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Leishmania panamensis: Comparative inhibition of nuclear DNA topoisomerase II enzymes from promastigotes and human macrophages reveals anti-parasite selectivity of fluoroquinolones, flavonoids and pentamidine

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Abstract

Certain model inhibitors exerted selective action against the catalytic activity of nuclear DNA topoisomerase II (TOPII) of *Leishmania panamensis* promastigotes. The second-generation fluoroquinolones enoxacin and ciprofloxacin exhibited extraordinarily high anti-parasite selectivity displaying 582- and 40-fold greater potencies against *L. panamensis* TOPII as compared with the human macrophage enzyme. The flavonoids quercetin and ellagic acid showed inverse specificities, the former being 161-fold more potent against *L. panamensis* TOPII, and the latter 15.7-fold more active against macrophage TOPII. The protoberberine coralyne was a potent inhibitor of both *Leishmania* and macrophage TOPII. Bis-benzimidazoles and the diamidine diminazene aceturate exhibited uniformly high potencies against parasite and host TOPII, but a second diamidine pentamidine showed 17.6-fold greater specificity for *Leishmania* TOPII. The antimonial sodium stibogluconate was an ineffective inhibitor of parasite TOPII showing 4.3-fold greater potency against the macrophage enzyme. These findings suggest that the leishmanicidal activities of certain fluoroquinolones and pentamidine may be mediated partly through TOPII inhibition.

Index Descriptors and Abbreviations: Leishmania panamensis; DNA topoisomerase II; Enzyme inhibition; Fluoroquinolone; Pentamidine; Flavonoid; Protoberberine; Bis-benzimidazole

1. Introduction

The intracellular protozoan parasite *Leishmania* spp. presents a major threat to human health in tropical regions as the vector-borne etiological agent of a clinically diverse disease known as leishmaniasis (Herwaldt, 1999). A combination of factors including the development of parasite resistance to drug therapies, the absence of a vaccine, problems with vector control and opportunistic *Leishmania* infections in AIDS patients (Montalban et al., 1989), have contributed to the rising incidence of leishmaniasis, and current estimates indicate that 12–15 million people are affected by the cutaneous, mucocutaneous or fatal visceral forms of the disease [Herwaldt, 1999; WHO Fact Sheet No. 116, 2000; WHO Report, 1999 (http://www.who.org)].

Treatment options for leishmaniasis are limited. The previous front-line agents, pentavalent antimonials, have been rendered obsolete in several endemic regions of the Old World (e.g., India) by the emergence of antimony-resistant parasites (Sundar, 2001). Antimonial treatment failure has also been reported in South America (Velez

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et al., 1997; Palacios et al., 2001), and conclusive proof of a link with drug resistance has recently been established (Rojas et al., 2004). The former anticancer agent miltefosine provides an effective oral therapy for Old World visceral leishmaniasis (Jha et al., 1999), but exhibited variable efficacies in trials against New World (muco)cutaneous syndromes caused by parasites of the *Leishmania Viannia* subgenus (Soto et al., 2004). This grave situation, exacerbated by the limitations of existing second-line drugs (e.g., pentamidine, amphotericin B) (Herwaldt, 1999), underlines the urgent need to validate biochemical targets for the rational development of novel antileishmanials and treatment strategies. The DNA topoisomerases (TOPs) of trypanosomatid parasites (*Leishmania* spp. and *Trypanosoma* spp.) have risen to prominence as potential targets for selective inhibition. TOPs play pivotal roles in modulating DNA topology during replication, transcription, recombination and repair and are established sites of action for clinical antitumour and antibacterial agents (Liu, 1989; Hooper, 1998). TOPI (EC 5.99.1.2) and TOPII (EC 5.99.1.3) of trypanosomatids exhibit significant structural and biochemical variations from the corresponding human enzymes, and perform critical functions in organizing the kinetoplast DNA (kDNA) network unique to these parasites (Burri et al., 1996; Cheesman, 2000; Das et al., 2001; Villa et al., 2003). Moreover, recent studies have revealed differential interactions of parasite and human TOPs with classical inhibitors including poisons and DNA minor groove-binding ligands (MGBLs), thus supporting the concept of designing trypanosomatid-specific TOP inhibitors (Burri et al., 1996; Cheesman, 2000). TOP poisons stabilize the enzyme-substrate intermediate (''cleavable-complex'') thus inducing TOP-mediated DNA cleavage, replication arrest and apoptosis. MGBLs act by interfering with substrate binding and catalysis, and apart from a few notable exceptions (e.g., bis-benzimidazoles), do not promote DNA cleavage (Burri et al., 1996; Cheesman, 2000). The prototype TOPI poison camptothecin and the bis-benzimidazoles Hoechst-33342 and -33258 (MGBLs) were shown to target trypanosomatid TOPs causing DNA fragmentation and cell death, but the relative sensitivities of parasites and host mononuclear cells varied considerably at the enzyme and/or cellular levels (Shapiro et al., 1989; Bodley and Shapiro, 1995; Marquis et al., 2003a; Walker and Saravia, 2004; Jean-Moreno et al., 2006). Specific protoberberines (Marquis et al., 2003b), bis-napthoquinones and flavonoids (Ray et al., 1998; Mittra et al., 2000) have been reported as *Leishmania*-selective TOP poisons at the enzyme and/or cellular levels, although inter-species variability is already evident (Jean-Moreno et al., 2006). Trypanosomatid TOPs have also been implicated as targets for current antitrypanosomal and antileishmanial drugs, including diminazene aceturate (Berenil®) (Shapiro and Englund, 1990; Basselin et al., 1998), pentamidine (Fox et al., 1990; Jean-Moreno et al., 2006) and the pentavalent antimonial sodium stibogluconate (Chakraborty and Majumder, 1988; Walker and Saravia, 2004; Jean-Moreno et al., 2006). Most recently, we demonstrated that fluoroquinolones (FQs), a class of TOPII poisons used as antibacterial agents, exert selective cytotoxic action *in vitro* against amastigotes of *Leishmania panamensis* (Romero et al., 2005).

In this study, we have identified specific FQs (enoxacin and ciprofloxacin), flavonoids (quercetin) and diamidines (pentamidine), as highly specific inhibitors of *L. panamensis* TOPII, thereby further validating *Leishmania* TOPs as candidate targets for rational drug design.

2. Materials and methods

2.1. Inhibitors

Additive-free preparations of the pentavalent antimonial drug sodium stibogluconate (SSG) (lot no. BL06916) were obtained as powdered formulations from the Walter Reed Army Institute. SSG concentrations were calculated in terms of μM of pentavalent antimony (Sb^V) using the following molecular weight (M_r) data: Sb^V, M_r 122; SSG, M_r 746. Ciprofloxacin was purchased from ICN Biomedicals (Aurora, Ohio), and all other inhibitors were obtained from Sigma (St. Louis, Missouri). Coralyne, quercetin and pentamidine were solubilized in dimethylsulphoxide (DMSO), enoxacin, lomefloxacin and ellagic acid were dissolved in 0.5 M NaOH, and all remaining compounds were prepared in ultra-pure water. Stock drug solutions were sterilized by filtration and either used immediately or stored at −70 °C.

2.2. Biological material

The human promonocytic cell line U-937 (ATCC CRL-159302) was cultured at 37 °C in a 5% (v/v) CO_2 atmosphere, using RPMI 1640 medium containing 1% (w/v) glutamine, 10% (v/v) foetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin (Sundstrom and Nilsson, 1976). Monocytes were harvested in the logarithmic stage of growth (48 h after subculture) by centrifugation (800*g*, 10 min, 4 °C), quantified by microscopy, then transferred to standard medium containing 100 ng/ml phorbol myristate acetate (PMA; Sigma). Following incubation for 72 h (37 °C, 5% CO_2) to induce adherence and differentiation (Bosque et al., 1998), macrophages were harvested by centrifugation (as above), washed in PBS, and stored in liquid nitrogen for subsequent preparation of nuclear extracts.

Promastigotes of *Leishmania (Viannia) panamensis* (strain MHCOM/CO/86/1166) were cultured at 25 °C by standard procedures (Bosque et al., 1998) in Schneider's Drosophila medium containing 10% (v/v) heat-inactivated foetal bovine serum (Gibco, Grand Island, New York), 1% (w/v) glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin (Bosque et al., 1998). Logarithmic phase parasites (at a mean cell density of 0.5×10^6 promastigotes/ml) were collected at 72 h after subculture by centrifugation, washed in PBS, and stored in liquid nitrogen.