In vitro and in vivo antileishmanial efficacy of a new nitrilquinoline against Leishmania donovani

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Abstract

The in vitro activity of a new analogue of 2-alkenylquinoline (2-nitrilquinoline or NQ) against Leishmania donovani was compared to oral reference drug miltefosine (HePC). IC50 of NQ was found at 38.6 μM against promastigotes and 2.4 μM against intramacrophage amastigotes. In vivo evaluation in the L. donovani Balb/c mice model indicated that oral treatments at 12.5 and 25 mg/kg for 10 consecutive days significantly reduced the parasite burden in the liver by 68.9 and 68.5%, respectively. This activity was similar to those of HePC at 7.5 mg/kg for 10 days which reduced the parasite burden in liver by 72.5%. The present study shows the positive contribution of a nitril substitute being added into the alkenyl chain branched at the 2-position of the quinoline ring to the antileishmanial activity. In addition, any apparent toxicological disorder was observed during the experiments.

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1. Introduction

Parasitic diseases such as leishmaniases have significant impacts in developing countries, with infections spread over several hundred millions of people. Conventional chemotherapies are often inadequate, toxic or are becoming less effective due to emergence of resistances as it is observed for antimicrobials [1,2].

2-alkenylquinolines and 2-arylquinolines prepared by total synthesis [2], have been investigated by us as new drug candidates. These low-molecular weight compounds exhibit pharmacological properties such as antiprotozoal activity (e.g. against Leishmania sp., Trypanosoma sp.), and were found to inhibit the human immunodeficiency virus of type-1 (HIV-1) as well as the proliferation of HTLV-1 transformed cell lines (HUT-102) [3,4].

In a recent study, we reported that various 2-substituted quinolines have shown interesting activity in Leishmania donovani infected-BALB/c mice when administered orally [5]. From structure-activity relationship especially based on the nature of the alkyl chain branched at the 2-position of quinoline, we have prepared by a simple low-cost method a new 2-substituted quinoline with a nitril function on the C3-alkenyl chain, (E)-3-Quinolin-2-yl-acrylonitrile (2-nitrilquinoline, NQ). The objective of the present study was to evaluate the activity of this compound on L. donovani promastigotes and amastigotes in vitro compared with oral reference drug miltefosine (HePC), and experimentally in L. donovani infected mice treated orally NQ compound and compared with oral HePC treatment.
2. Materials and methods

2.1. Chemicals

Miltefosine (HePC, 1-O-hexadecylphosphocholine, n° 000761) was provided by Zentaris (Frankfurt, Germany).

The 2-nitrilquinoline (NQ) was synthesized in the Laboratory of Pharmacognosy, Faculty of Pharmacy, Châtenay-Malabry, France by procedures described elsewhere [6]. Physical and spectral data (proton and 13C nuclear magnetic resonance and mass spectrometry) were used to check the purity of 2-substituted quinoline. It was shown to have in vitro antileishmanial activity against amastigote forms of L. amazonensis and L. donovani.

2.2. In vitro evaluation

2.2.1. Against L. donovani promastigotes

Leishmania donovani (MHOM/ET/L82/LV9) promastigotes were kindly provided by Pr. S.L. Croft, from the WHO collection at the London School of Hygiene and Tropical Medicine. The test was performed as previously described [7]. Briefly, promastigotes were grown at 27 °C and cultivated in HEPES (25 mM)-buffered M199 medium containing 10% fetal calf serum (FCS) and 50 µg/mL gentamycin. The test was performed in 96-well microtitre plates maintained at 27 °C under a 5% CO2 atmosphere. Two hundred µL of culture medium was placed in the well containing the maximum concentration of compound (C1), and 100 µL in the followings (C2 to C7 and controls); 2 µL of compound solution of 20 mg/mL in DMSO was added in C1 and a serial dilution in the wells were performed. After 1 h at 27 °C under a 5% CO2 atmosphere, we added 100 µL of culture medium complemented with 1.75x10^6 Leishmania/mL from a logarithmic phase culture. Biological tests were performed three times, and each tested concentration in duplicate. The viability of parasites was evaluated by the tetrazolium-dye (MTT) colorimetric method. The results are expressed as the concentration inhibiting parasite growth by 50% (IC50 ± S.D.) after a 72 h incubation period. The initial concentration for screening was 100 µg/mL. HePC was the reference drug.

2.2.2. Against intramacrophage amastigotes

Concerning the amastigote in vitro model, murine peritoneal macrophages withdrawn from CD1 mice were infected after a 24 h incubation period with amastigotes form of L. donovani (MHOM/ET/67/HU3) wild type (WT) purified from the spleen of an infected golden hamster at a ratio of five parasites per macrophage, to obtain 87% of infected macrophages and 10 ± 3 amastigotes per macrophage. At 18 h after the amastigotes had entered macrophages, free amastigotes were eliminated and intramacrophage amastigotes were treated at various concentrations of the compounds. HePC was used as reference compound. Each experiment was performed in triplicate. The experiment was stopped after an incubation time of 48 h, and the percentage of infected macrophages was evaluated microscopically after Giemsa staining. The IC50 were determined by linear regression analysis, and expressed in µM ± S.D.

2.2.3. In vivo evaluation against L. donovani

Six- to height week-old BALB/c mice (Elevages Janvier, France) were infected intravenously with 10^7 L. donovani amastigotes derived from spleen hamsters and randomly sorted into groups of seven. The treatment was started one week after infection and was continued for 10 days. In these experiments, the drugs given orally by gavages were formulated in 50 µl PBS and 5 µl of miglyol (Miglyol 812, Dymanol Nobel). One group received the oral reference drug, HePC, at 7.5 µg/kg of body weight; NQ was orally administered at two different doses, 25 or 12.5 µg/kg for 10 days. All doses were administered consecutively on days 7 to 17 after infection. At day 24, all groups were sacrificed and livers were weighted. Parasite numbers were determined by counting the number of amastigotes/500 liver cells in Giemsa-stained impression smears, prepared from the liver and multiplying that value by the weight of the liver (in milligrams).

2.3. Statistical analysis

The mean of parasites per gram of liver of treatment groups and controls were compared using Student's t-test or the Kruskal–Wallis non-parametric analysis of variance test for comparing two groups. Significance was established for a P value <0.05 [8].

2.4. Toxicological data

The behaviour of mice was monitored daily throughout the experiment and mice were weighed at the end of experiment in order to compare the weight of treated mice with that of untreated ones.

3. Results

The in vitro antileishmanial effects of NQ and reference drug HePC on the growth of L. donovani promastigotes are presented in Table 1. These results indicate that NQ was 16 times more active on L. donovani amastigote than on promastigote forms with IC50 of 2.4 and 38.6 µM, respectively.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µM)</th>
<th>L. donovani promastigotes</th>
<th>IC50 (µM)</th>
<th>L. donovani amastigotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-3-Quinolin-2-yl-acrylonitrile (NQ)</td>
<td>38.6 ± 2.8</td>
<td>2.4 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HePC</td>
<td>5.30 ± 0.56</td>
<td>7.5 ± 0.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean of IC50 (µM) ± S.D. for four determinations.
whereas HePC exhibited similar activity on both parasite stages (IC_{50} in a range from 5.3 to 7.5 μM). This result shows that NQ was able to enter the macrophage and cross over the phagolysosomal membrane to reach the parasite. Perhaps, NQ was biotransformed within the macrophage to give a more active compound. The absence of toxicity on mouse intraperitoneal macrophages at 25 μM indicates a therapeutic index superior to 10 justifying in vivo experiment on the L. donovani Balb/c mice model.

Oral treatment with NQ at 12.5 mg/kg for 10 days produced a significant reduction of parasite burden in liver in L. donovani infected mice (68.9%; P < 0.002). This efficacy was similar to the oral treatment with the reference drug, HePC at 7.5 mg/kg for 10 days (72.5%; P < 0.001). With NQ drug dose of 25 mg/kg, we observed a similar reduction (68.5%; P < 0.002) (Fig. 1). This non-dose-effect was previously observed with other 2-substituted quinolines [5].

4. Discussion

Inconvenience, toxicity and cases of resistance are main problems associated with the parenteral drug therapy for the leishmaniases [1]. In this study, we evaluated a new, orally administered 2-substituted quinoline antileishmanial drug, NQ, against L. donovani. During the experiments we have not observed any apparent toxicological disorder. In the present study, we observed, the oral efficacy of NQ against L. donovani infected mice. Its chemical synthesis is easily transposable in endemic emerging countries with a pharmaceutical company which could prepare the batches on a large scale. Studies on the mechanism of action, pharmacokinetics and toxicological studies are now in process in our laboratory. In previous studies, the potential importance of metabolites in this chemical series has been mentioned, for example the metabolites of 2-propylquinoline have been identified and exhibited strong in vitro antileishmanial activity whereas in vivo activity was weak [9].

Likewise, the present study shows, the positive advantage of a nitril substitute being added into the alkyl chain branched at the 2-position of quinoline. Hence, newer 2-alkenyl derivatives of the quinolines have to be designed in order to resolve the structure-activity relationship in this series leading to the development of more effective antileishmanial drugs.

References


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