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In vivo activity of ursolic and oleanolic acids during the acute phase of *Trypanosoma cruzi* infection

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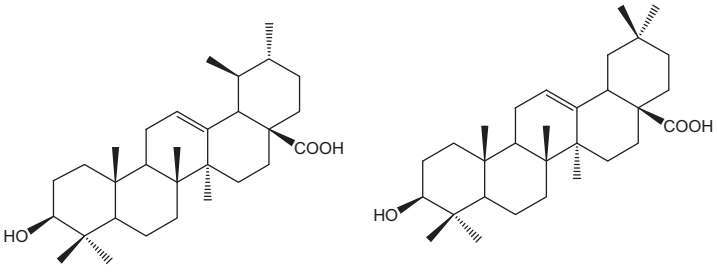
HIGHLIGHTS

- Infected animals were treated with ursolic and oleanolic acids.
- Oral treatment provided the most significant decrease of parasitemic levels.
- Intraperitoneal route did not affect the biological activity.
- The γ-IFN levels diminished considerably by the intraperitoneal treatment.
- Therefore, an immunosuppressive effect can be suggested for triterpenes.

GRAPHICAL ABSTRACT



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ABSTRACT

Reduction in the parasitemic levels of the Y strain of *Trypanosoma cruzi* in mice treated with oral or intraperitoneal ursolic (UA) and oleanolic (OA) acids was evaluated during the acute phase of Chagas' disease. Oral administration of UA and OA (50 mg/kg/day) provided the most significant reduction in the parasitemic peak, while intraperitoneal administration of UA and OA did not significantly affect the biological activity of the Y strain of *T. cruzi*. Interleukin levels in mice treated by the intraperitoneal route were compared to untreated chagasic mice. Reduced γ-IFN levels and enhanced IL-10 concentrations potentially explain the exacerbated parasitemia. Our data suggests an immunosuppressive effect for UA and OA, which could interfere with host control of parasitemia. Optimal results were achieved with oral administration. This observation may be explained by the low intestinal absorption of UA and OA, could cause a reduced immune response and promote parasite control. Taken together, these data demonstrate that triterpenes could be interesting compounds to develop therapeutically for the treatment of Chagas' disease.

1. Introduction

A century after its discovery, Chagas' disease still represents a health threat to an estimated 28 million people in the Americas, as the second most prevalent disease among neglected tropical illnesses. Infection by the protozoan *Trypanosoma cruzi* can be transmitted by blood-sucking triatomine bugs, blood transfusion, transplacental transmission, or foodstuffs contaminated with infected triatomine feces (WHO, 2007; Coura and Dias, 2009).

The main drug available for treatment of Chagas' disease is benznidazole (LAFEPE®), whose action eliminates *T. cruzi* parasites. However, this compound has limited efficacy and a high degree of toxicity (Cançado, 2002; Garcia et al., 2005). Great advances are being made in parts of South America to control *T. cruzi* transmission by insect vectors or via blood transfusion, but additional

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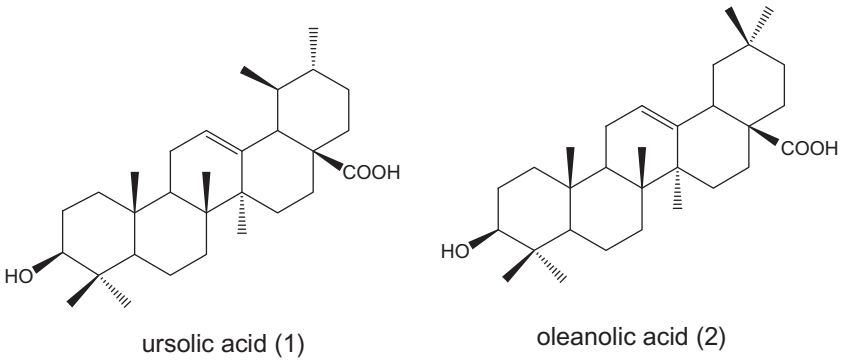

Fig. 1. Chemical structures of oleanolic acid (1) and ursolic acid (2).

priorities of Chagas' disease research should focus on identifying new drugs with shorter treatment courses and fewer side effects, as well as the design of pediatric formulations for these pharmaceuticals (Saùde-Guimarães and Faria, 2007; Moreno et al., 2010).

Several natural products from different structural classes have proven to be active against *T. cruzi*, and plant extract screening is a valid strategy that is currently being exploited for the discovery of natural trypanocidal products (Lirussi et al., 2004; Croft et al., 2005). Oleanolic and ursolic acids are ubiquitous triterpenoids in the plant kingdom, particularly in medicinal herbs, and are an integral part of the human diet (Liu, 2005). A variety of novel pharmacological effects produced by triterpenoids have been reported including pro-cardiovascular (Somova et al., 2003), anti-inflammatory (Vasconcelos et al., 2006), antimutagenic (Resende et al., 2006), antibacterial (Cunha et al., 2007), and anticancer activities (Liu, 2005; Ikeda et al., 2008). Previous studies reported by our group showed significant *in vitro* trypanocidal activity of triterpene acids (Cunha et al., 2003, 2006; Ferreira et al., 2010).

In the present study, a mouse model of acute Chagas' disease has been employed to investigate the effects of treatment with ursolic and oleanolic acids (Fig. 1). Interleukin levels were compared between treated and untreated chagasic mice. All of the biological assays performed herein concern the Y strain of *T. cruzi*.

2. Materials and methods

2.1. Extraction and isolation of ursolic acid and oleanolic acid

Ursolic acid and oleanolic acid were isolated from the plant species *Miconia fallax* as described by Cunha et al. (2003) (Fig. 1).

Benznidazole tablets (100 mg) were obtained from LAFEPE (Pharmaceutical Laboratory of Pernambuco, Recife, Pernambuco, Brazil).

2.2. Animals

Male BALB/C albino mice weighing approximately 25 g, provided by the University of São Paulo, were used. The animals were housed in clean cages at room temperature (22–25 °C) with both food and water *ad libitum* and controlled 12 h light/dark cycle. All procedures were approved by the Animal Research Ethics Committee of the University of São Paulo.

2.3. Trypanocidal activity in vivo

Mice were randomly divided into four groups of five animals each, as follows: group I (negative control) infected animals treated daily with 5% DMSO, 2.5% TWEEN, and 5% ethanol the same concentration employed for the dissolution of the investigated compounds UA and OA; group II (positive control) infected animals treated daily with benznidazole (LAFEPE®); group III- infected animals treated daily with UA; group IV- infected animals treated daily with OA. Animals were infected by intraperitoneal inoculation with approximately 2×10^4 trypomastigotes 48 h prior to drug administration. Drugs were administered using by both the oral and intraperitoneal route, with respective daily doses of 20 and 50 mg of the target drug/kg for 20 days. Parasitemia was evaluated by counting the trypomastigote forms of the parasite in 5 μl collected from animal's tail, starting at the second day of infection, following the method described by Brener (1962). The *in vivo* trypanocidal activity was expressed as the percentage of lysis of the trypomastigote form of *T. cruzi*. Animals were assessed for their survival time and level of infection.

2.4. Cytokine assays

For the cytokine assays, mice were divided into six groups of five animals each as follows: group I (IC) – control mice infected with 2×10^4 trypomastigotes of the Y strain of *T. cruzi*; group II (UC) – uninfected control; group III (UAT) – infected mice treated with 20 mg/kg UA; group IV (OAT) infected mice treated with 20 mg/kg OA; group V (UC UA) – uninfected control treated with 20 mg/kg UA; and group VI (UC OA) – uninfected control treated with 20 mg/kg OA. Test compounds conducted were administered by the intraperitoneal route.

Using plasma samples obtained 7 days post-infection, γ-IFN and IL-10 concentrations were measured by specific two-site enzyme-linked immunosorbent assay (ELISA) with reference standard curves according to the manufacturer's instructions. Samples were processed individually and assayed in duplicate. Plates were read at 450 nm, and all of the steps of the assay were carried out at room temperature. The antibody pairs for detection of the cytokines were acquired from R&D Systems (Minneapolis, MN, USA).

2.5. Statistical analysis

Statistical analysis was accomplished using the Prism program v. 4.0. Groups were compared using one-way ANOVA with Dunnett's multiple test for evaluation of parasitemia, or Bonferroni's multiple comparision test for cytokine assays.

3. Results

Intraperitoneal delivery of 20 mg/kg UA and OA did not significantly affect the Y strain of *T. cruzi* compared to control groups (Fig. 2A). Despite the shift in the parasitemic peak, UA treatment