REVERSAL OF HALOFANTRINE RESISTANCE IN
PLASMODIUM FALCIPARUM BY PENFLURIDOL
IN VITRO

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Abstract

Penfluridol, a neuroleptic drug, considerably reversed halofantrine resistance in T950 HF halofantrine resistant strain of Plasmodium falciparum (originally chloroquine sensitive parasites), but not in K, HF halofantrine resistant strain (originally chloroquine resistant parasites).

Introduction

The rapid spread of chloroquine-resistant strains of Plasmodium falciparum in most of the malarious areas has led to a need for new antimalarial drugs. A number of authors have shown that halofantrine (a 9-phenanthrene methanol) is highly active against both chloroquine-sensitive and chloroquine-resistant strains of P. falciparum (3,5,6,13,14), although some recrudescences have been reported by a number of investigators (3,11,13).

The development of resistance to halofantrine in chloroquine-resistant K, and chloroquine-sensitive T950 of P.falciparum in vitro indicates that extension of resistance of P. falciparum to halofantrine is most anticipated (10).

In this study we have demonstrated that penfluridol, a potent non-acting neuroleptic drug (8), was capable of reversing halofantrine resistance in T950 HF halofantrine resistant parasites.
Materials and methods

a) Parasites and cultivation: Three halofantrine-resistant K, K', and T. and two halofantrine-sensitive K, and T. strains of Plasmodium falciparum were used during this study. Cultivation of the parasites was conducted as described by Tonge and Jensen with some modifications (15). Briefly, the parasites were cultured in a 5% haematocrit containing complete culture medium including 10.4 g RPMI-1640 in 1 litre of double distilled water, 0.2% (w/v) sodium bicarbonate, 50 mg hypoxanthine in 1 litre of culture, 25 mM HEPEs buffer, 10 mg/ml gentamicin and 10% human AB type serum and human O+blood. Cultures were maintained in 50 ml flask (Nunc, Denmark). Fresh media were added until the desired haematocrit was achieved. Subsequently, the cultures were flushed with a gas mixture consisting of 3% O2, 4% CO2 and 93% N2 (Boc, special gases). All cultures were regassed daily after changing the media. Parasitaemia was monitored every day using Giemsa-stained thin blood smears. The cultures were usually diluted to a parasitaemia of 1% every 2-3 days, or when the parasitaemia exceeded about 8%.

b) Assessment techniques: The drugs employed in this study (i.e.: halofantrine, mefloquine, quinine, chloroquine, and penfluridol) were prepared in 70% ethanol. The drugs were dissolved in the solvent to a concentration of 10^2 M. The stock solution of the desired drug was diluted in the complete culture medium to give appropriate concentrations. The dilutions of drug were arranged in triplicate wells starting from lowest towards highest. Two-fold triplicate control wells were set up near the centre of the plate. Each test well received 10 μl of a 50% infected erythrocyte suspension producing a 4.5% haematocrit in complete culture medium at 1% parasitaemia (mostly ring stages). After preparation as above, the plates were covered, shaken gently, then placed in a modular incubator chamber (Billups - Rothenberg) and flushed with a gas mixture of 3% O2, 4% CO2 and 93% N2. The gas chamber was then incubated at 37°C for 24 hours. After the first incubation period, 0.5 μCi of [3H]-hypoxanthine (Amersham) was added to each well. The radiolabelled plates were then briefly shaken, regassed and incubated as before. After the second incubation period, the contents of the wells were collected onto a glass fibremat (Skatron - Ltd) using a TriTek cell harvester (Skatron, AG). After drying, each of the individual discs was suspended into 4 ml of optiphase se'f scintillation fluid (K.B) and then prepared for scintillation counting.

Results

1- Comparative dose response of K, K', and T. parasites to penfluridol (PF), halofantrine (HF), mefloquine (MF), quinine (QN) and chloroquine (CQ).

The IC50 values for PF, HF, MF, QN, and CQ against K, K', and T. strains are tabulated in Table 1. The K, K', and T. parasites showed a significant decrease in sensitivity to penfluridol, halofantrine, mefloquine, and quinine. A significant increase in sensitivity to chloroquine exhibited by the K, K', and T. parasite.

2- Combinations of halofantrine, mefloquine, quinine and chloroquine with penfluridol against K, K', and T. parasites.

The results are tabulated in Table 2. A combination of penfluridol with halofantrine or chloroquine exhibited an antagonistic effect against the K, K', and T. strain. The combination of penfluridol with halofantrine was associated with antagonism against K, K' parasites similar to the findings for penfluridol plus quinine. The greatest antagonism occurred with a combination of penfluridol and chloroquine, particularly in the ratios of 70:30, 50:50 and 30:70 (penfluridol:chloroquine; with 12.07, 9.91 and 12.39 percent inhibition, respectively) against K, K' strain. An additive effect was observed for the combination of penfluridol with halofantrine and an antagonism for the combination of penfluridol with mefloquine against T. parasites.
Potentiation was obtained for the combination of penfluridol with halofantrine against T_{99}HF parasites especially at the ratios of 50:50 and 30:70 with 80.64 and 80.69 percent inhibition respectively, as such penfluridol increased the activity of mefloquine against the parasites. In contrast, the interaction between penfluridol and quinine was one of antagonism against T_{96}HF strain particularly at the ratios of 70:30 and 50:50 with 10.35 and 9.79 percent inhibition, respectively.

Discussion

Penfluridol R1634 ; 4-(4-chloro-trifluoro-m-toly)-1-[4,4-bis (P-fluorophenyl) butyl]-4-piperidinol is a potent and long-acting neuroleptic drug. This drug is a white micocrystalline tertiary amine. The pharmacology, pharmacokinetics and toxicity of penfluridol has been extensively studied (8).

It was demonstrated that penfluridol is relatively nontoxic in normal dosages. The occasional side-effects are restricted to common neurological symptoms (8).

The action of penfluridol on parasites is unknown. It was reported that penfluridol like chlorpromazine can inhibit the activation of erythrocyte cAMP transporting ATPase via calmodulin (7). In another study the effect of penfluridol on fast axonal transport in the nerve of the bullfrog was examined in vitro (9), and it was suggested that inhibition of axonal transport might be connected with inhibition of the action of calmodulin by penfluridol(9). However, it is, more or less, clear that penfluridol can antagonise the action of calmodulin, but the actual mechanism of this inhibition remains unsolved. Results obtained for the combination of halofantrine with penfluridol produced a slight antagonism against the K_{1}HF strain, but a considerable potentiation against the T_{99}HF parasites. Although the reason for this discrepancy is not clear, it may be speculated that the T_{99}HF and K_{1}HF strains have inherently different responses to the combination of halofantrine with penfluridol or to either drug alone. In agreement with this in 1990, 178 strains of P.falciparum were isolated with a wide range of sensitivity to halofantrine from thirteen African countries (12). Additionally, the variable response of P.falciparum to similar drug combinations has been reported by a number of investigators. For instance, using combinations of chloroquine with desipramine or cyproheptadine against chloroquine-resistant FCR3 parasites in 1989, and against cloned multidrug-resistant FCM29/cameroon in 1990, produced significantly different results (2, 1). It was suggested that the difference between these results may depend on genetic and biochemical differences, or different sensitivity levels in these strains to the drug combinations (1).

The results indicated that combination of mefloquine with penfluridol produced potentiation against the T_{99}HF parasites, but only an additive effect against T_{99}HF (which has a lower level of resistance to mefloquine than T_{99}HF and unpublished). The difference between T_{99}HF and T_{99}HF parasites in response to mefloquine + penfluridol combinations presumably depends on the level of resistance to mefloquine. In other words, the combination of mefloquine with penfluridol against the T_{99} (mefloquine-sensitive strain), T_{99}HF and T_{99}HF-produced antagonism, an additive effect and potentiation, respectively. Although penfluridol potentiated the activity of mefloquine and halofantrine against the T_{99}HF parasites, in combination with quinine, (another member of mefloquine functional group) it showed a remarkable antagonism. The reason for this discrepancy is not clear.

In conclusion penfluridol appears to potentiate the activity of halofantrine and mefloquine against mefloquine and halofantrine-resistant T_{99}HF parasites.
Table 1: Penfluridol, halofantrine, mefloquine, quinine and chloroquine IC50 value for K1 HF, K1, Tn30/HF and Tn30 parasites of *P. falciparum* in 48-hour [3H] hypoxanthine incorporation assays.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Penfluridol</th>
<th>Halofantrine</th>
<th>Mefloquine</th>
<th>Quinine</th>
<th>Chloroquine</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 HF</td>
<td>1849*</td>
<td>20 + 1.7</td>
<td>75 + 6.2</td>
<td>346.6 + 15.2</td>
<td>80 + 35.1</td>
</tr>
<tr>
<td>K1</td>
<td>395</td>
<td>2.2 + 0.46</td>
<td>29 + 3.6</td>
<td>183.3 + 92.9</td>
<td>346.6 + 126.5</td>
</tr>
<tr>
<td>Tn30/HF</td>
<td>1733 + 656*</td>
<td>22 + 2</td>
<td>106.6 + 49.3</td>
<td>112 + 59.3</td>
<td>29.3 + 2.3</td>
</tr>
<tr>
<td>Tn30</td>
<td>4933 + 4041</td>
<td>66 + 12</td>
<td>553 + 4.7</td>
<td>76.6 + 4.1</td>
<td>31 + 4.3</td>
</tr>
</tbody>
</table>

X: Mean value of two individual experiments
*: Mean value of three individual experiments

Table 2: Summary of results of drug combination against K1, Tn30, K1 HF and Tn30 HF parasites of *P. falciparum* using [3H] hypoxanthine incorporation 48-hour assays.

<table>
<thead>
<tr>
<th>Combination</th>
<th>K1</th>
<th>Tn30</th>
<th>K1 HF</th>
<th>Tn30 HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF + HF</td>
<td>An.</td>
<td>Ad.</td>
<td>An.</td>
<td>PO.</td>
</tr>
<tr>
<td>PF + MF</td>
<td>-</td>
<td>An.</td>
<td>-</td>
<td>PO.</td>
</tr>
<tr>
<td>PF + CQ</td>
<td>An.</td>
<td>-</td>
<td>An.</td>
<td>-</td>
</tr>
<tr>
<td>PF + QN</td>
<td>-</td>
<td>-</td>
<td>An.</td>
<td>An.</td>
</tr>
</tbody>
</table>

PF: Penfluridol, HF: Halofantrine, MF: Mefloquine
CQ: Chloroquine; QN: Quinine
An: Antagonism; Ad: Additive Effect; Po: Potentiation

References


