The Clinically Tested Gardos Channel Inhibitor Senicapoc Exhibits Antimalarial Activity

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Senicapoc, a Gardos channel inhibitor, prevented erythrocyte dehydration in clinical trials of patients with sickle cell disease. We tested the hypothesis that senicapoc-induced blockade of the Gardos channel inhibits Plasmodium growth. Senicapoc inhibited \textit{in vitro} growth of human and primate plasmodia during the clinical blood stage. Senicapoc treatment suppressed \textit{P. yoelii} parasitemia \textit{in vivo} in C57BL/6 mice. The reassuring safety and biochemical profile of senicapoc encourage its use in antimalarial development.

Intracellular ion homeostasis of erythrocytes is important in the pathogenesis of sickle cell disease (SCD) and malaria. Stemming potassium loss and cellular dehydration have been explored as therapeutic strategies to prevent sickling in SCD (1). During intraerythrocyte maturation of \textit{Plasmodium}, parasite swelling is coordinated with erythrocyte sodium gain and potassium loss, ultimately leading to erythrocyte rupture (2). Inhibitors of erythrocyte volume regulation have been hypothesized to function as antimalarial agents (3, 4).

The Gardos channel (KCNN4/IK-1) is a calcium-activated potassium channel abnormally active in sickle erythrocytes (5). In a phase 3 trial in patients with SCD, therapy with the Gardos channel inhibitor senicapoc improved physiological disease markers but failed to modify the frequency of vasoocclusive pain episodes (6). Senicapoc was well tolerated in human and animal studies for SCD (7, 8). Here, we report the effect of senicapoc on the growth of \textit{Plasmodium}. Repurposing this well-studied safe drug may be an expeditious path to a new antimalarial agent.

The \textit{in vitro} 50% inhibitory concentration (IC$_{50}$) values of senicapoc and other antimalarials for inhibition of multiple strains of \textit{Plasmodium falciparum} and \textit{Plasmodium knowlesi} are shown in Table 1. Senicapoc demonstrated antimalarial activity against blood-stage \textit{P. falciparum} 3D7 with an IC$_{50}$ of 6.7 \textmu M (Fig. 1A). Comparable micromolar antimalarial activity was demonstrated against \textit{P. falciparum} strains with varied antimalarial sensitivities. In all human \textit{Plasmodium} strains tested, the IC$_{50}$ was <2-fold higher than the IC$_{50}$ for reference antimalarials determined in parallel were in the nanomolar range (Table 1).

Senicapoc demonstrated a low micromolar IC$_{50}$ against the primate parasite \textit{P. knowlesi}. To identify a relationship between senicapoc activity and human erythrocytes, \textit{P. knowlesi} H1 cultured in rhesus erythrocytes and \textit{P. knowlesi} YH-1 adapted for culture in human erythrocytes were each treated with senicapoc. There was no difference between the senicapoc IC$_{50}$s for H1 and YH-1 ($P = 0.14$) (Table 1). Senicapoc was similarly effective against parasitized human and rhesus erythrocytes.

To determine senicapoc’s activity through the 48-hour asexual blood stage of the parasite, growth inhibition of \textit{P. falciparum} 3D7 was characterized throughout the cycle by microscopy and flow cytometry. Parasitemia measured by flow cytometry was reduced by 48 h postinvasion (hpi) in all treated cultures compared to that in the control ($P < 0.008$) (Fig. 1B). Parasites within treated cultures appeared smaller and failed to form a digestive vacuole by 36 hpi, followed by rapid parasite death (see Fig. S1 in the supplemental material). Nucleic acid content measured by mean fluorescence intensity (MFI) was weaker in treated cultures at 36 h ($P < 0.008$), and the expected rise in MFI through schizontogenesis was absent (Fig. 1C). Schizonts were not seen by microscopy (see Fig. S1 in the supplemental material). These data suggest that senicapoc was effective during late, metabolically active stages of parasite development.

We examined several senicapoc congeners and identified no relationship between the potency of Gardos inhibitory activity and antimalarial activity (Fig. 1D). Thus, senicapoc may not specifically target the Gardos channel for its antimalarial activity. Interestingly, halogenation of the triaryl methyl group was related to inhibition of both parasite growth and the Gardos channel. \textit{Para}-position halogens were associated with more potent inhibition of parasite growth and of the channel (Fig. 1E). Unhalogenated compounds demonstrated the weakest antipa-
### Table 1: Antimalarial activity of senicapoc against *Plasmodium knowlesi* and *a*

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Res</th>
<th>SEN (μM)</th>
<th>CQ (μM)</th>
<th>MQ (μM)</th>
<th>DHA (μM)</th>
<th>CLT (μM)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em></td>
<td>3D7</td>
<td>6.74</td>
<td>0.25</td>
<td>70.8</td>
<td>0.12</td>
<td>2.73</td>
<td>5.67</td>
<td>[2.07, 2.46]</td>
</tr>
<tr>
<td></td>
<td>7G8</td>
<td>10.88</td>
<td>2.30</td>
<td>31.36</td>
<td>3.94</td>
<td>2.86</td>
<td>9.52</td>
<td>[8.92, 10.11]</td>
</tr>
<tr>
<td></td>
<td>W2mef</td>
<td>9.24</td>
<td>3.06</td>
<td>11.79</td>
<td>1.82</td>
<td>4.86</td>
<td>0.02</td>
<td>[1.24, 15.55]</td>
</tr>
<tr>
<td></td>
<td>H1</td>
<td>17.54</td>
<td>6.74</td>
<td>31.36</td>
<td>3.94</td>
<td>8.92</td>
<td>0.02</td>
<td>[12.30, 155.33]</td>
</tr>
<tr>
<td></td>
<td>YH-1</td>
<td>10.89</td>
<td>1.19</td>
<td>31.36</td>
<td>3.94</td>
<td>8.92</td>
<td>0.02</td>
<td>[12.30, 155.33]</td>
</tr>
</tbody>
</table>

- Mean IC₅₀ of senicapoc (SEN) against a panel of *P. falciparum* with varied resistance (Res) patterns (3D7, 7G8, W2mef) and *P. knowlesi* (H1, YH-1) are shown. 95% confidence intervals determined from at least three independent experiments performed in triplicate. CQ, chloroquine; CLT, clotrimazole; MQ, mefloquine; DHA, dihydroartemisinin.

To establish in *vivo* efficacy against blood-stage parasites, C57BL/6 mice infected with *P. yoelii* 17X-NL (nonlethal strain) were treated with vehicle or senicapoc. (All experiments were performed under protocols approved by the institutional animal care and use committees of the Harvard School of Public Health and of Beth Israel Deaconess Medical Center.) The expected immune-mediated clearance of 17X-NL was observed in vehicle-treated mice beyond postinoculation day 15. C57BL/6 mice exhibited partial suppression of parasite growth during treatment. When senicapoc was given as prophylaxis on postinoculation days 2 to 10, treated mice demonstrated significantly lower parasitemia by day 10 than vehicle-treated mice (4.3% versus 12%; *P* = 0.04). Parasitemia increased after the drug was stopped (Fig. 1F). When senicapoc was given as treatment on postinoculation days 8 to 15, treated mice demonstrated lower parasitemia beyond day 13, but the difference was not statistically significant (*P* = 0.058) (Fig. 1G).

Considering that the shorter half-life of senicapoc in mice compared to humans may impact its effect in the murine model (8), these data demonstrate antiparasite activity of senicapoc in *vivo* consistent with our *in vitro* results.

To test whether senicapoc targets the Gardos channel in malaria-infected erythrocytes, we assessed the ability of *P. yoelii* to grow in the presence and absence of the channel in *vivo* using a Gardos knockout (IK-1⁻/⁻) mouse model (10, 11). Unexpectedly, accelerated parasite growth was evident in IK-1⁻/⁻ mice by postinoculation day 7 (3.6% versus 2.2% in IK-1⁺/⁺ mice; *P* = 0.03) (Fig. 1H). The discrepant kinetics of parasite growth in IK-1⁻/⁻ and IK-1⁺/⁺ mice is not understood but may reflect impaired immunity in addition to absence of the ion channel from erythrocytes. For example, absent Gardos channels in some T-cell subsets might contribute to delayed parasite clearance, counteracting the effect of Gardos channel inhibition on parasite growth in *vivo* (12). We then assessed the effectiveness of senicapoc in the presence and absence of the channel in *vivo*. *P. yoelii*-infected IK-1⁻/⁻ and IK-1⁺/⁺ mice were treated with senicapoc 400 mg/kg twice daily. Senicapoc was active against *P. yoelii* in IK-1⁻/⁻ mice, suggesting activity independent of the channel (Fig. 1I).

Although senicapoc’s promising 12-day half-life in humans, activity across the parasite life cycle, and rapid cytocidal activity support its further exploration as an antimalarial, we identified potentially important limitations. The whole-blood concentration required for *in vitro* inhibition of parasite growth against *P. falciparum* was an order of magnitude higher than the peak concentrations achieved in human trials for SCD to date (337 nM [13]). However, in unpublished industry safety data in primates, daily doses of 1,000 mg/kg yielding a peak serum concentration of 5.3 μM were well tolerated for 9 months. Canines developed cardiotoxicity when serum concentrations exceeded 18 μM (Douglas Krafte, Pfizer, Inc., unpublished data). Thus, despite an effective antiparasite serum concentration exceeding that achieved to date in human trials of senicapoc, animal studies suggest that senicapoc is safe at
Identification of a congener with an IC<sub>50</sub> in the high nanomolar range may yield an effective antimalarial with a promising safety profile.

Our in vitro data support the concept of antimalarial activity of senicapoc through a mechanism independent of the Gardos channel. As senicapoc appears to inhibit intracellular growth of the parasite, other ion channels may be involved in the mechanism of the drug and should be investigated. Building on the safety profile and antimalarial activity of senicapoc for the identification of more potent senicapoc congeners may facilitate repurposing these compounds as antimalarial agents.

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We declare that we have no conflicts of interest.

REFERENCES


