Effects of Mefloquine on the Release of Marker Enzymes from the Rat Liver Crude Lysosomal Fraction

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Accepted February 23, 1990

Abstract—The effects of the antimalarial agent mefloquine on the release of marker enzymes, acid phosphatase and beta glucuronidase, from the rat liver lysosomes in a crude lysosomal preparation were investigated and compared with that of chloroquine whose membrane effects have been well-documented in the literature. At 10 μM, mefloquine decreased significantly the release of marker enzymes when compared to the control, but at the higher concentrations of 100 μM and 500 μM, it markedly accelerated the release of enzymes. This suggested that mefloquine exerted a concentration-dependant biphasic effect of membrane stabilization and labilization. In comparison, chloroquine diminished the release of enzymes over the concentration range of 10–500 μM, that is showing a membrane stabilizing effect similar to other reports. Since serum levels of 1.6–3.2 μM are reported after a weekly dose of mefloquine, the present results suggest that a predominant stabilization effect should prevail under these conditions. However, higher drug concentrations may result in labilization with undesirable consequences.

The emergence of strains of Plasmodium falciparum resistant to commonly used antimalarial agents has led to the development of new drugs which are effective against the drug resistant malarias (1). One of the most promising new antimalarials is the quinoline methanol mefloquine, which is effective in the suppression and cure of malaria caused by multiple drug resistant strains of P. falciparum (2). Mefloquine has a high affinity for membrane phospholipids (3) and has been shown to accumulate in both parasitized and non-parasitized red cells (4). Although Brown et al. (5) have proposed that mefloquine is a membrane active drug, little is known of the membrane effects of mefloquine on normal cells. Such information would be of particular interest in view of its use in the prophylaxis of malaria. This has prompted the present study that looks at the effect of mefloquine on the release of lysosomal marker enzymes acid phosphatase (AP) and beta glucuronidase (BG) from the crude lysosomal fraction of rat liver, using chloroquine for comparison.

Materials and Methods

Drugs and chemicals: Mefloquine [±-erythro-2,8-bis(trifluoromethyl)-α-(2-piperidinyl)-4-quinolinemethanol hydrochloride was a gift from Roche Pharmaceuticals (Republic of Singapore). Chloroquine phosphate was obtained from Aldrich Chemical Company (Wisconsin, U.S.A.). p-Nitrophenyl phosphate, p-nitrophenyl glucuronide, reference enzymes acid phosphatase and beta glucuronidase were obtained from Sigma Chemical Company (Montana, U.S.A.). All other chemicals were of analytical grade.

Procedure for assay of marker enzymes released from rat liver crude lysosomal fraction: The rat liver crude lysosomal fraction was prepared according to the method of Ignarro (6). The release of marker enzymes, AP and BG, from the liver suspension in the presence of mefloquine (1, 10, 100, 500 μM) and chloroquine (10, 100, 500 μM) was investigated. For solubility reasons, stock solutions (10 mM) of mefloquine were prepared in 20% dimethylsulfoxide (DMSO) in 0.18 M
sucrose, and subsequently diluted with 0.18 M sucrose to obtain the above stated concentrations. Although chloroquine is readily soluble in water, it was prepared in a similar manner for comparison with mefloquine. The use of DMSO as the solvent did not interfere with the release of marker enzymes (6), which was also observed in this investigation. Concentrations of mefloquine and chloroquine higher than 500 /uM were not used because of drug solubility problems at the alkaline pH required for the assay of marker enzymes. Such problems were not encountered at lower concentrations.

Aliquots of liver suspension were added to the following sets of solutions contained in pyrex glass tubes (3 tubes per set): (i) 1.8 ml of 0.18 M sucrose in 0.04 M tris acetate buffer (pH 7.4) plus 0.2 ml drug solution, (ii) 2.0 ml of the above buffer, (iii) as in (ii) but aliquots were added just before centrifugation, (iv) 2 ml of 0.2% Triton X-100 in 0.04 M tris acetate buffer (pH 7.4), and (v) 1.8 ml of the buffer used in (iv) plus 0.2 ml drug solution. In (i), (ii) and (iii), a 0.2-ml aliquot was used, whereas in (iv) and (v), a 0.05-ml aliquot was used. The tubes were incubated (37°C for 15 min) in a Gallenkamp metabolic shaker at 75 agitation cycles/min. Incubation was terminated by centrifugation (27,000xg, 15 min, 4°C). The clear supernatant was decanted into glass tubes and kept at 4°C until assayed for marker enzymes.

AP activity was determined by measuring the formation of p-nitrophenol from p-nitrophenyl phosphate. One milliliter of the supernatant and 1.0 ml of distilled water were added to 1.0 ml of 0.3 M citrate buffer, pH 4.8. Incubation (at 37°C, 20 min) was initiated by addition of 0.04 ml of freshly prepared substrate (60 mg/ml) and terminated by addition of 0.2 ml of 4 M sodium hydroxide. The absorbance of p-nitrophenol at 405 nm was measured with a Perkin Elmer Lambda 4A spectrophotometer. BG activity was similarly determined using p-nitrophenyl glucuronide as the substrate. The presence of Triton X-100 did not interfere with enzyme activities under the assay conditions. This was established by comparing AP and BG activities in the presence and absence of Triton X-100.

The release of marker enzymes was expressed as a % of the total enzyme released in the presence of Triton X-100 (set iv) and corrected for % enzyme released at 0 min (set iii). The effects of varying concentrations of mefloquine and chloroquine on the release of lysosomal enzymes were subsequently expressed as % increase or decrease of that obtained in the absence of drug, which acted as the reference control of the release of marker enzymes. For each drug concentration, the number of sets of experiments done varied from 5 to 10.

The protein content of the liver suspension was routinely determined by the method of Lowry et al. (7) and found to be 905 /ug/0.05 ml aliquot (S.E.M., ±50.8 /ug).

Statistical analysis: Since each complete set of experiments was done using the same lysosomal fraction, the paired t-test was employed to calculate the statistical significance of the effects of drugs on the release of marker enzymes. The level of P<0.05 was considered significant.

Results

The effects of mefloquine and chloroquine on the release of marker enzymes AP and BG from the crude liver fraction are shown in Fig. 1. The release of AP was approximately the same as that of BG at each drug concentration. Mefloquine at 1 ,uM caused a negligible decrease in the release of enzymes. However, at 10 ,uM concentration, mefloquine caused a significant decrease in the release: AP activity was decreased by 22.35% (±3.86, n=10, P<0.025), whereas BG activity was lowered by 24.45% (±6.66, n=8, P<0.025). In contrast, at 100 ,uM and 500 ,uM concentrations mefloquine demonstrated a sharp 200–300% increase in AP and BG release (n=5–10, P<0.025) when compared to the control, which is indicative of membrane labilization. In the case of chloroquine, only membrane stabilization was observed over the concentration range from 10 ,uM to 500 ,uM. The levels of decrease for both marker enzymes were approximately 66%, 49% and 21% in the presence of 500 ,uM, 100 ,uM and 10 ,uM chloroquine respectively. The decrease in enzyme release were significant (P<0.025, for n=5).

A comparison of the total enzymes re-
leased in Triton X-100 in the absence (set iv) and presence of drug (set v) showed that less enzyme was released in the latter set under similar conditions. This was suggestive of interference of drugs with the enzyme assays. Corrections for these interfering effects on the assays were made before calculation of the values for statistical analyses and presentation in Fig. 1.

Discussion

The liver suspension used in this investigation is a crude liver fraction containing lysosomes, heavy mitochondria and other organelles. Preparations of this kind have been widely used for in vitro investigations on lysosomal membrane stabilization and labilization by drugs (6, 8, 9). Moreover, releases of both marker enzymes AP and BG were consistently similar. This observation suggests that the enzymes were released from the lysosomes and not from contaminating organelles in the experimental incubation mixtures.

Mefloquine produced a concentration-dependent biphasic effect, with stabilization observed at 10 μM and labilization at 100 and 500 μM. Brown et al. (5) have also reported that mefloquine at concentrations greater than 56 μM lysed spheroplasts and bacteria. Chloroquine, on the other hand, showed membrane stabilization of the present lysosomal preparation at all concentrations (10–500 μM) tested, which concurred with the findings of Ignarro (6) and Weissmann (10) using a similar lysosomal preparation. Interestingly, membrane effects of chloroquine have been reported to be either stabilizing or labilizing depending on the experimental conditions and procedures employed (11, 12). Inglot and Wolna (13) have reported a biphasic effect of stabilization and labilization of chloroquine on human erythrocytes in vitro, which is similar to our findings for mefloquine in this study. One micromolar mefloquine in our investigations and 1 μM chloroquine as reported by Ignarro (6) showed negligible lysosomal stabilization. This lack of significant effect for both drugs at 1 μM concentration may be attributed to the unknown binding capacities of these drugs to other organelles present in the in vitro crude lysosomal preparation.

Much work has been done on the lysosomotropic effects of chloroquine. The accumulation of chloroquine and other weak
bases in lysosomes (14–16) caused a rapid increase in the intralysosomal pH possibly by inhibiting the energy-dependent active transport of H⁺ (proton pump) which maintains the low intralysosomal pH (17). This effect seemed attainable at concentrations easily achieved in patients on chloroquine therapy, where usual therapeutic plasma concentrations ranged from 0.1 to 0.6 μM. The buffering effect on the lysosomes is thought to be one of the contributing mechanisms to its pharmacological actions as an antimalarial and antiinflammatory agent. The high intralysosomal pH inhibits the penetration of intracellular parasites (18) and it is likely that its rheumatologic effectiveness is related primarily to the lysosomal action (19). Although not a 4-aminoquinoline, mefloquine is an amphipathic molecule with weak basic properties like chloroquine. Clinically as an antimalarial, plasma concentrations of 600–1200 ng/ml (1.6–3.2 μM) have been obtained after a weekly dose of 250 mg for prophylaxis (20). Franssen et al. (21) reported the mean plasma and blood mefloquine concentration to be about 1900 ng/ml (5 μM) after one 1250 mg dose given in divided doses for acute malaria. Extrapolating from our findings, at these concentrations, lysosomal membrane stabilization may be expected and would confer to mefloquine actions similar to that of chloroquine; that is, apart from being an antimalarial agent, it may have antiinflammatory action as well. Although the total plasma concentrations of mefloquine attained after therapeutic doses are much higher than that for chloroquine (1.6–5 μM as compared to 0.1–0.6 μM, respectively), it should be noted that mefloquine is more extensively bound to plasma proteins (98%) than chloroquine (60%) (22). Therefore, if free drug concentrations are considered, both are effective at about similar free drug concentrations.

The effect of mefloquine on the lysosomes was quite different from that of chloroquine at concentrations higher than 10 μM. Investigations at higher concentrations of 100 μM and 500 μM showed that mefloquine was a very potent labilizer, in contrast to the stabilizing effects observed with chloroquine at these same concentrations as reported by Ignarro (6) and observed here. The significant difference in the behavior of these closely related antimalarial agents suggest differing modes of interactions of these agents with lysosomal membranes. Thus far, mefloquine has not been used at such high concentrations therapeutically. Nevertheless, some knowledge of its behavior at high concentrations is important from the toxicological point of view. Thus, it may be prudent to be cautious if mefloquine is to be used for a prolonged period of time since problems with drug accumulation may become prominent in view of its long elimination half-life of 20 days (21). The possibility of other therapeutic potentials and toxicological implications of mefloquine warrants further investigation of the effects of this relatively new antimalarial agent on lysosomes.

References


