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Canine visceral leishmaniasis: Comparison of in vitro leishmanicidal activity of marbofloxacin, meglumine antimoniate and sodium stibogluconate

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Abstract

The control of canine leishmaniasis largely depends on the success of treatment. Drugs currently available to treat this disease are toxic and partially effective. The curative effect of marbofloxacin, a third-generation fluoroquinolone developed for veterinarian individual treatment, was evaluated in vitro in the presence of *Leishmania infantum* promastigotes and dog-monocyte-derived macrophages; meglumine antimoniate and sodium stibogluconate were used as comparative treatments. We observed that the killing of *Leishmania* promastigotes and intracellular amastigotes by marbofloxacin was dose-dependent. We demonstrated that successful treatment of canine infected macrophages for 48 h was possible with 500 μg/ml of marbofloxacin. Leishmanicidal activity acted through a TNF-α and nitric oxide pathway and correlated with the generation of nitric oxide (NO_2) production by monocytes derived macrophages from infected (23 ± 5 μM) or healthy (21 ± 6 μM) dogs, in comparison with NO_2 concentration in infected/non-treated macrophages (<3 μM, $P < 0.01$). This significant induced parasiticidal effect correlated with extensive elimination of amastigotes by macrophages derived from infected (11 ± 5) and healthy dogs (6 ± 2), when compared to infected/non-treated macrophages (530 ± 105 and 472 ± 86 amastigotes, respectively, $P < 0.01$). Marbofloxacin was shown to be non-toxic at 500 μg/ml in vitro and no cell apoptosis was observed. The molecule was able to induce a parasitic process after significant elimination of amastigotes in leishmania-infected dog macrophages. We propose that marbofloxacin, compared to standard chemotherapeutic agents (meglumine antimoniate and sodium stibogluconate), could be an effective and pragmatic oral route alternative to treat canine leishmaniasis.

Keywords: Visceral leishmaniasis; Dogs; Marbofloxacin; In vitro; Nitric oxide pathway

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1. Introduction

Visceral leishmaniasis (VL) is a disease that affects both humans and dogs in Mediterranean countries. The World Health Organization estimates that this anthropozoonosis causes 12 million cases of leishmaniases worldwide, with approximately 350 million people in 88 countries threatened by this disease and over 500,000 new cases of visceral leishmaniasis occurring each year (WHO, 2000). *Leishmania* promastigotes are transmitted by sandfly vectors of the *Phlebotomus* genus. After multiplication within mononuclear cells, intracellular amastigotes spread to other phagocytes of the reticuloendothelial system, causing a chronic and fatal disease (Peters and Killick-Kendrick, 1987). There is a long-held assumption that in VL the success of any chemotherapeutic regimen with a direct antiparasitic activity is dependent on the potential immunological response of the host, a response, which is probably mediated by macrophages activated by T-cell-derived cytokines, such as IFN-γ (Murray, 1988). Dogs represent an important reservoir of *Leishmania* parasites. The bio-molecular mechanism involved in the macrophage-induced killing of pathogens during leishmanial course is unclear (Green et al., 1991). During the last decade, the L-arginine-nitric oxide (NO) pathway has been shown to contribute to the macrophage-induced elimination of *Leishmania* spp. (Liew et al., 1990; Vouldoukis et al., 1995; Pinelli et al., 2000).

Control of the development of canine VL caused by *Leishmania infantum* parasites largely depends on the efficacy of treatment. Drugs currently used for the treatment of canine VL are only partially effective (Slappendel, 1988). Relapses are typical and toxicity and the emergence of resistance to antimony, amphotericin B and pentamidine therapies often limit their efficacy. Moreover, the presence of parasites in clinically cured dogs is common (Oliva et al., 1995, 1998; Riera et al., 1999). In recent years, canine VL has dramatically increased in the Mediterranean basin, with seroprevalence reaching up to 30–40% in some areas (Marty et al., 1994). It can thus be considered as an emergent disease in some endemic areas in Europe that show a serological prevalence of up to 63% in dogs (Solano-Gallego et al., 2001). Moreover, canine VL has recently emerged in the United States (Rosypal et al., 2003, 2005a,b). Consequently, improved chemotherapy of canine leishmanial infection is still necessary and the need for new molecular targets on which to base future treatment strategies appears justified.

Fluoroquinolone antibiotics demonstrate activity against several bacteria and are frequently used in the treatment of a wide range of Gram-positive and Gram-negative bacterial infections (Gooding et al., 1992; Schneider et al., 1996; Bryskier, 1999; Prescott et al., 2000; Meunier et al., 2004a,b). These antimicrobial agents act on DNA gyrase and/or topoisomerases. They have been demonstrated to be potential chemotherapeutic agents for antitumour and antiparasitic pathogens (Burri et al., 1996). Leishmanial DNA topoisomerases may well provide a good target for the successful curative effect of antileishmanial drugs (Ray et al., 1997; Chakraborty and Majumder, 1988). The *L. donovani* type I enzyme resembles bacterial topoisomerase I in its requirement for divalent cations and its inability to relax positively supercoiled DNA (Chakraborty et al., 1993).

Marbofloxacin (Marbocyl®) is a third-generation fluoroquinolone developed by Vétoquinol Laboratory for veterinary use (Fig. 1). It is used in the treatment of canine subjects and demonstrates remarkable pharmacokinetic characteristics in dogs. Oral bio-availability is close to 100% and plasma protein binding is low (Schneider et al., 1996). In addition, the molecule shows a very high volume of distribution, which is widely diffused throughout the organism. Its elimination half-life (14 h) facilitates a single daily administration (Schneider et al., 1996). Moreover, given their ability to reserve fluoroquinolones intracellularly, it has been suggested that immune phagocytic cells act as transporters to diffuse the drug to infected tissues (Gladue et al., 1989). These characteristics and abilities show the potential to test fluoroquinolones,

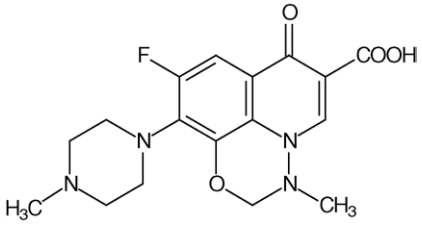

Fig. 1. Chemical structure of marbofloxacin (Marbocyl®; Vétoquinol Laboratory).