Anti-leishmanial and anti-trypanosomal activity of the 8-aminoquinoline tafenoquine

Vanessa Yardley, Francisco Gamarro, Simon L. Croft

Abstract

The 8-aminoquinoline, tafenoquine, showed significant \textit{in vitro} activity against \textit{Leishmania} species, including \textit{L. donovani} amastigotes in macrophages with IC$_{50}$ values between 0.1 – 4.0 µM for both Sb$^V$-sensitive and Sb$^V$-resistant strains, and by oral administration in BALB/c mice with ED$_{50}$ values of 1.2 – 3.5 mg/kg x 5. Tafenoquine was less active against intracellular \textit{Trypanosoma cruzi} amastigotes with an IC$_{50}$ value of 21.9 µM.

The neglected tropical diseases, leishmaniasis, Chagas disease and human African trypanosomiasis (HAT), caused by trypanosomatid parasites, have a limited number of drugs for treatment and control, all with limitations of toxicity, variable efficacy, long dosing regimens, and/or parenteral administration. Recent reviews have outlined the advances made in the chemotherapy of these diseases in the past decade, for visceral leishmaniasis [VL] (1), for cutaneous leishmaniasis [CL] (18), Chagas disease (22) and human African trypanosomiasis [HAT] (2).

The search for new treatments for these diseases has adopted various strategies, including rational design of drugs (7, 15), screening libraries of synthetic and natural products (11) and therapeutic switching. The more rapid development of a new treatment by the latter approach has been recently demonstrated for Chagas disease with ergosterol biosynthesis inhibitors (22) and for leishmaniasis with miltefosine and paromomycin (8, 20). The 8-aminoquinolines (Figure 1) have a long history as anti-protozoal drugs, in particular as anti-malarials. Since the 1950’s, several have also been reported as being active against \textit{Leishmania} and \textit{Trypanosoma} parasites (13, 21). Interest in the activity of this class of compounds for these diseases has been kept in focus by the clinical trials of sitamaquine (WR6026) for VL (12, 23). Sitamaquine also has anti-	extit{T.cruzi} activity (6). Research on another 8-aminoquinoline, NPC1161, has identified an enantiomer with significant anti-leishmanial activity and a lower toxicity profile (17). Tafenoquine (TFQ) (WR238605) developed, like many of this class by the Walter Reed Army Institute of Research (WRAIR), is now in clinical trials for the radical...
cure of *P. vivax*, with GSK and the Medicines for Malaria Venture (MMV) (16). We present here the results of the *in vitro* and *in vivo* activity of TFQ against *Leishmania donovani* and *Trypanosoma cruzi*. Studies on the mechanism of action of TFQ against *Leishmania* and activity against *Trypanosoma brucei* spp will be reported elsewhere.

Early tests of TFQ against the promastigotes of different *Leishmania* species demonstrated IC$_{50}$ values below 3µM (data not shown). Of more clinical relevance, TFQ (GSK, UK) activity was evaluated, *in vitro*, against intracellular amastigotes of *L. donovani* MHOM/ET/67/HU3 (from East Africa), *L. donovani* MHOM/IN/82/DD8 (from India), and *L. donovani* BHU1 and BHU3 (antimony resistant strains from India generously donated by Prof. Shyam Sundar). Infected murine peritoneal macrophages were exposed to the drug as previously described (24). The % infection was calculated and IC$_{50}$ values derived (Prism™). Subsequently, TFQ was further evaluated in the BALB/c mouse-*L. donovani* model of infection (9). 8-week old, female mice (Charles River, UK) were infected with amastigotes harvested from a donor animal. After 7 days, the mice were treated with TFQ formulated in 10% Tween-80/EtOH 70:30/ddH$_2$O, at 5mg/kg, by the oral route, for 5 consecutive days. At day 14 the mice were euthanised and liver impression smears made at necropsy. The amastigote burden was calculated (LDU’s) (4) and the % inhibition derived and ED$_{50}$ values were calculated. TFQ hydrochloride (racemate batch # R146390, +ve enantiomer batch # R206420, -ve enantiomer batch # R206422) and Sitamaquine tosylate (batch # SLV3L004) used were donated by GSK. Miltefosine was donated by Astra Zeneca, UK and Fungizone (amphotericin B deoxycholate) was purchased from a commercial supplier. All *in vivo* experiments were carried out under license at LSHTM according to UK Home Office regulations.

The efficacy of TFQ against *T. cruzi* (Tulahuen-LacZ strain) (5) strain was tested against amastigotes *in vitro*. Peritoneal macrophages were infected with *T. cruzi* harvested from feeder cell layers, and exposed to TFQ. β-galactosidase activity was measured by the addition of Nonidet P40 (detergent) and CPRG (developer). 96-well assay plates were read at 570k. and IC$_{50}$ values calculated. Benznidazole (Roche, Switzerland) was used as a positive control.

Both the racemate and +ve and –ve enantiomers of TFQ were active against intracellular amastigotes of all the *L. donovani* strains tested – see Table 1 for IC$_{50}$ values – and compared favourably with the standard drugs tested alongside. In the BALB/c mouse model, TFQ was...
equally active against both antimony sensitive and antimony resistant strains (BHU1 and BHU3), with no difference seen between the racemate and enantiomers. At 5mg/kg TFQ achieved 99% inhibition against all *L. donovani* species, with the enantiomers performing similarly. In a subsequent dose-response experiment the ED$_{50}$ values ranged from 1.01 to 3.5 mg/kg (Table 2).

We have shown that TFQ, an 8-aminoquinoline in development for the treatment of malaria (21) has, like other drugs of the same class, potential as an oral anti-leishmanial agent. In both *in vitro* and rodent models of *Leishmania* infection TFQ had similar potency to sitamaquine, the drug currently in clinical development for VL, and NPC111B which is in pre-clinical development (21). The limitation of this class has been toxicity, especially concerns for G6PD deficient patients. The extensive anti-malarial safety data for TFQ, along with clinical data on sitamaquine for VL, could support the design of appropriate treatment regimes for VL with TFQ. TFQ might also be an oral partner of interest in the combination therapies for the treatment of VL (10, 19).

The activities of several series of 8-aminoquinolines against the causative pathogen of Chagas disease have been published (13). Some have undergone preclinical development (23), for example moxipraquine for Chagas disease (3). Sitamaquine showed potential for prevention of *T. cruzi* transmission through blood transfusion with activity against trypomastigotes at 4°C (6). We did not find TFQ to be as active *in vitro* against *T. cruzi* as other 8-aminoquinolines as others have previously reported (3, 13-14).

Acknowledgements

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References


Figure 1: Structures of tafenoquine, sitamaquine and primaquine.

![Structures of tafenoquine, sitamaquine and primaquine.](image)
Table 1: Activity of TFQ enantiomers against *L. donovani* SbV-sensitive strains: amastigote-PEM model

<table>
<thead>
<tr>
<th></th>
<th>IC₅₀µM (± 95% confidence interval)</th>
<th>Cytotoxicity µM (KB cells)</th>
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<tbody>
<tr>
<td></td>
<td>HU3*</td>
<td>DDB**</td>
</tr>
<tr>
<td>TFQ racemate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFQ +ve</td>
<td>1.75 (1.3)</td>
<td>1.52 (1.02)</td>
</tr>
<tr>
<td>TFQ -ve</td>
<td>2.26 (1.8)</td>
<td>3.28 (0.45)</td>
</tr>
<tr>
<td>Sitamaquine</td>
<td>1.03 (1.02)</td>
<td>1.86 (0.2)</td>
</tr>
<tr>
<td>ShV µg/ml</td>
<td>4.49 (1.83)</td>
<td>13.79 (0.45)</td>
</tr>
<tr>
<td>Miltefosine</td>
<td>2.01 (1.84)</td>
<td>4.85 (0.39)</td>
</tr>
<tr>
<td>Fungizone</td>
<td>0.03 (0.01)</td>
<td>0.04 (0.05)</td>
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*TFQ positive enantiomer; †TFQ negative enantiomer
Confidence Interval *Average of 4 tests; ** average of 2 tests; ***not done

Average of 4 tests; ** average of 2 tests; ***not done
Table 2: *in vivo* activity (% inhibition ± 95% confidence interval) of TFQ p.o. x 5 days, in *L. donovani*-BALB/c mouse models

<table>
<thead>
<tr>
<th><em>L. donovani</em> strain</th>
<th>TFQ R* 5mg/kg</th>
<th>TFQ +ve 5mg/kg</th>
<th>TFQ -ve 5mg/kg</th>
<th>Sitamaquine 5 mg/kg</th>
<th>Sb V 15mg/kg</th>
</tr>
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<tbody>
<tr>
<td>HU3</td>
<td>99.32 ± 0.31</td>
<td>99.12 ± 0.45</td>
<td>99.03 ± 0.52</td>
<td>94.48 ± 0.29</td>
<td>70.93 ± 10.7</td>
</tr>
<tr>
<td>BHU1</td>
<td>99.49 ± 0.66</td>
<td>-*</td>
<td>-*</td>
<td>-*</td>
<td>Inactive at 100mg/kg</td>
</tr>
<tr>
<td>BHU3</td>
<td>100</td>
<td>98.7 ± 0.6</td>
<td></td>
<td></td>
<td>Inactive at 100mg/kg</td>
</tr>
</tbody>
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<tr>
<th>ED50 mg/kg (± 95% CI)</th>
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<tbody>
<tr>
<td>DD8</td>
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<td></td>
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*Not done

*TFQ racemate; +TFQ positive enantiomer; -TFQ negative enantiomer

AmBisome @1mg/kg i.v. x 3 = 93% inhibition

**60% inhibition**
Table 3: *in vitro* activity of TFQ vs *T. cruzi*

<table>
<thead>
<tr>
<th></th>
<th>IC₅₀ (µM)</th>
<th>95% confidence interval</th>
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<tbody>
<tr>
<td></td>
<td>TFQ R⁺</td>
<td>TFQ +veᵇ</td>
</tr>
<tr>
<td><em>T. cruzi</em> Tulahuen Lac-Z</td>
<td>21.9 (3.6-40.3)</td>
<td>17.5 (5.5-29.5)</td>
</tr>
</tbody>
</table>

ᵃ TFQ racemate;ᵇ TFQ positive enantiomer;ᶜ TFQ negative enantiomer
ERRATUM

Antileishmanial and Antitrypanosomal Activities of the 8-Aminoquinoline Tafenoquine

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