Redox systems as conduits for antimalarial compounds

J Antimicrob Chemother 2001; 47: 122–124
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Sir,

At the turn of the nineteenth century, Ehrlich focused on the selective staining of malaria-infected cells over non-infected cells, with methylene blue and acridine orange. On reviewing this information, two simple questions arose: why do these compounds selectively target parasite infected cells and can this information be used against the parasite?

We propose the following: methylene blue is a cationic redox dye, and on examination of the physiological consequences of malarial infection, we can see why this compound has a high affinity for infected blood cells. Infected cells are under a high degree of endogenous oxidant stress stemming from the parasitic presence, and various physiological processes come into play to relieve this stress, primarily through provision of substrates for reduction in a process known as the hexose monophosphate shunt (HMS). Therefore, the introduction of an alternative reductive target into the system, here a redox dye, will prompt the uptake of this target by the infected cell. We decided to investigate this hypothesis and chose to utilize these cationic redox dyes to show that they could operate as infection-specific conduits for other antimalarial compounds.

We prepared and assayed two novel adduct compounds as proof of our hypothesis, coupling azure A and proflavine, respectively, to quinine, via a simple azo linkage. Research into the role of methylene blue as an antimalarial agent has demonstrated its high antimalarial potency and of its analogues azures A, B and C (components of the malarial stain Giemsa). We were also interested in acridine orange, another malarial stain, for the same reasons as outlined for methylene blue. We therefore concentrated on the free amine analogues of methylene blue and acridine orange respectively, azure A 1 and proflavine 2.

Although azure A has previously been assayed as an antimalarial, the circumstance for its trial was based on its presence in Giemsa stain, not as the pure compound, and to our knowledge no-one has examined the potential of proflavine as an antimalarial drug in its own right. Azure A 1 and proflavine 2 were therefore assessed as antimalarial candidates, assayed in vitro against the blood stage of a cloned parasite strain of human Plasmodium falciparum D6. The activities are reported as IC50s in the Table.

The theory behind this coupling strategy was that the dyes should enhance the uptake of 7-aminoquinine (compound 3) into infected cells, thus lowering the concentration of 7-aminoquinine required for inhibition. Compound 3 is the presumed product on in vivo hydrolysis of the azo link, a mode of action similar to the antimalarial drug prontosil, which contains an azo linkage, which when cleaved in vivo delivers sulphonilamide. The coupling of the quinine to the dyes should not serve to diminish the inherent activity of neither the dye nor 7-aminoquinine. Compounds 4 and 5 were prepared using simple synthetic transformations and assayed against the D6 cloned malarial strain. A sample of 7-aminoquinine was prepared by Zn/HCl reduction of 4 and assayed against D6. The IC50s obtained for compounds 3–5 are also given in the Table. The D6 inhibition studies were carried out four times for all compounds 1–5. In each case the activity exhibited by the dye–quinine adducts 4 and 5 against D6 is significantly higher than the individual components’ recorded activity, representing increases of 9 and 40%, respectively, over the potency recorded for the sole, more active, member of the pairing.

These initial data demonstrate the potential of dye–antimalarial coupling to produce adduct compounds with enhanced antimalarial activity over the individual adduct component molecules.

Acknowledgements

The authors wish to express thanks to Dr Robert Ridley and his staff in the Tropical Disease Research division of the World Health Organization for the initial in vitro evaluation of some of the compounds described in this work.

References

Correspondence

Table. Biological activities recorded for individual dyes, 7-aminoquinine and the dye-adduct compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>D6 IC_{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.040 ± 0.011</td>
</tr>
<tr>
<td>2</td>
<td>0.829 ± 0.028</td>
</tr>
<tr>
<td>3</td>
<td>0.064 ± 0.013</td>
</tr>
<tr>
<td>4</td>
<td>0.058 ± 0.012</td>
</tr>
<tr>
<td>5</td>
<td>0.024 ± 0.014</td>
</tr>
</tbody>
</table>


