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Effects of canthin-6-one alkaloids from *Zanthoxylum chiloperone* on *Trypanosoma cruzi*-infected mice

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Abstract

Canthin-6-one (**1**), isolated from *Zanthoxylum chiloperone* (*Rutaceae*), possesses a broad sprectum of antifungal and leishmanicidal activities. In this study, we have examined the antiparasitic effects of canthin-6-one (**1**), 5-methoxycanthin-6-one (**2**), canthin-6-one *N*-oxide (**3**), as well as that of the total alkaloids of *Zanthoxylum chiloperone* stem bark, in Balb/c mice infected either acutely or chronically with *Trypanosoma cruzi*. The compounds were administered orally or subcutaneously at 5 mg/kg/day for 2 weeks, whereas the alkaloidal extract was given at 50 mg/kg/day for 2 weeks. The antiparasitic activity was compared with that of benznidazole given at 50 mg/kg/day for 2 weeks. In the case of acute infection, parasiteamia was significantly reduced following oral treatment with canthin-6-one (**1**). Moreover, the total alkaloids of *Zanthoxylum chiloperone* stem bark led to high levels of parasitological clearance. Seventy days post-infection, the serological response in the acute model was significantly different between oral canthin-6-one (**1**) and benznidazole-treated mice. Chronic model of the disease showed that both canthin-6-one (**1**) and the alkaloidal extract at the above dosage induced 80–100% animal survival compared to untreated controls. These results indicate that canthin-6-one (**1**) exhibits trypanocidal activity in vivo in the mouse model of acute or chronic infection. This is the first demonstration of anti-*Trypanosoma cruzi* activity for a member of this chemical group (canthinones). Considering the very low toxicity of canthin-6-one (**1**), our results suggest that long-term oral treatment with this natural product could prove advantageous compared to the current chemotherapy of Chagas disease.

Keywords: *Zanthoxylum chiloperone*; Rutaceae; Paraguay; Canthin-6-one; Chagas disease; Experimental treatment

1. Introduction

Eighteen to 20 million people in South and Central America and 50,000–100,000 in the United State (WHO, 1997) are infected with *Trypanosoma* (Schizotrypanum) *cruzi*, while nearly 90 million persons live in zones where Chagas disease is endemic. Recent surveys indicate that there are ∼200,000 new cases and 21,000 deaths associated with this condition every year (Morel, 2000; OPS/HCP/HCT/140/99, 1999; WHO, 2002). Chemotherapy of Chagas disease remains unsatisfactory, and is based exclusively on nitroimidazoles. Belonging to this class of agents, benznidazole, developed by Roche three decades ago, shows efficacy in the acute and short-term (up to a few years) chronic phase of the disease (Vinhaes and Schofield, 2003). In recent years, great advances have been made in the control of the vectorial and transfusional transmission of the disease, particularly through the Southern Cone Initiative involving Brazil, Argentina, Paraguay and Uruguay. However, the question of the persons already infected remains of great concern. For a few years, Paraguayan and French groups have investigated natural products from plants used in traditional medicine, as to find active compounds against *Trypanosoma cruzi*. *Zanthoxylum chiloperone* var. *angustifolium* Engl. (syn. *Fagara chiloperone* Engl. Ex Chod. & Hassl.), *Rutaceae*) is a dioic

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tree (up to 15 m) growing in South America, which is called "*tembetary hu*", ("*tembé* = lip, *ità* = stone, *y* = abbreviation of "*yvyra* = tree, *hu* = black *sa'yjù*) in Guarani (Spichiger and Stutz de Ortega, 1987). A decoction of *Zanthoxylum chiloperone* var. *angustifolium* root bark is used traditionally in Paraguay for its antimalaric (Milliken, 1987), emmenagogue and antirheumatic properties (ethnobotanical data collected in the field). Due to its also known antiparasitic properties, we evaluated herein the bioactivity of various extracts of *Zanthoxylum chiloperone* var. *angustifolium* against *Trypanosoma cruzi*, which is responsible for Chagas disease. We had previously reported that three canthin-6-one-related alkaloids, isolated from the Paraguayan medicinal plant *Zanthoxylum chiloperone*, were active against 13 fungi (Thouvenel et al., 2003), and also effective in vivo against *Leishmania amazonensis* (Ferreira et al., 2002). As far as their mechanism of action is concerned, the antifungal and antiprotozoal properties of canthin-6-one alkaloids might show certain similarities with those of the antifungal triazole ravuconazole (Urbina et al., 2003b), albaconazole (Matta Guedes et al., 2004), and posazaconazole (Urbina, 2002), which have been shown to induce parasitological cure in murine models of either acute or chronic Chagas disease. In a preliminary biological screening, the alkaloidal extract of stem bark of *Zanthoxylum chiloperone* showed activity against epimastigote and trypanomastigote forms of *Trypanosoma cruzi* (unpublished data). In the present study, we compared the in vivo activity of canthin-6-one (**1**), 5-methoxycanthin-6-one (**2**), canthin-6-one *N*-oxide (**3**), as well as the total alkaloids of *Zanthoxylum chiloperone*, with that of the reference compound benznidazole, in mouse models of acute and chronic *Trypanosoma cruzi* infection.

2. Materials and methods

2.1. Compounds

The crude alkaloidal extract of *Zanthoxylum chiloperone*, canthin-6-one (**1**), 5-methoxycanthin-6-one (**2**) and canthin-6-one *N*-oxide (**3**) (see Fig. 1) were isolated as previously described (Schiplander and Mitscher, 1971; Thouvenel et al., 2003). Physical and spectral data (^{1}H and ^{13}C NMR and mass spectrometry) were used to determine the chemical structure of the compounds, and were in agreement with those of reference samples and literature values. Benznidazole (*N*-benzyl-1,2-nitro-1-imidazole-acetamide) was purchased from Roche (Buenos Aires, Argentina), and used as a reference drug.

2.2. Mice and parasites

Female and male Balb/c mice were purchased by the Faculty of Veterinary Sciences, National University (La Plata, Argentina), and bred at the Instituto de Investigaciones en Ciencias de la Salud (Asuncion, Paraguay). Animals were 6–8 weeks old when used. In all experiments, the CL clone of *Trypanosoma cruzi* (Cano et al., 1995) was used, generously supplied by Dr. B. Zingales (Sao Paulo, Brazil). Routine maintenance of the

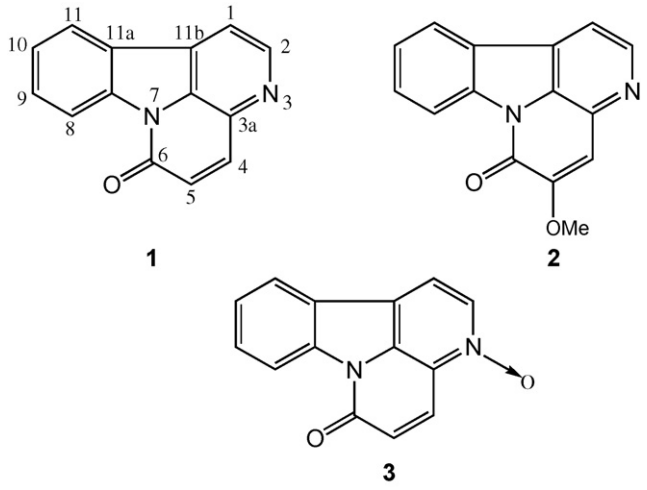

Fig. 1. Chemical structure of canthin-6-one alkaloids.

Trypanosoma cruzi strain was carried out in BALB/c mice inoculated intraperitonally every 14 days.

2.3. Drug treatment

2.3.1. Acute infection

All mice were infected intraperitonally with 5000 trypomastigotes from the CL strain following separation from blood. For the acute or chronic infection study, two separate experiments were conducted to evaluate the effectiveness of the drugs in vivo. Treatments were started 12 days after inoculation of the parasites. The mice were randomly divided into groups of 8–12 mice. The reference drug benznidazole was used for all experiments. All tested drugs were sampled in 50 μl aliquotes of phosphate buffered saline (PBS). Benznidazole was administered to BALB/c mice orally at 50 mg/kg/day for 2 weeks. Canthin-6-one (**1**), 5-methoxy-canthin-6-one (**2**) and canthin-6-one *N*-oxide (**3**) were administered orally or subcutaneously at 5 mg/kg/day for 2 weeks. The total alkaloids of *Zanthoxylum chiloperone* root bark was administered orally or subcutaneously at 50 mg/kg/day for 2 weeks. Each experiment was performed in duplicate.

2.3.2. Chronic infection

Routine maintenance of the *Trypanosoma cruzi* strain was carried out in BALB/c mice following intraperitoneal infection every 11 days. All mice were infected with 1000 trypomastigotes of CL strain from blood, allowing a slow-developing parasitaemia peaking between 21 and 28 days. This infection was effectively controlled since 70–80% animals survived showing a negative or subpatent parasitaemia, in spite of a slow degradation of their general physical conditions.

For the murine model of long-term infection, treatments were started 60 days post-infection when parasitaemia was sub-patent in all mice. In this experiment, the mice were randomly divided into groups of 5–8 animals. All tested drugs were sampled in 50 μl aliquotes of phosphate buffered saline (PBS) and given to BALB/c mice by the oral route in regimen of 50 mg/kg/day