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REVERSAL OF HALOFANTRINE RESISTANCE IN *PLASMODIUM FALCIPARUM* BY PENFLURIDOL *IN VITRO*

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Key words: *Plasmodium falciparum, halofantrine, penfluridol, $T_{9.96}$ HF, K_1 HF*

Abstract

Penfluridol , a neuroleptic drug, considerably reversed halofantrine resistance in $T_{9.96}$HF halofantrine resistant strain of *Plasmodium falciparum* (originally chloroquine sensitive parasites), but not in K_1HF 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_1 and chloroquine - sensitive $T_{9.96}$ 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 long-acting neuropleptic drug (8), was capable of reversing halofantrine resistance in $T_{9.96}$HF halofantrine resistant parasites.

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Materials and methods

a) Parasites and cultivation : Three halofantrine - resistant K_1HF, $T_{9.96}HF$, $T_{9.96}HF_4$ and two halofantrine - sensitive K_1 and $T_{9.96}$ strains of *Plasmodium falciparum* were used during this study. Cultivation of the parasites was conducted as described by Trager and Jensen with some modifications (15). Briefly, the parasites were cultured in a 5% haematocrit containing complete culture medium including 10.4 g RPMI1640 in 1 litre of double distilled water, 0.2% (W/V) sodium bicarbonate, 50mg hypoxanthine in 1 litre of culture, 25mM HEPES buffre, 10 mg/ml gentamicine 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 haematocrite was achieved. Subsequently, the cultures were flushed with a gas mixture consisting of 3% O_2 , 4% CO_2 and 93% N_2 (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 ul 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% O_2 , 4% CO_2 and 93% N_2. The gas chamber then was incubated at 37°C for 24 hours. After the first incubation period, 0.5 uci of [G-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 Titertek cell harvester (Skatron, AG). After drying , each of the individual discs was suspended into a 4 ml of optiphase' safe' scintillation fluid (LKB) and then prepared for scintillation counting.

Radioactivity was determined by means of a scintillation counter (LKB 1219 Rock Beta) linked to an Olivetti personal computer M24 which was programmed to calculate DPMs (disintegration per minute). DPMs were calculated from CPMs (counts per minute) of each tube either control or drugged and background. The results were expressed as a percent of the control. The IC50 (50% inhibitory concentration) and IC90 values were determined visually on the basis of the graph of the log drug concentration against incorporation of radiolablelled hypoxanthine by parasites (as a percentage of control value). The interaction of penfluridol with halofantrine, mefloquine, quinine and chloroquine was assessed by means of the method described by Chawira & Warhurst with some modifications(4).

Results

1- Comparative dose response of K_1HF , $T_{9.96}HF$, K_1 and $T_{9.96}$ 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_1HF, $T_{9.96}HF$, K_1 and $T_{9.96}$ strains are tabulated in Table 1. The K_1HF and $T_{9.96}HF$ parasites showed significant decrease in sensitivity to penfluridol , halofantrine, mefloquine and quinine. A significant increase in sensitivity to chloroquine exhibited by the K_1HF parasite.

2- Combinations of halofantrine, mefloquine, quinine and chloroquine with penfluridol against K_1HF, $T_{9.96}HF$, K_1 and $T_{9.96}$.

The results are tabulated in table 2. A combination of penfluidol with halofantrine or chloroquine exhibited an antagonistic effect against the K_1 strain. The combination of penfluridol with halofantrine was associated with antagonism against K_1HF parasites similar to the findings for penfluridol plus quinine. The greastest antagonism occurred with a combination of penfluridol and chloroquine, particularly in the ratios of 70:30 , 50:50 and 30:70 (perfluridol: chloroquine ; with 12.07 , 9.91 and 12.39 percent inhibition, respectively) against K_1HF strain. An additive effect was observed for the combination of penfluridol with halofantrine and an antagonism for the combination of penfluridol with mefloquine against $T_{9.96}$ parasites.

Potentiation was obtained for the combination of penfluridol with halofantrine against $T_{9.96}HF$ 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_9.96HF strain particulariy at the ratios of 70:30 and 50:50 with 10.35 and 9.79 percent inhibition, respectively.

Discussion

Penfluridol R1634 ; 4-(4-chloro-trifiluoro-m-tolyl)-1- [4,4-bis (P-fluorophenyl) buty] - 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 demosntrated 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 ca $^{2+}$ - 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 antagonsim against the K_1HF strain, but a considerable potentiation against the $T_{9.96}HF$ parasites. Although the reason for this discrepancy is not clear, it may be speculated that the $T_{9.96}HF$ and K_1HF 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 chloroqine with desipramine or cyproheptadine against chloroquine - resistant FCR_3 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 $T_{9.96}HF$ parasites, but only an additive effect against $T_{9.96}HF_4$ (which has a lower level of resistance to mefloquine than $T_{9.96}HF$ unpublished). The difference between $T_{9.96}HF4$ and $T_{9.96}HF$ 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 $T_{9.96}$ (mefloquine - sensitive strain), $T_{9.96}HF_4$ and $T_{9.96}HF$ produced antagonism, an additive effect and potentiation, respectively. Although penfluridol potentiated the activeity of mefloquine and halofantrine against the $T_{9.96}HF$ parasites , in combination with quinine, (another member of methanolic 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 $T_{9.96}HF$ parasites.

Table 1- Penfluridol , halofantrine , mefloquine , quinine and chloroquine Ic 50 value for K1HF, K1 , $T_{9.96}$HF and $T_{9.96}$ parasites of *P.falciparum* in 48 - hour [3H] hypoxanthine incorporation assays.

Drug / strain	Penflueidol	Halofantrine	Mefloquine	Quinine	Chloroquine
K1 HF	1840*	20 + 1.7	75 + 6.2	386.6 + 15.2	80 + 35.1
K1	395	2.2 + 0.46	29 + 3.6	183.3 + 92.9	366.6 + 136.5
$T_{9.96}$HF	1733.3 + 6506*	22 + 2	166.6 + 49.3	112 + 59.3	29.3 + 2.5
$T_{9.96}$	493.3 + 40.41	6.6 + 1.2	58.3 + 4.7	76.6 + 4.1	31 + 4.3

x : Mean value of two individual experiments

* : Mean value of three individual experiments

Table 2- Summary of results of drug combination against K1 , $T_{9.96}$, K1HF and $T_{9.96}$HF parasites of *P.falciparum* using [3H] hypoxanthine incorporation 48 - hour assays

Strain / Combination	K_1	$T_{9.96}$	K_1HF	$T_{9.96}$HF
PF + HF	An.	Ad.	An.	PO.
PF + MF	-	An.	-	PO.
PF + CQ	An.	-	An.	-
PF + QN	-	-	An.	An.

PF : Penfluridol ; HF : Halofantrine ; MF : Mefloquine

CQ : Chloroquine ; QN : Quinine

An : Antagonism ; Ad : Additive effect ; Po: Potentiation

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