0.38 ± 0.12 | 0.55 ± 0.16 | 0.49 ± 0.12 | 0.55 ± 0.28 |
| AST | µkat/L | 0.59 ± 0.10 | 0.55 ± 0.07 | 0.55 ± 0.12 | 0.43 ± 0.07 | 0.40 ± 0.08 | 0.44 ± 0.08 |
| CK | µkat/L | 3.17 ± 0.45 | 2.28 ± 0.55 | 2.61 ± 0.83 | 2.05 ± 0.68 | 1.95 ± 0.52 | 1.75 ± 0.40 |
| GMT | µkat/L | 0.24 ± 0.03 | 0.23 ± 0.03 | 0.22 ± 0.02 | 0.24 ± 0.01 | 0.21 ± 0.01 | 0.23 ± 0.03 |
| ALP | µkat/L | 1.06 ± 0.37 | 1.54 ± 0.76 | 2.31 ± 1.49 | 1.11 ± 0.58 | 1.34 ± 0.73 | 1.40 ± 0.69 |
| TP | g/L | 61.2 ± 4.5 | 62.3 ± 5.3 | 63.2 ± 3.3 | 60.8 ± 2.5 | 60.3 ± 3.7 | 63.7 ± 4.8 |
| Alb | g/L | 11.7 ± 1.0 | 10.5 ± 1.0 | 9.00 ± 1.1 | 12.3 ± 1.4 | 11.2 ± 1.8 | 10.5 ± 1.8 |

Hematological and biochemical parameters measured before the first administration (D0), and after 7 (D7) and 13 (D13) days of treatment with Antimania (group A, n = 6) or Glucantime (group B, n = 6). Data are expressed as mean ± SD.

biochemical and hematological analysis were performed before the start of the experiment (Day 0), and before the first drug administration of the day on Day 7 and Day 13. The animals were fasted for 12-18 hours before blood sampling, while water was provided *ad libitum*. The measured hematology and biochemical parameters are reported in Table 1. Local tolerability was evaluated by observation and palpation of the injection area following each administration: appearance (e.g. erythema, hair loss, scaling, pigmentation, edema), pain, heat, induration and swelling were evaluated using specific rating scales 1, 2, 3 and 6 hours after the first and the last administrations, and 1 hour after all the other administrations. Blood samples (2 mL) were collected 10 minutes before each administration, and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 480, 600 and 710 minutes after the first and the last administrations of both tested products. Plasma was extracted and stored at -20 °C (< 3 weeks). After thawing plasma at room temperature, 200 µL were transferred into a new tube and 10 mL of a 0.5% nitric acid 0.2% Triton X 100 aqueous solution containing the indium ICP internal standard was added. Samples were then agitated on a vortex mixer for 10 seconds and frozen until analysis. Quantitative determination of the target analyte was performed by inductively coupled plasma mass spectrometry (ICP/MS), operating at a RF power of 1100 W and with lens voltage set at 9.00 V. The signals monitored for Sb and the internal standard were 120.904 *m/z* and 114.904 *m/z*, respectively. The method was successfully validated over the 0.5-100.0 µg/mL range prior to the study, showing good linearity (R2 > 0.98), as well as satisfying accuracy and precision (bias and CV% always < 10%), assessed in sextuplicate over four different concentrations. In order to evaluate any potential drug accumulation, peak concentration (Cmax), time of peak concentration (Tmax), elimination rate constant (Kel), half-life of elimination phase (t1/2), area under the curve to the last quantifiable concentration (AUCt), area under the curve to infinity (AUCi) and mean residence time (MRT) of antimony (sum of SbIII and SbV) in plasma were calculated by non-compartmental analysis. The accumulation factor for each dose was calculated using two different approaches: as AUCt (after the last administration)/AUCt (after the first administration), and as $1/(1\text{-e-Kel}\cdot\tau)$, where $\tau$ is the dose interval of 12 hours. Pharmacokinetic parameters of antimony were calculated with EquivTest 2 (Statistical Solutions, Cork, Ireland); Statistica v.8 (StatSoft, Tulsa, OK, USA) was used for the evaluation of hematological and biochemical parameters. All analyses were performed in blind.

## Results

According to clinical observations and physical examinations, all the animals were in good health condition throughout the whole study period, and did not show any toxicity or behavioral abnormalities.

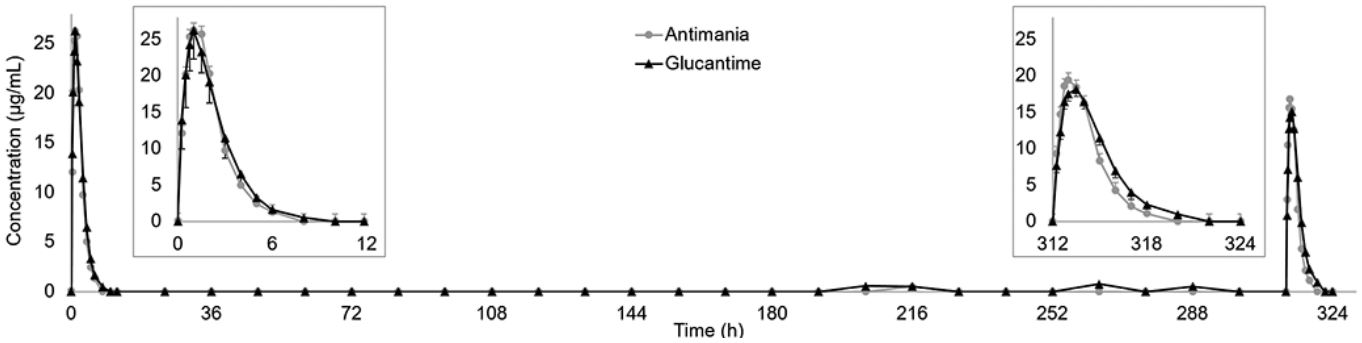
No considerable changes and differences between the groups were observed in hematological parameters, with total white blood cells count slightly increased in both groups from day 0 to day +13 (Table 1). In particular, statistically significant ($p < 0.05$) higher mean in neutrophils count was found in group A compared to group B on day +13; also, mean HGB and HCT were lower ($p < 0.05$) in group A on day +13, most likely influenced by the blood sampling schedule. However, individual and mean values of all the monitored hematological parameters varied within the physiological ranges. Normal or biologically insignificant findings were observed in biochemical parameters during the experiment. A modest increment of alkaline phosphatase (ALP), indicating an inflammation process, and a slight decrease of serum

**Table 2.** Pharmacokinetic parameters.

| | | Group A | | Group B | |
|---|---|---|---|---|---|
| | | **First** | **Last** | **First** | **Last** |
| Cmax | (µg/mL) | 28.2 ± 7.8 | 20.9 ± 4.3 | 26.3 ± 4.0 | 18.7 ± 4.9 |
| Tmax | (min) | 70.0 ± 22.6 | 77.5 ± 33.4 | 65.0 ± 12.2 | 90.0 ± 26.8 |
| Kel | (1/min) | 0.012 ± 0.001 | 0.010 ± 0.002 | 0.011 ± 0.002 | 0.008 ± 0.002 |
| t1/2 | (min) | 59.6 ± 7.0 | 69.7 ± 10.7 | 65.0 ± 10.0 | 87.2 ± 21.4 |
| AUCt | (µg min/mL) | 4276 ± 688 | 3367 ± 628 | 4435 ± 510 | 3874 ± 937 |
| AUCi | (µg min/mL) | 4348 ± 698 | 3429 ± 626 | 4525 ± 509 | 3980 ± 952 |
| MRT | (min) | 124 ± 19 | 132 ± 18 | 133 ± 15 | 167 ± 28 |

Pharmacokinetic parameters after the first and last administrations of Antimania (group A, n = 6) or Glucantime (group B, n = 6), corresponding at 50 mg meglumine antimoniate/kg b.w. Cmax (peak concentration); Tmax (time of peak concentration); Kel (rate constant of the elimination phase): t1/2 (half-life of elimination phase); AUCt (area under the curve to the last quantifiable concentration); AUCi (and area under the curve to infinity); MRT (mean residence time). Data are expressed as mean ± SD.

**Figure 1.** Plasma vs. time curve.




Mean plasma concentrations of antimony following the first and the last subcutaneous administrations of Antimania (group A, n = 6) or Glucantime (group B, n = 6) at 50 mg meglumine antimoniate/kg b.w. and before all the other administrations (total dosage 100 mg meglumine antimoniate/kg b.w./day). Error bars in the magnified pictures indicate SD.

albumin were found in two animals of group A on day +13. Yet, these findings did not influence mean values and did not generate statistically significant differences between the two groups. Urine analysis performed during the study revealed neither signs of kidney injury nor marked differences between the two groups. The ultrasonography confirmed the physiological aspect of abdominal organs: cortical and medullar structure of the kidney and general perfusion remained unchanged, as well as the systolic and diastolic blood flow from the renal artery measured with pulsed wave Doppler.

Local reactions at the injection sites were observed 12-84 hours after the administration. Only minor subcutaneous swelling (< 2 cm in diameter) was found by palpation at the injection sites in dogs from both groups (6 from group A and 4 from group B), and progressively disappeared. No other signs, such as erythema, hair loss, scaling, pigmentation, edema, pain, heat and induration, were recorded. No differences were found between Antimania and Glucantime for the main pharmacokinetic parameters following the first and the last administrations of 50 mg meglumine antimoniate/kg b.w. (Table 2), as also suggested by the relative concentration over time curves (Figure 1). Accumulation factor based on AUC was always < 1, while accumulation factor based on Kel was 1 ± 0.02 in all dogs, thus indicating no accumulation.

## Discussion

In consideration of the results of the present study, meglumine antimoniate administered for 14 days at the therapeutic daily dose of 100 mg/kg b.w., divided in two administrations, is well tolerated by healthy dogs. In addition, no relevant differences were observed in the pharmacokinetics and in the tolerability of the two tested products. As extensively reported in literature, most of the animals showed slight local swelling at the injection sites, which spontaneously disappeared after few days. This, together with the modest increase of the total white blood cells count, could be related to the local reactions in the injection sites. Therefore, the known adverse effects during treatment with antimony may be imputable to the health status of infected animals and not to the therapy.

However, the severity of the medical condition of a dog affected by leishmaniasis should be taken into account when treating with meglumine antimoniate, especially considering the severe renal dysfunction associated with this disease, which can greatly influence the drug pharmacokinetics and tolerability.

## Conclusions

Pentavalent antimony is the gold standard treatment for leishmaniasis in dogs. The two antimony based drugs tested were well tolerated by all the animlas enrolled in the present study, with no accumulation of the active principle, as demonstrated by the results of the pharmacokinetic analysis. In conclusion, there is no significant difference between the two tested products, and they can be considered safe when administered at the recommended dosage regimen in dogs

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