Malaria kills between 1 and 2 million people worldwide every year. The majority of malarial morbidity and mortality is caused by *Plasmodium falciparum*, with an estimated annual productivity loss of US$12 billion in Africa alone (1). Ineffective drugs continue to be used in many of the poorest regions despite very high levels of resistance and treatment failure.

The development of new, efficacious, cost-effective drugs remains crucial. Extensive searches for novel compounds have met with only limited success. Work by the Walter Reed Army Institute of Research showed that only 10 out of 350,000 compounds screened had anti-malarial potential (2).

In other experimental studies, methotrexate has been used to induce in vitro resistance in strains of *P. falciparum* but has not been considered anti-malarial itself. Fidock and Wellems (6) thus showed 100% inhibition of parasite growth with methotrexate concentrations of 100 nM, and a 50-83% inhibition at methotrexate concentrations of 50 nM in 5 falciparum strains tested (3D7, HB3, FCB, Dd2, and V1/S). These results are similar to values presented in the present study for the MDR K1 strain. Our findings are also consistent with an earlier report examining the clinical efficacy of methotrexate against *P. falciparum* infection, which showed that a 2.5-mg oral dose of methotrexate given daily for 3 days completely cleared parasitemia within 48 h, without causing any side effects (7). As is expected with *P. vivax* infections, a recrudescence (3 weeks after termination of methotrexate therapy) occurred unless primaquine was also administered for 14 days. This, together with our results, suggests that methotrexate may also be extremely effective in vivo against *P. falciparum*.

### Table 1. IC50 of methotrexate against T9-96 and K1 *P. falciparum* strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Methotrexate (nM) ± SD</th>
<th>Chloroquine (nM) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>T9-96 (CQS)</td>
<td>0.32 ± 0.05</td>
<td>8.89 ± 1.00</td>
</tr>
<tr>
<td>K1 (MDR)</td>
<td>48.02 ± 4.40</td>
<td>240.70 ± 11.50</td>
</tr>
</tbody>
</table>

CQS, chloroquine sensitive; MDR, multidrug-resistant.
From a broader perspective, methotrexate has many advantages. Its half-life (3-10 h) is shorter than those of currently used antifolates such as sulfadoxine-pyrimethamine (100-200 h) and chlorproguanil-dapsone (12-20 h). Thus, resistance to methotrexate may be slower and less likely to develop. Methotrexate is available in oral, intramuscular, and intravenous preparations, an advantage over many antimalarial drugs. As methotrexate is an off-patent drug, low-cost generic formulations are already available and widely used in malaria-endemic countries. Methotrexate’s advantage over ‘novel compounds’ is its well-documented history of clinical use, reducing the expensive and time-consuming process of drug approval.

Miltefosine, an anti-proliferative drug first developed for treating metastatic carcinomas, has been successfully trialled and adapted for the treatment of human leishmaniasis (8,9). Our results demonstrate that methotrexate has a more potent parasiticidal effect on both susceptible and resistant *P. falciparum* strains in vitro than miltefosine does on *Leishmania donovani* promastigotes (IC$_{50}$ 25 μM) (10).

The main concern in the use of methotrexate as an antimalarial would be its side-effects profile. It is promising that other potentially toxic anti-cancer agents, such as Miltefosine, are being successfully used as parasiticidal drugs. Side effects from long-term methotrexate use are numerous and well described (5). However, in a short-term regimen, even at the relatively high single dose of 50 mg/m2, as when methotrexate is used as an abortifacient, side effects are rare and mild. In a study on 178 women seeking first-trimester pregnancy termination, the only reported side effects attributable to methotrexate were two cases of self-limiting (within 48 h), mild stomatitis (3). Concurrent administration of folic acid or leucovorin may play a role in reducing side effects in higher risk patients. Our results, along with those of Sheehy and Dempsey (7), suggest that only the lowest currently used clinical doses of methotrexate would be required for a period of 24-72 h to eliminate parasitemia. Therefore, the risk of serious side effects is minimal.

Possible indications for methotrexate include treatment of both uncomplicated and complicated resistant falciparum malaria as well as emergency standby treatment in areas of known resistant falciparum malaria. In addition, travelers to malaria-endemic regions already on low-dose, long-term methotrexate therapy (rheumatoid arthritis or psoriasis patients) may not require supplementary malaria prophylaxis, as their methotrexate intake may suffice as anti-malarial chemoprophylaxis.

Further research to expand the work of this brief report should include testing methotrexate against a wider variety of resistant strains such as the 108N, 51I, 164L, and 59R triple and quadruple DHFR mutants in both in vitro and murine models. We also advocate the evaluation of methotrexate in combination regimens to test for possible synergy with other antimalarials, thus improving efficacy and reducing the risk of resistance developing. Ultimately, it would be of considerable clinical interest to examine the efficacy of methotrexate in human cases of falciparum malaria.

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**REFERENCES**