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Short Communication

THE BIOACTIVATION OF AMODIAQUINE BY HUMAN POLYMORPHONUCLEAR LEUCOCYTES *IN VITRO*: CHEMICAL MECHANISMS AND THE EFFECTS OF FLUORINE SUBSTITUTION

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Abstract—Amodiaquine, a 4-aminoquinoline antimalarial, has been associated with hepatitis and agranulocytosis in humans. Drug hypersensitivity reactions, especially agranulocytosis, have been attributed to reactive intermediates generated by the oxidants discharged from stimulated polymorphonuclear leucocytes (PMN). The metabolism of amodiaquine to both stable and chemically reactive metabolites by human PMN has been investigated *in vitro*. Incubation of [^{14}C]-amodiaquine with PMN resulted in irreversible binding of radiolabel to protein and depletion of intracellular reduced glutathione, which were enhanced by phorbol myristate acetate (PMA), a PMN activator. Two metabolites were identified: the C-5′ glutathione adduct of amodiaquine, derived from both endogenous and exogenous glutathione, and 4-amino-7-chloroquinoline, which was presumed to be formed by hydrolysis of amodiaquine quinoneimine. Desethylamodiaquine, the major plasma metabolite of amodiaquine in humans, also underwent bioactivation to a chemically reactive species in the presence of PMA-stimulated PMN. Substitution of the 4′-hydroxyl group in amodiaquine with fluorine significantly reduced irreversible binding to protein and abolished depletion of intracellular glutathione in the presence of PMA. These findings indicate that the bioactivation of amodiaquine by PMN is associated with the formation of a quinoneimine intermediate. Such a reactive metabolite, if produced in PMN or bone marrow *in vivo*, may be responsible for the drug's myelotoxicity.

Key words: amodiaquine; bioactivation; polymorphonuclear leucocytes; fluorine substitution

Amodiaquine is a 4-aminoquinoline antimalarial that has been withdrawn from prophylactic use because of a high incidence of agranulocytosis and hepatotoxicity [1, 2]. Direct dose-dependent toxicity has been suggested as a possible mechanism [3, 4], although a number of studies have indicated the involvement of an immune response, and that type II or type IV hypersensitivity reactions may be involved [5–7]. Amodiaquine is too small a molecule to be immunogenic per se, but when conjugated with a high molecular weight protein, it is capable of initiating an immune response: Rats administered amodiaquine [8, 9] and the majority of patients suffering from adverse reactions to the drug [10] have detectable IgG anti-amodiaquine antibodies.

In vitro studies have shown that amodiaquine readily undergoes oxidation to a protein-reactive quinoneimine in the presence of either hydrogen peroxide or hypochlorous acid, and this bioactivation is enhanced by the presence of a peroxidase [11]. Formation of such a reactive species *in vivo* and subsequent binding to cellular macromolecules could affect cell function either directly or indirectly by immunological mechanisms.

In humans, the major plasma and urinary metabolite of amodiaquine is desethylamodiaquine [12], which, like the parent compound, has been shown to accumulate in peripheral white cells *in vitro* [13]. Since activated leucocytes are able to transform a variety of xenobiotics to stable and chemically reactive metabolites [14–16], it appeared likely, especially in view of the susceptibility of amodiaquine to oxidation, that PMN† could oxidase the drug. Bioactivation could be effected by either myeloperoxidase or the reactive oxygen species released during the oxidative burst of the PMN [17]. Preliminary studies [9] demonstrated that amodiaquine is metabolised by stimulated PMN to a reactive intermediate (i.e., a species that binds irreversibly to protein), but the structure of the reactive metabolite was not defined.

In the rat, the C-5′ glutathione conjugate of amodiaquine derived from a quinoneimine metabolite has been identified in bile [18]. The formation of the glutathione conjugate was reduced by prior administration of ketoconazole, indicating that the chemically reactive intermediate formed *in vivo* is generated, at least in part, by hepatic cytochrome P-450 [19]. Metabolic studies with fluorinated analogues of amodiaquine (Fig. 1) have shown that introduction of fluorine into the 4′ position, which increases the oxidation potential of amodiaquine, blocks bioactivation of the drug in the liver, as indicated by the absence of thioether conjugates of the analogues from the bile of rats [20] and mice [21].

The aims of this study were, firstly, to investigate the chemical and biochemical aspects of the bioactivation of amodiaquine and desethylamodiaquine by human PMN, and secondly, to examine the effect of fluorine substitution on this activation.

Materials and Methods

Materials. Amodiaquine was a gift from Parke Davis (Ann Arbor, MI, U.S.A.). [quinoline-2-^{14}C]-Amodiaquine (sp.act 9.4 μCi/μmol; 95% pure by TLC) was prepared by Amersham International (Amersham, U.K.) and purified to >99% purity by HPLC prior to use.

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† Abbreviations: AQ, amodiaquine; AQQI, amodiaquine quinoneimine; DEAQ, desethylamodiaquine; DFAQ, 4′-deshydroxy-4′-fluoroamodiaquine; HDFAQ, hydroxy-4′-deshydroxy-4′-fluoroamodiaquine; ACQ, 4-amino-7-chloroquinoline; PMN, polymorphonuclear leucocyte; PMA, phorbol 12-myristate 13-acetate; HSA, human serum albumin; PBS, phosphate-buffered saline; CI, chemical ionisation; LCMS, liquid chromatography-mass spectrometry.

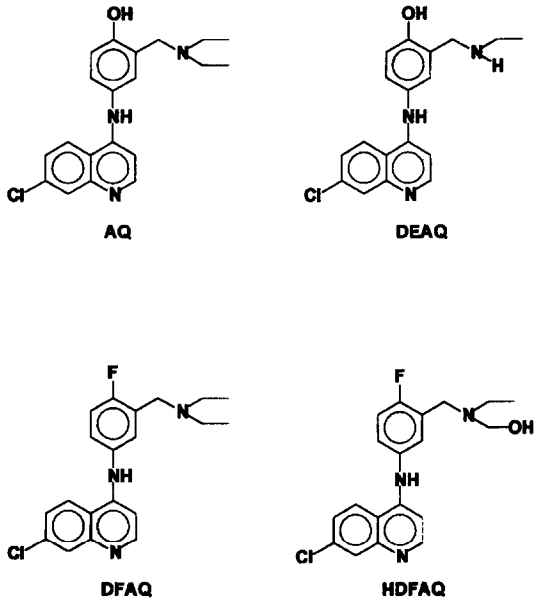

Fig. 1. Structure of amodiaquine (AQ), desethylamodiaquine (DEAQ), 4′-deshydroxy-4′-fluoroamodiaquine (DFAQ), and hydroxy-4′-deshydroxy-4′-fluoroamodiaquine (HDFAQ).

[G-^{3}H]-4′-Deshydroxy-4′-fluoroamodiaquine ([^{3}H]-DFAQ, sp.act 3.35 μCi/μmol) was synthesised according to a previously described method [20], and was >99% pure by radiometric HPLC.

The quinoneimine and the C-5′ glutathione adduct of amodiaquine were synthesised by the methods of Harrison *et al.* [18]. 4-Amino-7-chloroquinoline was synthesised by the method of Baker *et al.* [22]. Glutathione, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, $NADP^+$, phorbol 12-myristate 13-acetate (PMA), bromobimane, and human serum albumin (HSA) were obtained from Sigma Chemical Co. (Poole, U.K.).

Monopoly resolving medium (Ficoll Hypaque, density 1.114 g/mL) was obtained from ICN Biomedicals (Bucks, U.K.). Lymphoprep (density 1.077 g/mL) was a product of Nycomed (Oslo, Norway). All other reagents were from Fisons (Loughborough, U.K.), and were of analytical grade.

Preparation of [^{14}C]-desethylamodiaquine. [^{14}C]-desethylamodiaquine was generated by incubating [^{14}C]-amodiaquine (10 μM, 0.1 μCi) with human liver microsomes (2 mg protein/incubation) in the presence of an NADPH-regenerating system (glucose-6-phosphate 10 mM, NADP 0.5 mM, glucose-6-phosphate dehydrogenase 2 units). The total incubation volume was 2.5 mL. After 30 min, the reaction was terminated by the addition of methanol (3 mL) to precipitate the protein. The supernatant was analyzed by HPLC using acetonitrile (10–20% over 18 min) in ammonium acetate buffer (0.1 M, pH 3.8), and was found to contain exclusively [^{14}C]-desethylamodiaquine.

Synthesis of [^{3}H]-HDFAQ. 4,7-dichloro-[G-^{3}H]-quinoline (253 μmol, 1 mCi) and 4-fluoro-3-[2′-(ethylamino)ethanol]-methylaniline (383 μmol) in acidified ethanol (5 mL) were refluxed for 16 hr. The final product, [^{3}H]-hydroxy-4′-deshydroxy-4′-fluoroamodiaquine ([^{3}H]-HDFAQ) had a specific activity of 2.92 μCi/μmol, and was isolated in 20% yield by silica column chromatography (methanol:dichloromethane 1:9 v/v). It was characterized by CI mass spectrometry: m/z 374 (M + 1,100), 340 (39), 330 (11), 296 (10), 287 (12), 253 (15).

Isolation of polymorphonuclear leucocytes. Venous blood from healthy male volunteers (24–40 yr) was layered onto a dual-density gradient that consisted of Lymphoprep (4 mL) on monopoly-resolving medium (8 mL). After centrifugation (750 g, 25 min), PMN were removed from the resolving medium and washed twice with phosphate-buffered saline (PBS). PMN were resuspended in Dulbecco's phosphate-buffered saline, pH 7.4, and viability assessed by trypan blue dye exclusion.

Irreversible binding of radiolabelled material. PMN (5×10^6 cells) were incubated at 37°C with [^{14}C]-amodiaquine (10 μM, 0.075 μCi.), [^{14}C]-desethylamodiaquine (10 μM, 0.075 μCi.), [^{3}H]-DFAQ (10 μM, 0.105 μCi), or [^{3}H]-HDFAQ (10 μM, 0.088 μCi) in the presence or absence of PMA (10 ng/mL) in Dulbecco's PBS containing HSA (5 mg); the protein acted as a quantitation target for chemically reactive species. The total incubation volume was 2.5 mL. The incubation was terminated by addition of methanol (3 mL) after 60 min, which gave maximum bioactivation of amodiaquine by PMN [9]. Protein was allowed to precipitate overnight at –20°C. The protein precipitate was washed with methanol (2×5 mL) and 70% aqueous methanol (5 mL) before being dissolved in 1 M sodium hydroxide (2 mL). Aliquots (2×0.75 mL) of the solution were neutralised with glacial acetic acid (120 μL) and assayed for radioactivity after addition of scintillant (16 mL).

Analysis of stable metabolites. HPLC analysis was carried out using a Kontron 325 pump linked in series to a Spectra 1000 UV variable wavelength detector (Spectra Physics, San Jose, CA, U.S.A.) and an online Radiomatic A250 Flo-One/β radioactivity detector (Canberra-Packard, Berks, U.K.). The aqueous supernatant was reduced to dryness under a stream of nitrogen, and redissolved in methanol (200 μL). An aliquot (50 μL) of this solution was injected onto an octadecyl-bonded silica column (μBondapak, 10 μM, 3.9×300 mm, Waters). Compounds were eluted with a mobile phase that consisted of acetonitrile (10–20% over 18 min) and ammonium acetate (0.1 M, pH 3.8) flowing at 1.5 mL/min. Supernatants from incubations of [^{14}C]-amodiaquine with PMN and exogenous glutathione were analyzed with an alternative eluent comprised of acetonitrile (10–25% over 30 min, 25–40% over 15 min) and ammonium dihydrogen orthophosphate (10 mM, pH 4.6) containing heptane sulphonic acid (1.01 g/L).

Hydrolysis of synthetic amodiaquine quinoneimine. A solution of amodiaquine quinoneimine (0.5 mg/mL) was incubated with methanol: concentrated hydrochloric acid (4:1 v/v) for 24 hr at room temperature prior to analysis by LCMS.

Liquid chromatography-mass spectrometry (LCMS). Samples (in 10 μL methanol) were eluted from a μ Bondapak C_{18} column with a gradient of acetonitrile (10–20% over 18 min) in either ammonium acetate (0.1 M, pH 3.8) or ammonium formate (20 mM, pH 3.5). The flow rate was 1.5 mL/min. Two Jasco PU-980 pumps were linked to an HG-980-30 mixing module. Eluate passed through a Jasco UV-975 absorbance detector (254 nm), and thence, via a stream splitter, at approximately 50 μL/min, to the electrospray probe and interface of a Quattro II mass spectrometer (Fisons Biotech MS, Manchester). The splitter and probe were connected by 1.5 m of 75 μm fused silica capillary. Nebulising and drying gas (nitrogen) were delivered at 13 L/hr and 300 L/hr, respectively. The interface temperature was 60°C; the capillary voltage, 4×10^3 V. Compressed centroid spectra were acquired between m/z 100–850 with a scan duration of 4.91 sec; the photomultiplier voltage was 530 V. Fragmentation of analyte ions was enhanced by increasing the cone voltage.

Determination of intracellular glutathione concentration. PMN (0.5×10^6 cells) were incubated with compound (0, 10, 30, 100, or 300 μM) in the absence or presence of PMA (10 ng/mL) in Dulbecco's PBS. The total incubation volume was 1 mL. After the incubation period (60 min at 37°C), levels of reduced glutathione were determined by the method of Cotgreave and Moldéus [23]. Bromobimane (3 mM) in N-ethyl morpholine (3 mM, pH 8.0) (100 μL) was added, and the mixture left in the dark for 5 min. Protein was precipitated with trichloroacetic acid (10 μL) and sedimented by centrifugation (750 g, 3 min). An aliquot (50 μL) of the supernatant was injected onto an HPLC column (5 μm Hypersil BDS C_{18}, 4.5×150 mm). Glutathione adducts were eluted with a mobile phase that consisted of 100% A (0.25% acetic acid:9% acetonitrile, pH 3.7) for 7 min followed by 100% B (75% aqueous acetonitrile) for 4 min; each elution was succeeded by re-equilibra-

tion with 100% A over 5 min. The flow rate was 1 mL/min. Eluate was monitored with a fluorescence detector (Hitachi 1080) set at $Ex_{394\,nm}$:$Em_{480\,nm}$. A standard curve was constructed between 0 and 2 mmol glutathione.

Statistical analysis. All values given in the text are mean ± *SD;* values in figures are mean ± *SEM.* Data were compared using Student's *t*-test for nonpaired data. Where necessary, data were subjected to analysis of variance. A difference was deemed significant when $p < 0.05$.

Results

Metabolism and irreversible binding of amodiaquine and analogues. Increasing the PMN numbers from 0.5×10^6 to 5×10^6 cells in the presence of PMA increased the amount of [^{14}C]-amodiaquine irreversibly bound to HSA after 60 min from 126.5 to 177.1 pmol/mg (Fig. 2). However, in the absence of PMA there was no significant change in irreversible binding with increasing cell numbers. Five million cells were used in subsequent incubations for the determination of irreversible binding, as this gave sufficient activation of substrate for the quantification and characterisation of stable and protein-reactive metabolites. The presence of PMA significantly increased irreversible binding of [^{14}C]-amodiaquine ($p < 0.001$), [^{14}C]-desethylamodiaquine ($p < 0.05$), and [^{3}H]-HDFAQ ($p < 0.05$) to protein over 60 min (Fig. 3). There was some irreversible binding of [^{3}H]-DFAQ, but it was not significantly greater than in the absence of PMA. Covalent binding of [^{14}C]-amodiaquine was six-fold greater than that of [^{3}H]-DFAQ, and almost ten-fold greater than the binding of [^{3}H]-HDFAQ ($p < 0.001$) in the presence of PMA.

No metabolites of either [^{3}H]-DFAQ or [^{3}H]-HDFAQ were detected by reversed-phase HPLC. In the presence of PMA, [^{14}C]-amodiaquine was converted to 4-amino-7-chloroquinoline (11.9 ± 4.5% of incubated radioactivity) and trace amounts of the C-5′glutathione adduct of amodiaquine that were identified by co-chromatography with authentic standards and LCMS. The 4-amino-7-chloroquinoline metabolite yielded pseudomolecular ions ($[M + 1]^+$) at m/z 179 (100%, ^{35}Cl-isotope form) and m/z 181 (33%, ^{37}Cl), and fragmented, at a cone voltage of 70 V, by loss of chlorine to give m/z 144 (25%). The ion chromatograms for m/z 661 (^{35}Cl) and m/z 663 (^{37}Cl), the pseudomolecular ions of glutathionyl amodiaquine, contained coincidental peaks of relative intensities 100:24, at the retention time of the authentic adduct (Fig. 5C). This ratio of peak intensities of the two ion currents corresponds approximately to the natural chlorine isotope ratio (3:1). Neither metabolite was detected by LCMS in the absence of PMA. [^{14}C]-desethylamodiaquine (10 μM) was also metabolised to a polar metabolite (9.4 ± 3.5% of incubated radioactivity) that co-chromatographed with 4-amino-7-chloroquinoline.

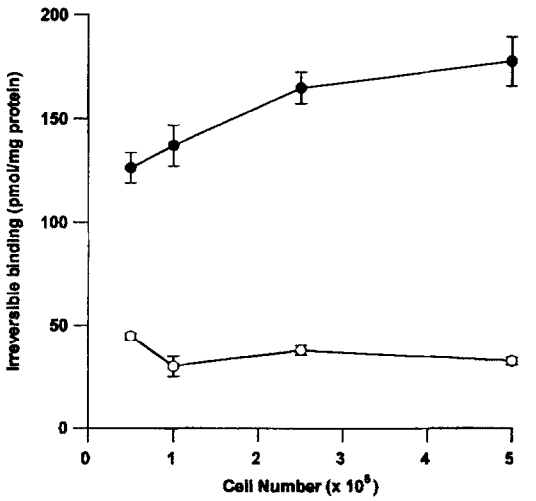

Fig. 2. Effect of polymorphonuclear leucocyte numbers on the irreversible binding of amodiaquine *in vitro* to protein in the absence (○) and presence (●) of the cell stimulator PMA. Values shown are mean ± *SEM* ($n = 4$).

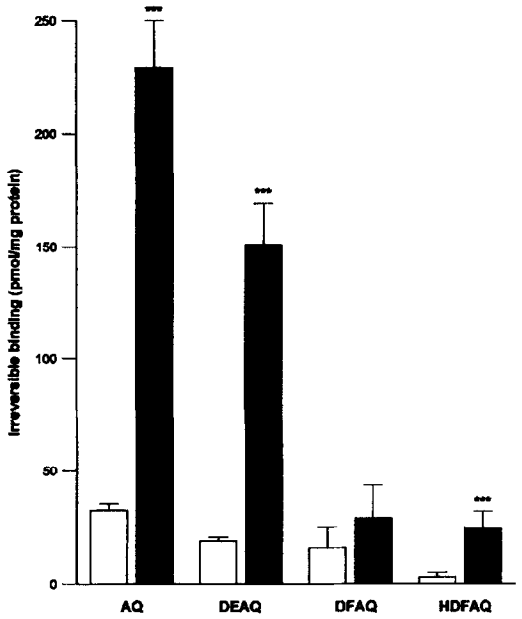

Fig. 3. Irreversible binding of amodiaquine (AQ), desethylamodiaquine (DEAQ), 4′-deshydroxy-4′-fluoroamodiaquine (DFAQ), and hydroxy-4′-deshydroxy-4′-fluoroamodiaquine (HDFAQ) to protein in the absence (open bars) and presence (hatched bars) of PMA. Incubations contained 5×10^6 cells. Values shown are mean ± *SEM* ($n = 4$). ***$P < 0.001$ compared with control (analogue − PMA).

Effect of glutathione on the irreversible binding of [^{14}C]-amodiaquine. Inclusion of glutathione (1 mM) resulted in a 67% ($p < 0.05$) decrease in the irreversible binding of [^{14}C]-amodiaquine to protein in the presence of PMA. Radiometric HPLC and LCMS analyses of supernatants revealed enhanced production of 4-amino-7-chloroquinoline (18.2%) and C-5′glutathione adduct of amodiaquine (6.3%) in the presence of PMA (Fig. 4). The 4-amino-7-chloroquinoline yielded the spectrum described above. Detection of diagnostic fragments of the C-5′glutathione adduct (Fig. 5C) was only possible in the presence of exogenous glutathione, and the adduct was assigned from $[M + 1]^+$ ions at m/z 661 (100) and 663 (42), and from fragments at m/z 588(36) and 590(23), generated at a cone voltage of 50 V, which are attributable to loss of the diethylamino moiety [18].

Depletion of intracellular glutathione. Incubation of amodiaquine with PMN in the absence of PMA resulted in a small, but significant ($p < 0.001$), concentration-dependent decrease in intracellular reduced glutathione, which reached a minimum of 87.3 ± 2.1% of the control value (2.4 ± 0.1 nmol glutathione/10^6 cells) with 300 μM amodiaquine. Addition of PMA in the absence of aminoquinoline resulted in a marked reduction in glutathione levels in all experiments ($n = 4$), but the extent of the reduction varied between experiments (mean reduction of 28.5 ± 9.3%). In the presence of PMA, there was enhanced depletion of glutathione by amodiaquine, which was concentration-dependent (Fig. 6) and reached a minimum of 46.7 ± 7.1% of solvent control values (1.9 ± 0.1 nmol glutathione/10^6 cells). Incubation of desethylamodiaquine with PMN did not result in glutathione depletion in the absence of PMA, but a significant reduction occurred at 10, 30, and 100 μM (though not at 300 μM) desethylamodiaquine when PMA was added (Fig. 6). DFAQ and HDFAQ did not deplete glutathione in the absence

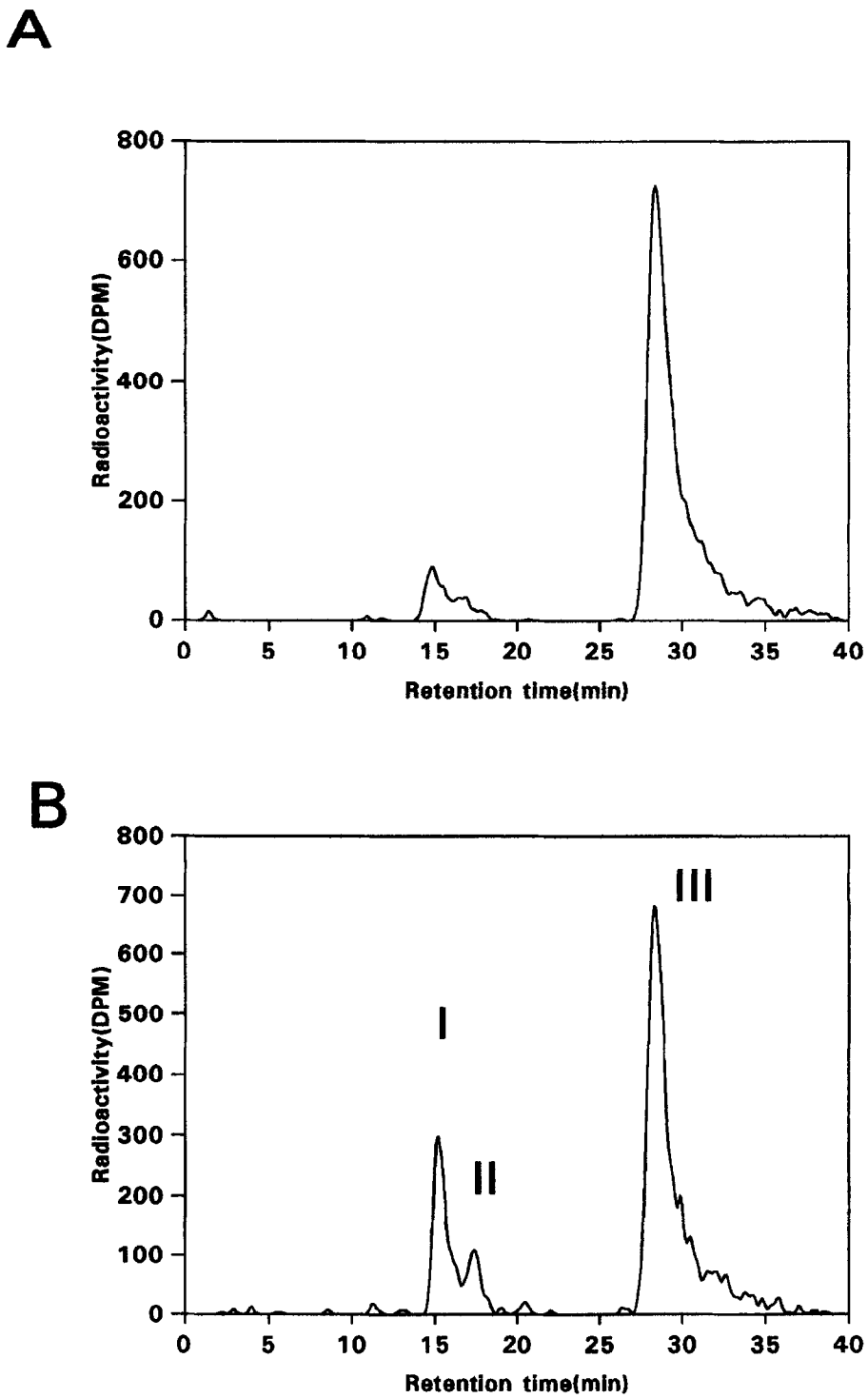

Fig. 4. Reversed-phase HPLC trace of amodiaquine and metabolites following incubation of drug with 5×10^6 PMN and exogenous glutathione (1 mM), in the absence (A) or presence (B) of PMA. Peak I was identified as 4-amino-7-chloroquinoline, whilst peak II is the C-5′ glutathione conjugate of amodiaquine. Peak III is parent compound.

of PMA, and appeared to cause a concentration-dependent reduction in the depletion of glutathione in the presence of PMA (Fig. 6).

Hydrolysis of amodiaquine quinoneimine (AQQI). After 24 hr, the methanol-water solution of AQQI was found to contain amodiaquine ($[M + 1]^+$ at m/z 356/358; fragments at m/z 283/285), 4-amino-7-chloroquinoline ($[M + 1]^+$ at m/z 179/181; fragments at m/z 144 [M + 1-Cl] and m/z 117 [m/z 144-HCN]); and an incompletely characterised product, of R_t 25 min when eluted with acetonitrile-ammonium acetate, which yielded pseudomolecular ions at m/z 299/301 for a monochlorinated compound (relative intensities 100:33) and fragment ions at m/z

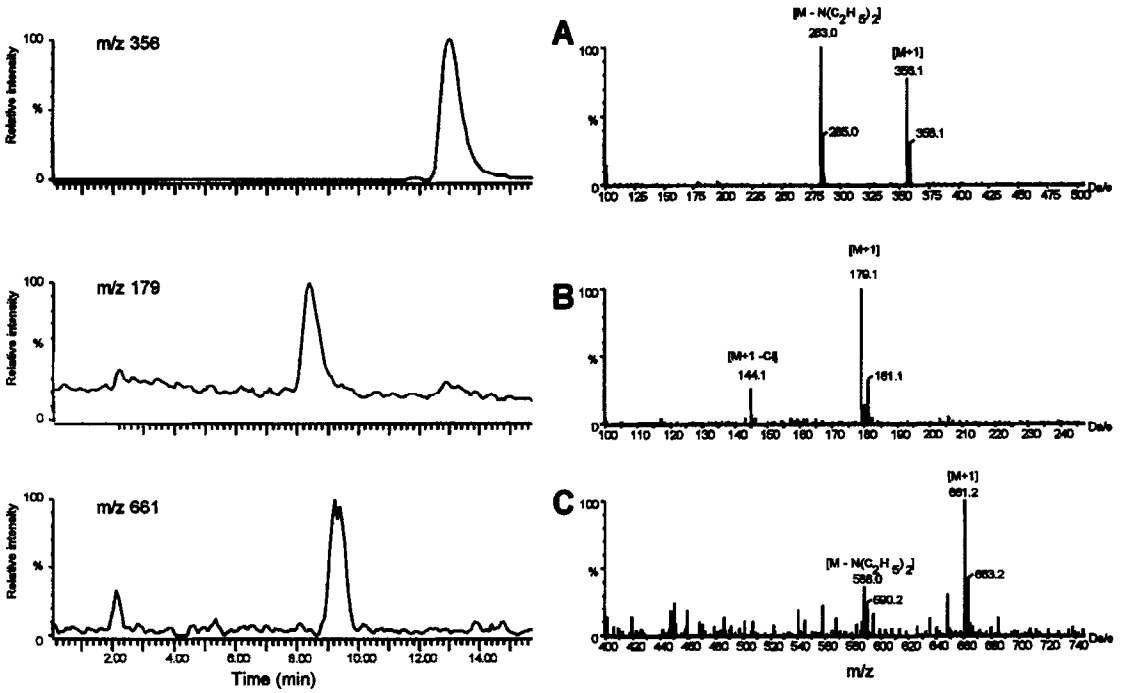

Fig. 5. LCMS (electrospray) analysis of metabolites of amodiaquine formed by activated PMN in the presence of exogenous glutathione (1 mM): pseudomolecular ion chromatograms (left) and spectra (right) of amodiaquine (A), 4-amino-7-chloroquinoline (B) and the C-5'glutathione adduct of amodiaquine (C). Concentrated supernatants of PMN incubations were eluted with acetonitrile-ammonium formate. Fragmentation was accomplished by a cone voltage of either 50 V (B and C) or 70 V (A).

269/271 ([M-HCO]). In the absence of HCl, AQQI was not hydrolysed to 4-amino-7-chloroquinoline, and only amodiaquine and the third product were detected.

Discussion

Amodiaquine undergoes bioactivation to a protein-reactive metabolite(s) in the presence of stimulated PMN. The ready activation was presumed to be a consequence of the facile oxidation of the hydroxyaniline side chain to a quinoneimine by either myeloperoxidase or any of the reactive oxygen species released from the PMN during the oxidative burst of the PMN [17, 24]. This was confirmed in the present study by identification of the C-5'glutathione conjugate of amodiaquine, the principal isomer generated when synthetic amodiaquine quinoneimine is reacted with glutathione [18], and 4-amino-7-chloroquinoline. 4-amino-7-chloroquinoline was shown to be a product of the acid-hydrolysis of amodiaquine quinoneimine.

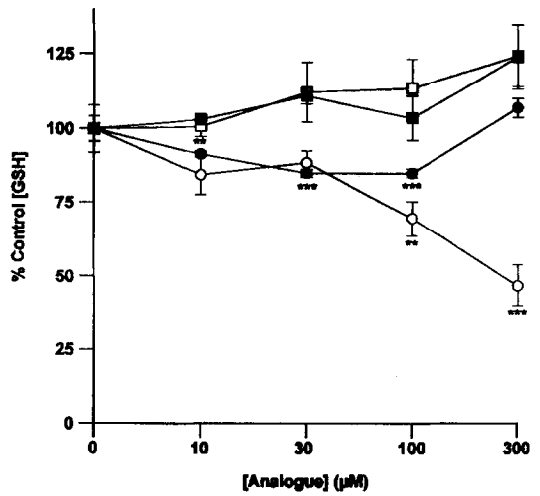

Fig. 6. Depletion of intracellular glutathione by amodiaquine (○), desethylamodiaquine (●), DFAQ (□), and HDFAQ (■) in the presence of PMA (10 ng/mL)). Values shown are mean ± *SEM* ($n = 4$). $^{**}P < 0.01$; $^{***}P < 0.001$ compared with control (solvent + PMA) glutathione concentrations (1.9 ± 0.1 nmol/10^6 PMN cells).

Addition of glutathione significantly reduced the irreversible binding of amodiaquine to protein. This would appear to be consistent with the extracellular generation of a chemically reactive metabolite. However, the observed formation of a glutathione conjugate from endogenous glutathione, and the PMA-enhanced depletion of cellular levels of glutathione, suggest a degree of intracellular bioactivation of amodiaquine. Such depletion of reduced glutathione may reflect not only conjugation with reactive drug metabolite(s), but also redox cycling between the aminophenol and quinoneimine, as observed with paracetamol [25]. If the acidic conditions necessary for the hydrolysis of the quinoneimine occur only within the PMN, it is conceivable that the formation of 4-amino-7-chloroquinoline specifically indicates intracellular oxidation of amodiaquine. Such an oxidation might be effected by hypochlorous acid, the principal oxidant generated by PMN [26], as shown previously [11].

Stimulated PMN release superoxide, which undergoes spontaneous conversion to hydrogen peroxide. Myeloperoxidase catalyses the oxidation of chloride to hyperchlorous acid by hydrogen peroxide. Although this process occurs predominantly outside the cell, under certain circumstances hyperchlorous acid is formed intracellularly [27]. Extracellular production of hyperchlorous acid in the present incubations would have been facilitated by the presence of chloride ions in the cell medium (Dulbecco's PBS). Intracellular bioactivation of amodiaquine may offer a chemical mechanism for the cell-directed toxicity associated with amodiaquine administration to humans.

Amodiaquine accumulates in PMN *in vivo* because of its basic properties [28], and could undergo bioactivation in either bone marrow or peripheral cells stimulated by, for example, infection. Under these circumstances, the reactive quinoneimine might exert toxic effects in a manner analogous to that of the reactive metabolite of paracetamol [29]. Alternatively, it could either migrate to the surface of the cell to form a neoantigen or, if it is released from the cell, haptenate extracellular proteins and lead to immune-mediated toxicity.

Desethylamodiaquine is the major plasma metabolite of amodiaquine in humans, and is thought to be largely responsible for the drug's antimalarial activity *in vivo*, because amodiaquine itself has a short plasma half-life [28]. Like amodiaquine, desethylamodiaquine is a basic compound that accumulates in peripheral white cells *in vivo* [28]. This metabolite underwent significant bioactivation to a protein-reactive species in the presence of PMA-stimulated PMN, although to a significantly ($p < 0.001$) lesser extent than amodiaquine. The depletion of intracellular glutathione caused by desethylamodiaquine was also less than that observed with amodiaquine. The lack of glutathione depletion observed at 300 μM desethylamodiaquine may reflect the ability of the metabolite to inhibit PMN function more effectively than amodiaquine itself at high concentrations [30].

Substitution of the 4′-hydroxyl group in amodiaquine with a fluorine, by preventing oxidation to the quinoneimine, completely abolished the depletion of intracellular glutathione. Furthermore, at high concentrations, both DFAQ and HDFAQ appeared to reduce the depletion of glutathione in the presence of PMA, which may reflect inhibition of cellular function. The lack of bioactivation was also reflected in the significantly ($p < 0.001$) lower irreversible binding of DFAQ and HDFAQ in the presence of PMA. No stable metabolites of either derivative could be detected by HPLC. Thus, the 4′-fluoro group blocks not only the hepatic bioactivation of amodiaquine [20], but also activation within the granulocytes. A hydroxyl group was incorporated into one of the ethyl side chains of DFAQ to provide a moiety for glucuronidation. It has been demonstrated that introduction of a hydroxyl group into chloroquine decreases the accumulation of the parent compound by enabling formation of a glucuronide, which increases the rate of excretion in the rat [31]. Thus, the slight bioactivation of HDFAQ observed *in vitro* may be reduced still further *in vivo* by clearance through glucuronidation, which would preclude phase I activation.

In conclusion, we have shown that both amodiaquine and its major plasma metabolite, desethylamodiaquine, undergo extensive bioactivation by PMA-stimulated PMN to quinoneimines, which results in glutathione depletion, glutathione conjugate formation, irreversible protein binding, and ring excision to a hitherto unknown metabolite of amodiaquine, 4-amino-7-chloroquinoline (Fig. 7). Substitution of the 4′-hydroxyl group in the molecule with a fluorine atom significantly reduces the irreversible binding and abolishes the glutathione depletion in PMN observed with amodiaquine. Such fluorinated compounds will be of use to investigate further the role of bioactivation in the immunotoxicity of amodiaquine.Furthermore, since 4′-fluoro-substituted amodiaquine analogues are active *in vitro* against the malaria parasite *Plasmodium falciparum* [20], these compounds may provide a lead for the synthesis of safer antimalarials.

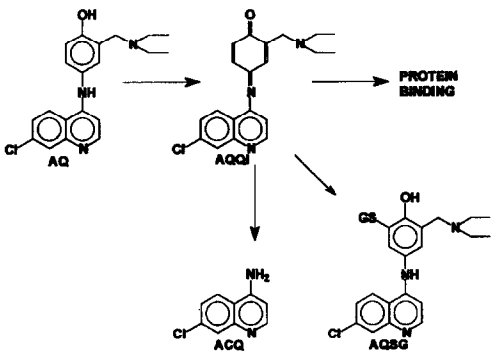

Fig. 7. Scheme for the metabolism of amodiaquine (AQ, $C_{20}H_{22}N_3OCl$, $[M + 1]^+$ m/z 356) by activated PMN *in vitro* to yield amodiaquine quinoneimine (AQQI, $C_{20}H_{20}N_3OCl$, $[M + 1]^+$ m/z 354) that can be hydrolysed to 4-amino-7-chloroquinoline (ACQ, $C_9H_7N_2Cl$, $[M + 1]^+$ m/z 179), react with protein, or form the C-5′glutathione conjugate of amodiaquine (AQSG, $C_{30}H_{37}N_6O_7SCl$, $[M + 1]^+$ m/z 661).

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