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Antiparasitic activity of nerolidol in a mouse model of schistosomiasis

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ABSTRACT

Schistosomiasis is a major public health problem worldwide, especially in poor communities. Since praziquantel is currently the only drug available to treat schistosomiasis, there is an urgent need to identify new antischistosomal drugs. Nerolidol is a sesquiterpene present as an essential oil in several plants that has been approved by the FDA. This study evaluated the in vivo antischistosomal activity of nerolidol in a mouse model of schistosomiasis infected with either adult or juvenile stages of *Schistosoma mansoni*. A single dose of nerolidol (100, 200 or 400 mg/kg) administered orally to mice infected with adult schistosomes resulted in a reduction in worm burden and egg production. Treatment with the highest nerolidol dose (400 mg/kg) caused significant reduction in a total worm burden of 70.06% ($P < 0.001$). Additionally, the technique of quantitative and qualitative oograms showed that a single 400 mg/kg nerolidol dose achieved an immature egg reduction of 84.6% ($P < 0.001$). In faecal samples, the Kato–Katz method also revealed a reduction of 75.2% in eggs/g at a dose of 400 mg/kg ($P < 0.001$). Furthermore, scanning electron microscopy revealed that nerolidol-mediated worm killing was associated with tegumental damage. In contrast to activity against adult *S. mansoni* infection, oral treatment with nerolidol 400 mg/kg had low efficacy in mice harbouring juvenile schistosomes. Since nerolidol is already in use globally as a food additive and has a proven safety record, evaluation of this natural compound's potential for treatment of schistosomiasis could be entirely cost effective in the near future.

1. Introduction

Schistosomiasis is a major neglected tropical disease caused by blood-dwelling flatworms of the genus *Schistosoma* [1]. It affects more than 200 million people in tropical and subtropical regions of the world, especially in poor communities without access to safe drinking water and adequate sanitation [1,2]. The economic and health effects of schistosomiasis are considerable, and the disease disables more than it kills. In children, schistosomiasis can cause anaemia, stunting and a reduced ability to learn. Chronic schistosomiasis may affect people's ability to work and in some cases can result in death [3]. The current estimate of yearly disability-adjusted life-years (DALYs) for schistosomiasis is 3.4 million [2]. Human infection is due to three main species, namely *Schistosoma mansoni* and *Schistosoma japonicum*, which causes intestinal/hepatic schistosomiasis, and *Schistosoma haematobium*, which results in urinogenital disease [1].

Over the last several decades, chemotherapy using praziquantel (PZQ) has been a widely used strategy for the control and treatment of schistosomiasis. However, the drug does not prevent reinfection and, owing to use for more than three decades, the emergence of PZQ-resistant schistosomes is a constant threat. In addition, there is a critical deficiency in its therapeutic profile as it lacks activity against juvenile parasites [4]. Therefore, the search for a new chemotherapy is crucial to effectively control schistosomiasis in the future.

Natural products, especially from medicinal plants, present a diversity of molecules and have been a reliable source of chemotherapeutic agents, including in anthelmintic drug discovery [5,6]. Artemisinin and chloroquine are examples of plant-derived products with important therapeutic value. In recent years, an increasing number of studies have shown that nerolidol, an aliphatic sesquiterpene alcohol found in essential oils of several plants,

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exhibits a variety of biological properties, such as antimicrobial, antitumour, antioxidant, antinociceptive, antiulcer, anti-inflammatory and insecticidal properties (for review see [7]). In addition, nerolidol has demonstrated activity against several species of parasites, including *Plasmodium* [8], *Babesia* [9], *Trypanosoma* [10] and *Leishmania* [11]. It is important to note that nerolidol is used in many food and scented products and has been approved by the US Food and Drug Administration (FDA) [12,13].

Our group previously demonstrated that nerolidol at 31–62 μM possesses schistosomicidal activity against ex vivo *S. mansoni* adult worms [14]. We also showed that nerolidol caused morphological alterations in the tegument of parasites in a concentration-dependent manner. On the other hand, in vivo studies to determine the chemotherapeutic potential of nerolidol in the treatment of schistosomiasis have not yet been described. The present study investigated the in vivo antischistosomal activity of nerolidol administered by the oral route in mice infected with either adult or juvenile stages of *S. mansoni*.

2. Materials and methods

2.1. Animals

BALB/c mice were obtained from the Universidade Estadual de Campinas (Campinas, Brazil). For in vivo studies, 3-week-old BALB/c mice were infected with 80 cercariae of *S. mansoni* (BH strain). All animals were kept under environmentally controlled conditions (temperature 25 °C, humidity 70%) and had access to water (municipal tap water supply) and rodent food ad libitum.

2.2. Ethics statement

Procedures involving animals were carried out in accordance with Brazilian legislation. The protocol for maintenance of the *S. mansoni* life cycle was approved by the local ethics committee on animal experimentation.

2.3. Experimental design

In the first step, to study the dose–response relationship of nerolidol (a mixture of *cis*- and *trans*-nerolidol; Sigma-Aldrich, St Louis, MO) in adult *S. mansoni* infection (patent infection), mice were treated with a single oral dose of nerolidol at 49 days post-infection (p.i.). Nerolidol was mixed in corn oil and was administered to rodents in 100 μL by the oral route. The control group received an equal volume of corn oil only. Mice harbouring a chronic schistosome infection were divided into four experimental groups, with each group consisting of 10 animals treated with a single oral dose as follows: Group I, vehicle-treated control group; Group II, group treated with nerolidol at 400 mg/kg; Group III, group treated with nerolidol at 200 mg/kg; and Group IV, group treated with nerolidol at 100 mg/kg.

Subsequently, on the basis of their in vivo activity against adult schistosomes, nerolidol was tested in mice harbouring juvenile *S. mansoni* (pre-patent infection). In this case, a group of 10 mice was ere treated with a single 400 mg/kg oral dose of nerolidol at 21 days p.i. (pre-patent infection). Ten untreated mice served as controls.

2.4. In vivo drug assessment

At 2 weeks post-treatment, mice were euthanised and surviving schistosomes residing in the mesenteric veins and liver were counted and sexed as previously described [15]. Assessment of therapeutic efficacy was also based on the technique of quantitative and qualitative oograms using a fragment of the ascending colon (10 mm) [16] as well as the Kato–Katz method for quantitative faeces examination [17]. In the oogram pattern, eggs were scored as immature, mature or dead [16].

2.5. Scanning electron microscopy (SEM) studies

To determine whether nerolidol can cause morphological alterations in adult schistosomes recovered from mice, two additional mice were orally treated with 400 mg/kg nerolidol and were dissected at 24 h and 48 h post-treatment [15]. Worms were extracted from the mesenteric veins and liver as described above, were rinsed twice in phosphate-buffered saline and were fixed in 1 mL of 2.5% glutaraldehyde (Merck-Millipore, Cotia, SP, Brazil) for 3–24 h at room temperature. Samples were prepared as previously described [18]. Briefly, specimens were air-dried, were mounted on stubs and were metalised with gold using a Desk II sputter coater (Denton Vacuum LLC, Moorestown, NJ). Samples were then visualised using a JEOL JSM-6460LV high-resolution scanning electron microscopy (JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 20 kV.

2.6. In vitro drug assessment

In vitro drug assessment was performed as previously reported [19–21]. Briefly, juvenile (21-day-old) or adult (49-day-old) schistosomes, freshly recovered from a rodent host, were washed and were placed in a 24-well culture plate (TPP, St Louis, MO) containing RPMI 1640 medium (Vitrocell, Campinas, SP, Brazil) supplemented with 10% fetal bovine serum (FBS), and 200 μg/mL streptomycin and 200 IU/mL penicillin (Vitrocell) at 37 °C in a 5% CO2 atmosphere. Newly transformed schistosomula were obtained by mechanical transformation using a vortex mixer. Parasites were cultivated for 3 h prior to the bioassay in 169 medium (Vitrocell) containing antibiotics and supplemented with 10% FBS at 37 °C in a 5% CO2 atmosphere. For in vitro bioassays, schistosomula were transferred to 24-well culture microplates (TPP) containing ca. 50 parasites/well and were cultured in medium 169. All stages of *S. mansoni* were maintained continuously in medium (with or without drugs) for 72 h at 37 °C in a 5% CO_2 atmosphere. The following drug concentrations were evaluated: 6.25, 12.5, 25, 50, 100, 200 and 400 μM. All worms were monitored using an inverted microscope.

2.7. Statistical analysis

Statistical analyses were performed using GraphPad Prism v.6.0 software (GraphPad Software Inc., La Jolla, CA). Dunnett's test was used to analyse the statistical significance of differences between mean experimental and control values [15]. A P-value of <0.05 was considered significant. The 50% lethal concentration (LC_{50}) was also calculated using sigmoid dose–response curves, along with the 95% confidence interval (CI) [5].

3. Results

First, the dose–response relationship of nerolidol in adult *S. mansoni* infections was evaluated. Groups of 10 mice were treated orally with single doses of nerolidol (100, 200 or 400 mg/kg) at 49 days p.i. Then, on the basis of their in vivo activity against adult schistosomes (patent infection), nerolidol was tested in mice harbouring juvenile *S. mansoni* (pre-patent infection). Furthermore, schistosomula, juveniles and adult *S. mansoni* were each incubated in vitro with nerolidol over a wide concentration range (6.25–400 μM).