Leisguard® enhances the leishmanicidal activity of canine macrophages

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Introduction

Leisguard® is a domperidone based product recently approved by the Regulatory Authorities of several European countries for the treatment and prevention of canine Leishmaniosis. Its clinical efficacy has been demonstrated in several clinical trials (Gómez-Ochoa et al., 2009, The Vet.Journal 179: 259–263; Llinás et al., 2011, SEVC-AVEPA Congress, Barcelona;). It has been published that its administration to dogs stimulates their cell-mediated immunity leading to a statistically significant activation of phagocytic cells responsible for parasite killing

(Gómez-Ochoa et al., 2012, Veterinary Immunology and Immunopathology 146: 97-99, Sabaté et al., 2013, SCIVAC Congress, Pisa: 117-119). The aim of the present study was to confirm that the stimulatory effect of Leisguard® on the canine phagocytic cells results in an increase of their leishmanicidal activity.

Material and methods

The study was performed with 10 crossbreed healthy dogs of different sex, age and weight, seronegative to Leishmania. All dogs were administered 1ml/10kg/day of Leisguard® (equivalent to 0.5mg domperidone/kg/day) during 30 consecutive days. Blood samples from each animal were obtained at days 0 (before treatment initiation) 15 and 30. For each sample, mononuclear cells were isolated and incubated during 10 days to obtain a macrophage monolayer. Promastigotes of Leishmania infantum were then added to the culture medium (ratio of 10:1) and allowed to rest in contact with macrophages during 2 hours. Two days later, the percentage of infected macrophages and the number of intact parasites inside macrophages were assessed in each preparation by means of fluorescence staining of macrophage DNA. Finally, the percentage of activated macrophages in each preparation was assessed by using the Nitroblue Tetrazolium (NBT) Test (Gomez-Ochoa et al., 2010, Vet.Parasitology 172: 135–138).

Results

Mean percentage of infected macrophages in preparations of day 0 was 89.0% thus confirming the validity of the experimental model. Values of preparations from days 15 and 30 were 27.2% and 24.4% respectively, being differences vs. day 0 statistically significant. These results correspond to a reduction of 70-73% in the percentage of infected macrophages. In addition, mean number of intact parasites inside macrophages among preparations from days 15 and 30 were also significantly lower than those from day 0 (9.3 and 11.3 vs. 44.4; p<0.05), corresponding to a reduction of 75-80% in this parameter. On the other hand, while mean percentage of activated macrophages at day 0 was 2.0%, these percentage significantly raised up to 61.1% and 62.2% in preparations from days 15 and 30 (p<0.05), coinciding with the lower infection rates. A statistically significant correlation (r = -0.93; p<0.01) was found between the % of infected macrophages and the % of activated macrophages. Finally, microscopic evaluation of infected macrophages in preparations from days 15 and 30 revealed the presence of fragmented DNA corresponding to destroyed amastigotes inside the cytoplasm, therefore confirming that parasites had been eliminated once phagocyted by these cells.

Conclusions

The results of this study confirm that Leisguard® stimulates the dog's cell-mediated immunity leading to a statistically significant activation of macrophages which is correlated with a statistically significant increase of their leishmanicidal activity.