Antimalarial Activities of Polyhydroxyphenyl and Hydroxamic Acid Derivatives

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Received 5 February 1998/Returned for modification 13 May 1998/Accepted 1 July 1998

Several known mammalian ribonucleotide reductase inhibitors featuring a polyhydroxyphenyl and/or hydroxamate moiety as the active group were screened for potency in inhibiting growth of the malaria parasite Plasmodium falciparum. Compounds containing a 2,3- or 3,4-dihydroxyphenyl group as well as benzohydroxamate appear to be the most effective inhibitors of the malaria parasite.

There is an urgent need to develop antimalarials targeted against new metabolic targets as the drugs available to fight the disease are rapidly losing their efficacy. Ribonucleotide reductase (RNR) catalyzes the reduction of ribonucleotides to deoxyribonucleotides, the first and rate-limiting step for de novo synthesis of 2'-deoxyribonucleoside 5'-triphosphates (16). RNR activity has been shown to be closely correlated to the rate of tumor growth (5, 19); consequently, inhibitors directed against RNR have been used for many years in cancer chemotherapy (7, 11, 12). The central role of the ubiquitous enzyme RNR in DNA metabolism also makes this enzyme an excellent target for chemotherapy of malaria. We have previously reported that an oligodeoxynucleotide phosphorothioate complementary to RNR small subunit sequences showed antimalarial activity (2). We have now initiated a systematic investigation to test known RNR inhibitors for possible antimalarial activity. Several antitumor and antiviral compounds exist which are specific inhibitors of RNR (15). The hydroxamate groups of RNR inhibitors, such as hydroxyurea are electron reductants and destroy the tyrosyl radical (6). This paper reports evaluation of hydroxamic acid and polyhydroxyphenyl derivatives as potential antimalaria- lars.

We evaluated three known RNR inhibitors, hydroxyurea, acetohydroxamate, and benzohydroxamate, for potential antimalarial activities in synchronous Plasmodium falciparum Dd2-infected erythrocyte culture according to the method of Desjardins et al. (3). Ring-stage cultures were incubated with [3H]hypoxanthine (1 μCi/μl; 1 μCi = 37 kBq) and various concentrations of inhibitors for 24 h at 37°C. The radiolabeled genomic DNA was isolated by sodium dodecyl sulfate-proteinase K treatment (14) and counted with a liquid scintillation spectrometer. The data were analyzed by logistic regression (11) since experimental variance is not constant for all drug concentrations, which makes the standard least squares technique inappropriate. This type of analysis was conducted for each drug evaluated to produce curves from which the drug concentration inhibiting 50% of the parasite growth (IC50) was calculated.

As can be seen in Table 1, both hydroxyurea and hydroxam-
ical quenching potency (4). Hence, the mechanism by which polyhydroxyphenyls inhibit RNR is now believed to be free radical scavenging. Table 2 provides the structure and a summary of test results for each drug tested. VF149 and VF147, the two vicinal dihydroxybenzohydroxamates tested, outperformed the other drugs as inhibitors of *P. falciparum* growth. Trihydroxyphenyl-containing compounds are more effective mammalian RNR inhibitors than are compounds which con-

<table>
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<th>Table 1. Antimalarial activities of hydroxamic acids</th>
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<tr>
<td>Compound and structure</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Aminohydroxamate (hydroxyurea)</td>
</tr>
<tr>
<td>Acetohydroxamate</td>
</tr>
<tr>
<td>Benzohydroxamate</td>
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<td>VF 149</td>
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<td>VF 147</td>
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<td>VF 236</td>
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<td>VF 268</td>
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<td>VF 282</td>
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* RNR inhibitors synthesized in one of our laboratories (H.L.E.) were tested in an in vitro culture system for potential antimalarial activities according to the method of Desjardins et al. (3).
tain one fewer hydroxyl group but are otherwise identical (4, 7, 8, 17). Yet we found the reverse to be true when these drugs were tested as antimalarials. It could be argued that the trihydroxyphenyls were at a disadvantage in our test program since they are not associated with the hydroxamate group. But testing on mammalian systems demonstrated that the hydroxamate functional group is relatively unimportant for antitumor activity and that the polyhydroxyphenyl group is the primary source of activity (6). This appears to be further evidence of a difference between mammalian and malarial forms of RNR, since hydroxamate-containing agents were the best antimalarials. Of the drugs tested, vicinal dihydroxyphenyl-substituted hydroxamic acids are the most effective antimalarials. The inhibitory effect though was reversible at the IC50. At four times the IC50 the effects of these inhibitors were found to be irreversible (data not shown). Didox (VF147, 3,4-dihydroxybenzo-hydroxamate) has been in clinical trials as an anticancer agent since 1988 (18). It exhibits low toxicity to the extent that hydroxamate-containing agents were the best antimalarials. Efforts are under way to characterize the mechanisms of action of these inhibitors in in vitro assays with recombinant malarial RNR.

We thank Michelle Fluegge and Cherie DeVecchio for technical assistance.

This investigation was supported by a grant (AI40692) from the National Institutes of Health.

REFERENCES