

Pharmacokinetic and Tolerance Study of Meglumine Antimoniate after subcutaneous administration in dog

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Introduction

Pentavalent antimony products (Sb^V) are the first choice for the treatment of Leishmaniasis disease, a zoonosis caused by more than 20 different species of the protozoan *Leishmania* that are transmitted by the bite of infected sandflies (*Phlebotominae*) (WHO, 2016).

Dog is a reservoir host for leishmaniasis, which represents a serious veterinary and public health problem also because the therapeutic arsenal cannot completely eliminate the infection in this species, and remission of clinical signs is only temporary.

The mechanism of action of Sb^V 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 (Oliva et al., 2010).

The therapy outcome is influenced by the individual immune response and by the susceptibility of the parasite strain to the drug (Amusatgeui et al., 1998; Carrió and Portús, 2002). It is known that nephrotoxicity, cardiotoxicity and hepatotoxicity are associated with antimonial therapy in humans (Ikeda-Garcia et al., 2007). In dogs, common adverse effects include apathy, anorexia, vomiting, diarrhea and pain at the site of injection (Noli, 1999; Baneth and Shaw, 2002); pancreatitis has also been associated with N-methyl glucamine treatment (Aste et al., 2005). 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 pharmacokinetic of plasma antimony (sum of Sb³⁺ and Sb⁵⁺) and tolerance after long-term (14 days) administration of the two meglumine antimoniate based veterinary products available across Europe

Study design and methods

The study was performed in blind and with parallel design in 12 adult healthy Beagle dogs. The dogs 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 Test Item (TI)(FATRO S.p.A., Ozzano dell'Emilia, Bologna, Italy); group B was treated with Glucantime 300 mg/mL Reference Item (RI)(Merial Laboratorios s.a. Barcelona, Spain).

Each animal has received a daily dose of 0.33 ml of solution for injection/kg b.w. equivalent to 100 mg meglumine antimoniate/kg b.w., divided in two separate subcutaneous administrations performed with a 12-hours interval (morning and evening).

The trial was conducted in compliance with Good Laboratory Practice requirements.

Blood samples 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 last administrations. For each animal, the following pharmacokinetic parameters were determined: AUC_t, AUC_∞, residual area, C_{max}, T_{max}, k_{el} and t_{1/2} and parameter C_{min} was evaluated for steady state or accumulation evaluation.

The plasma samples were analysed for the determination of antimony, using ICP/MS validated analytical method. 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.

General tolerability was evaluated by clinical observation of the animal health status, physical examination, biochemical and hematological analysis, urine analysis and abdominal ultrasonography (focusing on the kidney) throughout the study.

Local tolerability was evaluated by observation and palpation of the injection area following each administration 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

Results and discussion

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.

Pharmacokinetic parameters (C_{max}, T_{max}, k_{el}, t_{1/2}, AUC_t, AUC_∞, MRT and residual area) were calculated for antimony, using the analytical data within the validation range of the analytical method, after the first and the last administration.

The calculated mean values after the first and last administrations of 50 mg meglumine antimoniate/kg b.w. were, respectively: in group A, C_{max} 28.23 and 20.89 µg/mL, T_{max} 70.0 and 77.5 min, k_{el} 0.01 and 0.01 min, t_{1/2} 59.55 and 69.69 min, AUC_t 4275.8 and 3366.7 µg·min/mL, AUC_∞ 4348.3 and 3428.7 µg·min/mL, MRT 124.48 and 132.18 min; in group B, C_{max} 26.29 and 18.66 µg/mL, T_{max} 65.0 and 90.0 min, k_{el} 0.01 and 0.01 min, t_{1/2} 65.02 and 87.23 min, AUC_t 4435.0 and 3874.1 µg·min/mL, AUC_∞ 4524.6 and 3979.7 µg·min/mL, MRT 133.02 and 166.72 min. In Figure 1 and Figure 2 are shown, respectively, the overlay plot of plasma concentration vs time curve.

Figure 1 - Antimony Plasma Concentration-time Curve – Overlay Plot –ANTIMANIA.

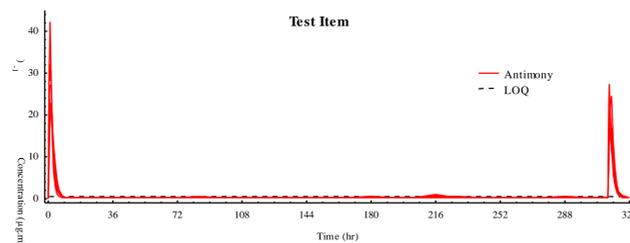
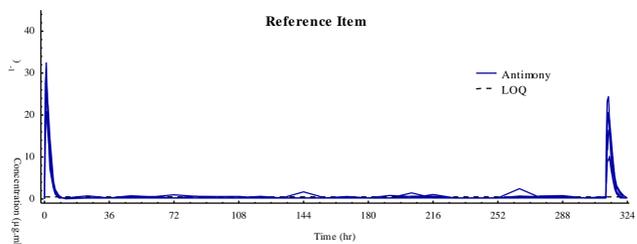


Figure 2 - Antimony Plasma Concentration-time Curve – Overlay Plot – GLUCANTIME.



No difference was found between TI and RI for main pharmacokinetic parameters after the first and the last administration.

Table 1. - Evaluation of Difference between Test and Reference Item.

		SW test p	F-test p	df	t-test p	N	MW test p
C _{max} (µg·mL ⁻¹)	Test Item	0.215					
the first dose	Reference Item	0.986	0.161	10	0.599	12	0.873
AUC _t (µg·min·mL ⁻¹)	Test Item	0.997					
the first dose	Reference Item	0.348	0.528	10	0.659	12	0.749
AUC _∞ (µg·min·mL ⁻¹)	Test Item	0.997					
the first dose	Reference Item	0.370	0.505	10	0.628	12	0.873
C _{max} (µg·mL ⁻¹)	Test Item	0.615					
the last dose	Reference Item	0.451	0.797	10	0.419	12	0.522
AUC _t (µg·min·mL ⁻¹)	Test Item	0.090					
the last dose	Reference Item	0.443	0.400	10	0.296	12	0.262
AUC _∞ (µg·min·mL ⁻¹)	Test Item	0.088					
the last dose	Reference Item	0.419	0.379	10	0.264	12	0.200

Accumulation factor based on AUC were lower than 1, accumulation factor based on k_{el} were round 1 therefore any accumulation was not approved for TI and RI.

Normal or biologically insignificant findings in biochemistry parameters evaluated were observed during the study and no considerable differences in serum chemistry parameters were identified between the Groups. Examination of urine samples and abdominal ultrasonography (focused on kidney) performed during the study revealed neither signs of kidneys' injury nor marked differences between the Group A and B.

The statistical evaluation confirmed that the variability of the haematological, biochemistry and urinary data, mainly due to animal effect, led to differences which were statistically significant for some parameters both within and between Groups. Anyway, these differences must be evaluated considering that, almost in all cases, the data moved within the physiological ranges of the parameters.

No mortality, signs of toxicity, ill health or behavioural abnormalities were observed in the animals and the results of clinical examinations supported good general tolerance. Only slight local reactions swelling (< 2 cm in diameter) at the injection sites were found by palpation 12-84 hours after the administration and persisted to the end of the study or progressively disappeared in both Groups. 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, as also suggested by the relative concentration over time curves

On the base of the evaluations of the pharmacokinetic, haematological and biochemistry results, it can be concluded that no difference was found between the two products for main pharmacokinetic parameters after the first and the last administration. Also, no relevant in the tolerability of the two tested products.

It can be concluded that 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 and any accumulation occurs.

As extensively reported in literature, most of the animals showed slight local swelling at the injection sites, which spontaneously disappeared after few days. Therefore, the known adverse effects during treatment with antimony may be imputable to the health status of infected animals and not to the therapy.

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