Mefloquine: an antimalarial drug interacting with the b/c region of bacterial respiratory chains

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The effects of mefloquine, a quinoline-4-methanol antimalarial drug, on respiration by various bacterial strains endowed with branched electron transport pathways, have been examined. It is concluded that mefloquine strongly interacts with cytochrome b in the b/c segment of the chain. Unlike myxothiazol, mefloquine does not inhibit oxidant-induced b-type reduction but does induce an extra cytochrome b reduction when used in combination with antimycin A. The data reported are interpreted as evidence that mefloquine inhibits a ubiquinol oxidase site in common to both the alternative oxidase and the b/c oxidoreductase complex of the chain.

Mefloquine   *Electron transport*   *Alternative oxidase*   Rhodopseudomonas capsulata
Rhodopseudomonas sphaeroides   Pseudomonas cichorii

1. INTRODUCTION

Mefloquine (fig.1) is a quinolmethanol blood schizontocide which is highly effective against drug-resistant as well as drug-sensitive *Plasmodium vivax* and *Plasmodium falciparum*, has a high therapeutic index and has been accepted for clinical use (review [1]). Mefloquine is also bactericidal, and causes exponential loss of viability in cultures of *Escherichia coli* [2]. Loss of viability is accompanied by decrease in turbidity which suggests lytic properties of mefloquine. Mefloquine (MEF) binds to both phospholipids and iron porphyrin components such as

MEFLOQUINE (WR142490)

Fig.1. The structure of mefloquine (WR 142,490). DL-*erythro*-2-Piperidyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol hydrochloride.

plasmodial hemozoin [3,4] and affects the integrity of *E. coli* inner membrane in which has been proposed to inhibit NADH oxidation through interaction with membrane-bound b-type cytochromes [5]. This latter hypothesis has been verified and more thoroughly analyzed here by examining the effects of mefloquine on various bacterial respiratory chains.

2. MATERIALS AND METHODS

Mefloquine was a gift from Dr A.D. Wolfe (Walter Reed Army Institute of Research, Washington DC). The bacterial species used in this investigation were *Pseudomonas cichorii*, *Rhodopseudomonas capsulata* MR126 and *Rhodopseudomonas sphaeroides* R26 [6–8]. Aerobic cultures of *R. capsulata* MR126 were obtained as described [7] using a synthetic medium containing malate [9]. *R. sphaeroides* R26 was grown photosynthetically in the same medium supplemented with yeast extract (Difco, 2 g/l). *P. cichorii* was grown aerobically as described by Zannoni [6]. Plasma membrane fragments were
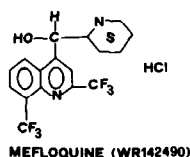
prepared according to standard procedures [6]. Ubiquinones were reduced to ubiquinols as in [10]. Rates of oxygen consumption were measured polarographically at 30°C with a Clark oxygen electrode. 2,6-Dichlorophenolindophenol (DCIP), cytochrome c and ubiquinone-1 reductases were measured spectrophotometrically (Hitachi model 100-40) at 30°C in the presence of 5 mM KCN. The absorption changes of membrane-bound cytochromes b and c were monitored in a dual-wavelength spectrophotometer (Sigma ZW-II) equipped with a rapid mixing apparatus (mixing time 0.2–0.3 s). Optical difference spectra were obtained with a Jasco UVIDEC-610 UV/VIS spectrophotometer. Proteins were assayed by using the method of Lowry et al. [11], and bacterio-chlorophyll was measured at 775 nm in acetone/methanol (7:2, v/v) extracts as in [12].

3. RESULTS

Fig.2 shows the influence of two concentrations of mefloquine upon $R.$ $capsulata$ MR126 growing photosynthetically in a malate minimal medium. A concentration of 25 μM mefloquine completely inhibited growth, and eventually caused a decrease in turbidity. More rapid and more extensive decrease in turbidity was observed at 50 μM mefloquine.
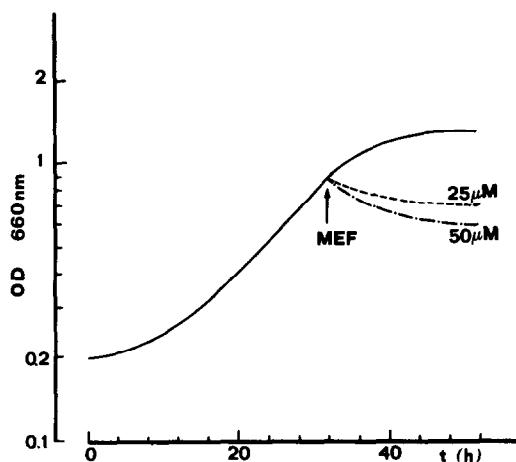
The effects of mefloquine on various respiratory

Fig.2. Effect of mefloquine on photosynthetic growth of *Rhodopseudomonas capsulata* MR126. Mefloquine (MEF), dissolved in dimethyl sulfoxide, was added at the time indicated by the arrow. An identical volume of DMSO was added to the control curve (solid line). MEF (25 μM, – – –); MEF (50 μM, – – – –).

activities by membrane fragments from aerobically grown $R.$ $capsulata$ MR126 are summarized in table 1. It is apparent that 50 μM mefloquine partially inhibited NADH oxidation (40% of inhibition), the residual activity completely blocked by 50 μM cyanide. As shown in [13], $R.$ $capsulata$ is endowed with a branched respiratory chain leading

Table 1

Effects of mefloquine on various respiratory activities by membrane fragments from *Rhodopseudomonas capsulata* MR126

Activities (inhibitors)	Control	+ Mefloquine (50 μM)
	(μequiv./h per mg protein)[a]	
(1) NADH oxidase	21.0	13.4
(2) (+ KCN 50 μM)	13.5	0.1
(3) NADH-DCIP reductase[b]	8.6	8.1
(4) NADH-Q-1 reductase[b]	8.5	8.5
(5) NADH-cyt. c reductase[b]	34.0	17.0
(6) Q_1H_2-cyt. c reductase[b]	30.0	25.0
(7) Cyt. c oxidase	13.0	12.0

[a] Expressed as μequiv. of either reduced electron acceptor or oxidized electron donor
[b] Measured in the presence of antimycin A (10 μM) plus KCN (5 mM). Cytochrome c oxidase was measured as ascorbate-DAD oxidase

Q-1, ubiquinone-1; DAD, diaminodurene; Q_1H_2, ubiquinol-1. Concentrations of electron acceptors and donors used were as follows: ascorbate, 10 mM; ubiquinone-1, 40 μM; ubiquinol-1, 150 μM; DCIP, 150 μM; DAD, 200 μM; NADH, 2 mM

to two cyt. *b*-type oxidases, one responsible for cyt. *c* oxidase activity and the other for the alternative (cyanide-insensitive) pathway of oxygen consumption. These oxidases differ in mid-point potentials ($E_{m,7}$ +410 and +260 mV, respectively) and in sensitivity to KCN (50% of inhibition with 5 μM and 0.5 mM KCN, respectively) [14]. Thus, expts 1–3 of table 1 suggest that mefloquine mainly affects the alternative (cyanide-insensitive) oxidase-dependent respiration. A second inhibitory site can also be identified at the *b/c* region of the chain. Indeed both cyt. *c* oxidase and NADH dehydrogenase activities were unaffected by mefloquine whereas the NADH-dependent cyt. *c* reduction was 50% blocked by the antimalarial drug. Expt 6 of table 1 shows that ubiquinol-1-cyt.*c* reductase is largely insensitive to mefloquine. This result may be due to a bypass of the endogenous electron transport chain operated by the exogenous artificial redox couple. Additional experiments revealed 50% inhibition of the alternative oxidase-dependent respiration at 15 μM mefloquine (not shown).

The traces in fig.3 represent the patterns of cytochromes *b* (560–575 nm) and *c* (551–540 nm) reduction in membranes from *R. capsulata* MR126 as a function of mefloquine, myxothiazol and antimycin A concentration. It is apparent that following the addition of ubiquinol-1 (150 μM) to membranes pretreated with 5 mM KCN and oxidized by 10 μM ferricyanide, reduction of a large amount of the total dithionite-reducible *b* and *c* cytochromes was achieved in about 0.2–0.3 s. In the presence of either antimycin A (20 μM), myxothiazol (18 μM), or mefloquine (20 μM) no appreciable change of the reduction kinetics of cytochromes *b* was observed whereas opposite synergistic effects could be induced by the presence of either antimycin A plus mefloquine or myxothiazol plus mefloquine. In the former case 20–30% extra *b*-type reduction was observed, and in the latter cyt. *b* reduction was drastically blocked. The effects of these inhibitors on the reduction kinetics of *c*-type cytochromes indicated that myxothiazol is highly effective in inhibiting reduction of cyt. *c* in contrast to antimycin A and
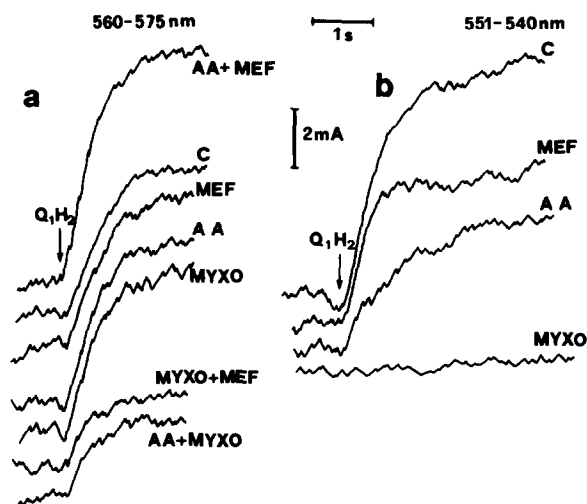

Fig.3. Effects of antimycin A, myxothiazol and mefloquine on the reduction kinetics of *b*- and *c*-type cytochromes in membrane fragments from *R. capsulata* MR126. The traces in a and b show reduction of cyt. *b* and *c* (monitored at 560–575 nm and 551–540 nm, respectively). C, control; AA, antimycin A; Q_1H_2, ubiquinol; MYXO, myxothiazol; MEF, mefloquine. Additions: AA, 20 μM; MYXO, 18 μM; MEF, 20 μM; Q_1H_2, 150 μM. Membranes ($\simeq$1 mg/ml) were suspended at pH 7.2 in 50 mM Hepes, 5 mM MgCl$_2$ buffer containing 5 mM KCN.
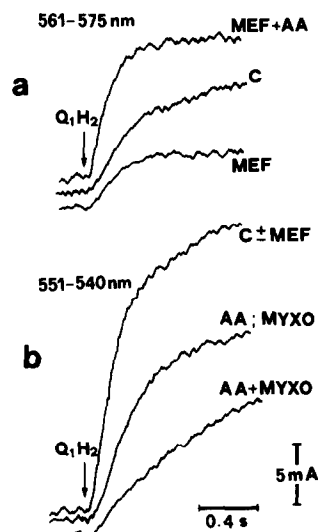

Fig.4. Effects of antimycin A, myxothiazol and mefloquine on the reduction kinetics of *b*- and *c*-type cytochromes in membranes from *Pseudomonas cichorii*. The traces in a and b show reduction of cyt. *b* and *c* (monitored at 561–575 nm and 551–540 nm, respectively). Non-standard abbreviations, inhibitors concentration and experimental conditions as given in fig.3. Protein concentration was 1.5 mg/ml.

mefloquinone which show the same inhibitory effect as percentage of cyt. *c* reduced but different kinetic patterns (cf. traces AA and MEF in fig.3). Slightly different results were obtained with plasma membranes from *P. cichorii*, a phytopathogenic obligate aerobe, in which a branched respiratory chain similar to that found in *R. capsulata* has recently been shown to operate [6]. As shown in fig.4a and b, mefloquine (20 μM) inhibited cyt. *b* reduction and induced an extra *b*-type reduction in the presence of antimycin A whereas it had no effect on cyt. *c* reduction.

It has been reported that in the presence of antimycin A the absorption maximum of dithionite-reduced cytochromes *b* is shifted by 2–3 nm to longer wavelengths ([15], but see also [16]). Fig.5 shows that membranes from *R. sphaeroides* reduced by dithionite at slightly acidic pH (pH 5.8) present a difference spectrum with a red-shift peaking at 562.5 nm (one minimum at 557 nm)
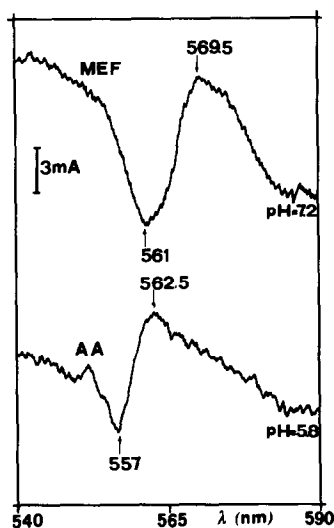

Fig.5. The spectral change induced by mefloquine and by antimycin A in dithionite-reduced *R. sphaeroides* R26 membrane particles. Membranes were diluted at a concentration of 0.1 mg bacteriochlorophyll/ml equivalent to approx. 3.5 mg protein/ml in either 50 mM Mes, 5 mM MgCl₂ (pH 5.8) buffer or 50 mM Hepes, 5 mM MgCl₂ (pH 7.2) buffer and reduced with addition of solid dithionite. After the baseline had been recorded (not shown) either antimycin A (20 μM) or mefloquine (25 μM) was added to the sample cuvette. Spectrophotometer settings were as follows: *t*, 25°C; bandwidth, 1 nm; time constant, 0.5 s; scanning speed, 20 nm/min; photometer scale, 0.03 A.

when treated with antimycin A (20 μM) (lower trace). The upper trace of fig.5 demonstrates that mefloquine (25 μM) induced a dramatic spectral variation of cytochromes *b* with a minimum at 561 nm and a maximum at 569.5 nm (pH 7.2). It is noteworthy that differently from antimycin A, the mefloquine-induced red-shift was not observed at acidic pH values.

4. DISCUSSION AND CONCLUSIONS

This study clearly shows that mefloquine, a quinoline-4-methanol antimalarial drug, is an effective inhibitor of electron transport in bacterial respiratory systems. Testing the sensitivity of several electron transport reaction steps toward this antimalarial drug, it has been found that only those systems comprising the ubiquinol-(cyanide insensitive) oxidase and the *b/c* segment of the chain were affected by mefloquine. The spectral change induced by mefloquine in dithionite-reduced membranes from *R. sphaeroides* suggested that the binding site(s) of the drug is probably cyt. *b* in the *b/c* complex (shown in fig.5). In both isolated *b/c* complex and chromatophore membranes from *R. sphaeroides*, a spectral shift induced by antimycin A of the peaks of cytochrome *b* has been linked to close association between the antibiotic and the heme of cyt. *b*-561, one of the two hemes of the ubiquinol-cyt. *c* oxidoreductase complex, i.e., cyt. *b*-561 and cyt. *b*-566 [17]. As shown in fig.5, the spectral change caused by mefloquine does not resemble that found with antimycin A. In addition, the red-shifts induced by mefloquine and antimycin A were best observable at quite different pH values (pH 7.2 and 5.8, respectively) and they seemed to be independent of each other (not shown). These results might therefore indicate a binding effect of mefloquine at a site perturbing the spectroscopic properties of *b*-566 heme. The latter suggestion is in line with the kinetic reduction patterns of cyt. *b* and *c* reported in figs 3 and 4. Indeed, in the absence of inhibitors, ubiquinol-1 rapidly reduced cyt. *c* (70%) and approx. 40% of the dithionite-reducible *b*. This *b* consists solely of cyt. *b*-562, owing to the relatively high potential of the quinone/quinol couple. Mefloquine together with antimycin A caused an increase in cyt. *b* reduction by ubiquinol-1 but largely blocked reduction of cyt. *b*

when used in combination with myxothiazol. Likewise, if myxothiazol was added together with antimycin A the combination of these inhibitors drastically blocked reduction of cyt. b. In this connection it has been shown [17] that myxothiazol, which contains β-methoxyacrylate as structural segment, binds to cyt. b-566 and blocks electron flow to both cyt. b-566 and Rieske iron-sulphur protein at a redox centre defined as Qo according to the protonmotive 'Q-cycle' scheme [18–20]. In contrast, antimycin A blocks reduction of ubiquinone by cyt. b-562 at a redox centre defined as Qi through binding to cyt. b-562 as indicated by a red-shift of the cyt. b-562 absorbance signal [15]. The experimental data described above suggest that the effects produced by mefloquine on respiration by various bacterial systems are different from those induced by antimycin A and myxothiazol. The hypothesis sustained here is that mefloquine interacts at a ubiquinol oxidase site which is probably shared by the alternative oxidase branch and the b/c complex of the chain. Unlike myxothiazol, mefloquine does not affect the formation of the ubisemiquinone $Q^{\cdot-}$ at the redox centre Qo, since it does not inhibit the oxidant-induced extra b-type reduction (not reported) but does bring about an extra reduction of b-type cytochromes only in combination with antimycin A. The latter phenomenon might reflect a mefloquine-induced shift to higher values of $E_{m,7}$ for cyt. b-566 so as to provide reduction of both cyt. b-566 and b-562 by the quinone/quinol couple as suggested by preliminary results from redox titrations of b-type cytochromes (Zannoni, in preparation). Thus, the effect of mefloquine on the electron transport through the b/c complex must be a consequence of the fact that the b/c redox components are relatively close to each other, and that their proteins may form the reaction site at which ubiquinol from the pool is oxidized [17–21]. Further studies will clearly be necessary to determine whether other redox components in addition to cyt. b-566, e.g., Rieske Fe-S centre, are spectrally and/or thermodynamically affected by mefloquine.

ACKNOWLEDGEMENTS

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