Comparative antiplasmodial, leishmanicidal and antitrypanosomal activities of several biflavonoids

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Received 26 July 2004; accepted 19 October 2004

Abstract

The antiplasmodial, leishmanicidal and antitrypanosomal activities of eight natural biflavonoids were estimated in vitro on a chloroquine-resistant strain of Plasmodium falciparum, axenically grown Leishmania donovani amastigotes and Trypanosoma cruzi trypomastigotes and Trypanosoma brucei rhodesiense bloodstream forms. Lanaroflavone showed the highest antiplasmodial activity (IC50 = 0.48 μM), isoginkgetin was the most active leishmanicidal compound (IC50 = 1.9 μM), whereas ginkgetin (IC50 = 11 μM) and isoginkgetin (IC50 = 13 μM) showed the best antitrypanosomal activity in our assays. The cytotoxicity and the selectivity indices for the most active compounds were also estimated. Lanaroflavone exhibited a high selectivity index value (SI = 159), indicating selective antiplasmodial activity.

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Keywords: Biflavonoids; Antiplasmodial activity; Leishmanicidal activity; Antitrypanosomal activity; Selectivity index

Introduction

Protozoal diseases, and particularly malaria, leishmaniasis, Chagas disease and African trypanosomiasis, represent major causes of mortality in various tropical and subtropical regions. The situation is compounded by increasing treatment failures by current drugs. With the increase in the resistance of Plasmodium falciparum to more and more chemotherapeutic agents (Wellem and Plowe, 2001), morbidity and mortality due to malaria remain an important health problem in many developing countries. The different forms of leishmaniasis require expensive treatments, and the currently used medicines, pentavalent antimonials and/or pentamidine salts, show toxicity together with numerous side effects (Barrett and Gilbert, 2002). Trypanosoma cruzi is a protozoan that causes Chagas disease (American trypanosomiasis), an illness that affects approximately 16 million people in tropical and sub-tropical Americas. The drugs currently in use against this disease, nifurtimox and benznidazole, present several side effects and have limited efficacy (Kirchhoff, 2003). African trypanosomiasis is a vector-borne parasitic disease which is causing major health and economic problems in rural sub-Saharan Africa. The drugs currently used to treat sleeping sickness are far from satisfactory because
of major side effects, increasing resistance and the unaffordable price for African countries (De Koning, 2001).

In an effort to discover new lead compounds for infectious diseases, several research groups screen plant extracts to detect secondary metabolites with relevant biological activities. Biflavonoids have recently come to the forefront of the polyphenol family for their antiviral and antituberculosis activities (Lin et al., 1999, 2001). In recent pharmacological studies, they are also reported to possess antinociceptive, antiinflammatory and cytotoxic activities (Bittar et al., 2000; Kim et al., 2000; Lin et al., 2000). Biflavonoids are characterized by two flavonoid monomeric units (flavone or flavanone) covalently linked either with C–C or C–O–C bonds. The monomeric units may be of the same or different structural types. Only few reports are available in relation with their antiprotozoal activity, but recently antiplasmodial activity was established for two compounds of this class (Ahmed et al., 2001; Suárez et al., 2003; Nunome et al., 2004). The aim of the present study was to examine eight biflavonoids for their antiplasmodial, leishmanicidal, and antitrypanosomal activities. Toxicity of the different compounds was also estimated on L-6 rat skeletal myoblast cells.

Materials and methods

Cupressus flavone, bilobetin, sciadopitysin and amento-flavone-4″,4‴,7,7‴-tetramethyl ether were obtained commercially (LGC Promochem, Molsheim, France). The other examined compounds were isolated from plant species: Lanaroflavone was isolated by bioguided fractionation from the methanol extract of the aerial part of Campnosperma panamense Standl. collected in May 2000 in Southern Colombia (Weniger et al., 2001); Amentoflavone was isolated from Viburnum pichinchense Benth. leaves collected in 1996 near Medellin, Colombia (Lobstein et al., 2003); Ginkgetin and isoginkgetin were isolated from Ginkgo biloba L. leaves from the Ginkgo plantation S.C.A., Domaine de Saint-Jean d’Illac, France (Joyeux et al., 1995).

All tested compounds were dissolved in 10% DMSO and working stock solutions of 10 mg/ml were prepared in culture medium. Stock solutions were then further diluted in culture medium, as indicated for each test.

Antiplasmodial activity

Quantitative assessment of antimalarial activity in vitro was determined by means of the microculture radioisotope technique based upon the method previously described by Desjardins et al. (1979) and modified by Ridley et al. (1996). The assay uses the uptake of [3H]hypoxanthine by parasites as an indicator of viability. Continuous in vitro cultures of asexual erythrocytic stages of P. falciparum were maintained following the methods of Trager and Jensen (1976). Compounds were tested on K1 strain (multidrug pyrimethamine/chloroquine-resistant strain; Thaithong and Beale, 1981). Initial concentration of the compounds was 20 μg/ml diluted with two-fold dilutions to make seven concentrations, the lowest being 0.32 μg/ml. After 48 h incubation of the parasites with the compounds at 37 °C, [3H]hypoxanthine (Amersham, UK) was added to each well and the incubation was continued for another 24 h at the same temperature. Chloroquine (Sigma C6628) and artemisinin (Sigma 36,159-3) were used as positive references. The values given in Table 1 are means of two independent assays; each assay was run in duplicate.

Leishmanicidal activity

Fifty microlitres of culture medium, a 1:1 mixture of SM medium (Cunningham, 1977) and SDM-79 medium (Brun and Schönberger, 1979) at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum (FBS), was added to each well of a 96-well microtiter plate (Costar, USA). Serial drug dilutions in duplicates were prepared covering a range from 30 to 0.041 μg/ml. Then 10⁵ axenically grown Leishmania donovani amastigotes (strain MHOM/ET/67/L82) in 50 μl medium were added to each well and the plate incubated at 37 °C under a 5% CO₂ atmosphere for 72 h. Ten microlitres of resazurin solution (12.5 mg resazurin dissolved in 100 ml distilled water) were then added to each well and incubation continued for a further 2–4 h. The plate was then read in a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm (Rätz et al., 1997). Fluorescence development was measured and expressed as percentage of the control. Miltefosin (Zentaris GmbH, Germany) was used as a positive reference.

Antitrypanosomal activity (T. cruzi)

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells per well per 100 μl in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, 5000 trypomastigotes of T. cruzi Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene (Buckner et al., 1996) were added in 100 μl per well with two times of a serial drug dilution covering