ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1996, p. 2345–2349 Vol. 40, No. 10
0066-4804/96/$04.00+0

Manipulation of the *N*-Alkyl Substituent in Amodiaquine To Overcome the Verapamil-Sensitive Chloroquine Resistance Component

SHAUN R. HAWLEY, PATRICK G. BRAY, PAUL M. O'NEILL, DEAN J. NAISBITT, B. KEVIN PARK, AND STEPHEN A. WARD*

Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, L69 3BX, United Kingdom

Received 9 May 1996/Returned for modification 3 July 1996/Accepted 8 August 1996

Aminoquinoline resistance correlates with lipid solubility at pH 7.2. Consequently, the in vivo dealkylation of amodiaquine, to the less lipid-soluble desethylamodiaquine, is a likely contributor to therapeutic failure in vivo. Therefore, 4-aminoquinoline drugs with lipid solubilities similar to that of amodiaquine, but which are not subject to side chain modification in vivo, should be superior antimalarial agents. In this study, we have identified amopyroquine and *N*-tertbutylamodiaquine as two such compounds. The values for the logarithms of the partition coefficients for amopyroquine and *N*-tertbutylamodiaquine are between those for amodiaquine and its dealkylated metabolite, desethylamodiaquine. Both amopyroquine and *N*-tertbutylamodiaquine possess levels of antimalarial activity greater than that of desethylamodiaquine and significantly reduced cross-resistance patterns; i.e., the former two compounds are not subject to the verapamil-sensitive resistance mechanism. Simple in vitro markers of direct toxicity and potential reactive metabolite formation suggest that these two compounds are no more toxic than amodiaquine and desethylamodiaquine.

The successful chemotherapy of human malaria infection is becoming increasingly difficult because of the widespread problem of parasite resistance to currently available antimalarial drugs (40). Parasite resistance to quinoline-containing antimalarial drugs such as chloroquine (CQ) is characterized by an inability of resistant parasites to accumulate as much drug, at steady state, as their susceptible counterparts (3, 13, 22, 38). This resistance phenotype shares similarities with mammalian multidrug resistance, the most striking of which is the fact that resistance can be reversed, at least partially, by a number of chemosensitizers, such as verapamil (VP) (2, 25, 27). One explanation for this reduced accumulation of drug is argued to be that the presence of an efflux process actively extrudes preaccumulated drug from within the parasite (22). From analogy with mammalian multidrug resistance systems, chemosensitizers such as VP would exert their effects by competing with the antimalarial drug for active drug efflux from within the parasite (14, 30, 34).

In comparison with CQ, the 4-aminoquinoline amodiaquine (AQ) (Fig. 1) exhibits greater parasite-specific accumulation and activity (12, 20) and a markedly reduced cross-resistance pattern in vitro (5, 15, 17, 33). These differences result from the fact that unlike CQ, AQ is a very poor substrate for the VP-sensitive resistance component. As such, VP, at concentrations which increase CQ accumulation and activity in resistant isolates, has no measurable effect on AQ accumulation or activity (4). However, AQ is extensively metabolized in vivo to its primary metabolite, desethylAQ (DesAQ) (Fig. 1) (8), which unlike AQ is a substrate for the VP-sensitive resistance component (4). DesAQ is therefore markedly less active against CQ-resistant isolates (4, 6). This probably explains why the superior in vitro characteristics of AQ over CQ are not always reflected in its in vivo antiparasitic efficacy and also why AQ resistance in vivo is not uncommon (24, 28, 32). We have previously put forward a hypothesis, based on the physicochemical properties of the aminoquinolines, which enables the identification of substrates of the VP-sensitive resistance component (4). This model predicts that the degree of parasite resistance would be greatest with CQ, less with DesAQ, and still less with AQ, because the lipophilicity of these compounds at pH 7.2 decreases in the order AQ, DesAQ, CQ. Therefore, the in vivo dealkylation of AQ by decreasing the lipid solubility of the compound converts a drug showing limited cross-resistance with CQ, namely, AQ, to a compound with a cross-resistance pattern which might limit efficacy in vivo, namely, DesAQ.

On the basis of the considerations outlined above, we have selected amopyroquine (AMOPYRO) and *N*-tertbutylamodiaquine (NTBAQ) (Fig. 1) compounds which should demonstrate pharmacological advantages over both AQ and CQ in vivo, for biological evaluation. Both drugs are predicted to show lipophilicity similar to that of AQ at pH 7.2 and therefore should be poor substrates for the VP-sensitive component of aminoquinoline resistance. Furthermore, the chemical alterations introduced into these molecules, i.e., an *N*-tertbutyl or pyrrolidine group, are metabolically less labile than the *N*-ethyl group of AQ (29, 31, 39), thus reducing the risk of metabolic conversion in vivo to metabolites with reduced lipid solubility and greater cross-resistance with CQ.

MATERIALS AND METHODS

Drugs used. AQ and VP were purchased from Sigma, Dorset, United Kingdom. DesAQ and AMOPYRO were obtained from Parke Davis Ltd., Hampshire, United Kingdom. NTBAQ was synthesized as follows.

4-Hydroxy-3-*tert*-butylaminomethylacetanilide. 4-hydroxyacetanilide (1.00 g, 6.6 mmol) was refluxed with *tert*-butylamine (0.73 g, 10 mmol) and aqueous formaldehyde (1.25 g, 14 mmol; 33% [wt/vol]) in ethanol (50 ml) for 24 h. The solvent was evaporated, and the product was purified by flash column (silica) chromatography using 1 to 15% methanol–dichloromethane as the eluent. The product was obtained as a white solid: ^{1}H nuclear magnetic resonance ($CDCl_3$, 200 MHz) δ 7.25 (1 H, s, NH), 7.20 (1 H, d, $J_{H\text{-}H}$ = 2.20 Hz, Ar-H), 7.02 (1 H,

* Corresponding author. Mailing address: Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, United Kingdom. Phone: 0151-794-8219. Fax: 0151-794-5540. Electronic mail address: saward@liverpool.ac.uk.

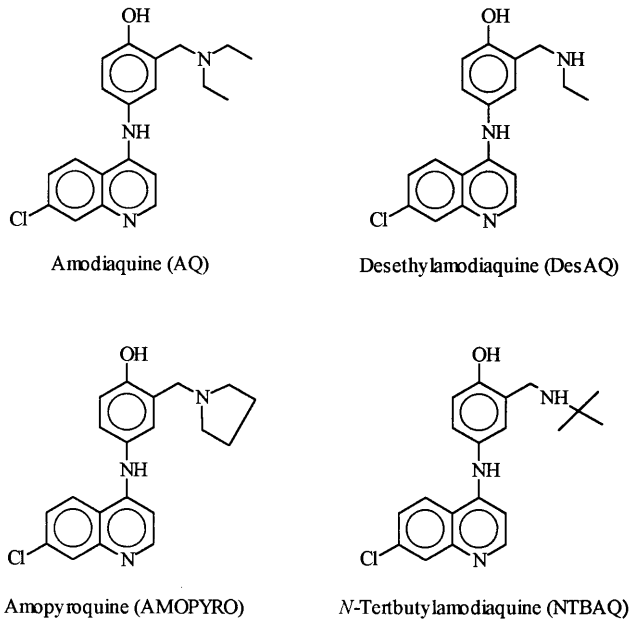

FIG. 1. Chemical structures of the compounds used in this study.

dd, $J_{H\text{-}H}$ = 8.80 and 2.20 Hz), 6.71 (1 H, d, $J_{H\text{-}H}$ = 8.80 Hz, Ar-H), 3.92 (2 H, s, $\underline{CH_2}N$), 2.13 (2 H, s, $NHCO\underline{CH_3}$), 1.20 (9 H, s, t-Bu); LC-MS (5 μM C_{18} column) (50:50 CH_3CN–CH_3COOH) *m/z* 297 (M^+ + AcH + 1, 18%), 278 (M^+ + AcCN + 1, 30%), 237 (M^+ + 1, 100%); infrared (CH_2Cl_2), 3,056, 2,988, 1,684, 1,534, 1,497, 1,421, 1,368, 1,265, 1,210, 896.

7-Chloro-4-(3′-*tert*-butylaminomethyl-4′-hydroxyanilino)quinoline (NTBAQ). The acetanilide (1.00 g, 4.2 mmol) was heated under reflux in 40% HCl (5 ml) for 1.5 h. Solvent was removed and the residue was dissolved in ethanol (20 ml). The pH was adjusted to 5.5 with aqueous ammonia, 4,7-dichloroquinoline (0.72 g, 4 mmol) was added, and the mixture was refluxed for 24 h. The solvent was evaporated under reduced pressure, and the product was purified by flash column (silica) chromatography using methanol-dichloromethane (1:4, vol/vol) as the eluent (0.71 g, 47% overall yield) as a yellow foam: ^1H nuclear magnetic resonance ($CDCl_3$, 200 MHz) δ 8.48 (1 H, d, $J_{H\text{-}H}$ = 5.50 Hz, Ar-H), 8.02 (1 H, d, $J_{H\text{-}H}$ = 2.20 Hz, Ar-H), 7.82 (1 H, d, $J_{H\text{-}H}$ = 8.80 Hz, Ar-H), 7.42 (1 H, dd, $J_{H\text{-}H}$ = 9.35 and 2.20 Hz, Ar-H), 7.10 (1 H, dd, $J_{H\text{-}H}$ = 8.25 and 2.20 Hz, Ar-H), 6.95 (1 H, d, $J_{H\text{-}H}$ = 2.20 Hz, Ar-H), 6.89 (1 H, d, $J_{H\text{-}H}$ = 8.25 Hz, Ar-H), 6.60 (1 H, d, $J_{H\text{-}H}$ = 5.50 Hz, Ar-H), 3.97 (2 H, s, $\underline{CH_2}N$), 1.25 (9 H, s, t-Bu); LC-MS (direct injection) (30:70 CH_3CN-CH_3COOH) *m/z* 356 (M^+ + 1, 100%), 300 (18%), 283 (6%); infrared (CH_2Cl_2), 3,045, 2,988, 1,490, 1,421, 1,330, 1,265, 1,215. NTBAQ requires C = 67.50, H = 6.23, N = 11.81. Found C = 67.20, H = 6.10, N = 11.60.

Parasite isolates: maintenance and preparation. Four isolates of *Plasmodium falciparum* were used in this study. 3D7 and HB3, CQ-susceptible isolates, and K1, a CQ-resistant isolate, were obtained from D. Walliker, University of Edinburgh, Edinburgh, United Kingdom. PH3, a CQ-resistant isolate, was obtained from M. Hommel, Liverpool School of Tropical Medicine, Liverpool, United Kingdom. Cultures, which were maintained by an adaptation of the method of Jensen and Trager (21), consisted of a 1 to 5% suspension of O-positive erythrocytes in complete culture medium (RPMI 1640 medium supplemented with 10% human AB serum, 25 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] buffer, and 23 mM $NaHCO_3$). These cultures were gassed with an atmosphere of 93% N_2, 3% CO_2, and 4% O_2. Cultures were synchronized by the method of Lambros and Vandenburg (23) 48 h before use.

Drug susceptibility assays. Drug potency was assessed by an adaptation of the standard 48-h microdilution technique described by Desjardins et al. (11). Parasites were exposed to serial dilutions of drugs, in the presence and absence of 5 μM VP, over 48 h, and growth was assessed by comparing the level of incorporation of [G-^{3}H]hypoxanthine by the parasites, at each drug concentration, with that of an appropriate control.

Measurement of drug lipophilicity. The relative lipophilicity, the logarithm of the partition coefficient, *D*, at pH 7.2, of each compound used in this study, was assessed as described by Zamora et al. (41) with minor modification. High-performance liquid chromatography (HPLC)-grade 1-octanol (Sigma) was presaturated with aqueous-phase buffer (0.1 M phosphate-buffered saline [PBS], pH 7.2), and conversely, buffered aqueous phase was presaturated with HPLC-grade 1-octanol before use. Each drug was dissolved in aqueous-phase buffer at a final concentration of 1×10^{-4} M, an equal volume of 1-octanol was added, and the tubes were then continuously inverted for 15 min. The final concentrations of drugs in both aqueous and octanol fractions were assessed by comparing the UV A_{340} with the absorbance of known drug standards. The partition coefficient, *D*, was determined by dividing the concentration of drug in 1-octanol by the concentration in the aqueous phase. Log *D* was used as a measure of lipophilicity.

Measurement of drug accumulation and absolute drug activity by inoculum effect analysis. It has been shown previously that, as a consequence of their high level of accumulation within the malaria parasite, the aminoquinoline antimalarial agents are subject to a significant inoculum effect (5, 16). As a result, the measured 50% inhibitory concentration (IC_{50}) of the drug increases as inoculum size increases (where inoculum size = parasitemia × hematocrit) because of drug depletion from the medium. In this study, the drug potency of the compounds studied was assessed at inoculum sizes ranging from 1 to 10 (fractional parasite volume, 0.0001 to 0.001). Over this range, the relationship between the measured IC_{50} of the drug and inoculum size is linear. Extrapolation of this line to an inoculum size of zero provides a measure of absolute drug potency based on the following equation: measured IC_{50} = absolute IC_{50} + (absolute IC_{50} × accumulation ratio × fractional volume of PRBC), where PRBC stands for parasitized erythrocytes. Furthermore, we have previously validated the use of this mathematical relationship in the determination of the cellular drug accumulation ratio (CAR) based on the following equation (16): CAR = (measured IC_{50} − absolute IC_{50})/(absolute IC_{50} × fractional volume of PRBC), where PRBC stands for parasitized erythrocytes and CAR is the ratio of the amount of drug in the infected cell pellet to the amount of drug in a similar volume of medium. This approach circumvents the need for direct measurement of accumulation using radioactively labelled drug.

Isolation of peripheral blood leukocytes. Human polymorphonuclear leukocytes (PMN) were isolated from venous blood of healthy male volunteers (21 to 40 years). Blood was layered onto a dual-density gradient of Lymphoprep (4 ml; Sigma) and Monopoly resolving medium (8 ml; Sigma). Following centrifugation (1,000 × *g*, 45 min), the band of PMN was removed from the resolving medium and washed with PBS (pH 7.4). Erythrocyte contamination was eliminated by repeating the resolving procedure. Purified PMN were resuspended in Dulbecco's PBS (pH 7.4; Sigma). This procedure resulted in a 99% yield of PMN with 95% viability as determined by the Wright staining technique.

Determination of direct cellular toxicity. Direct cytotoxicity was determined by trypan blue exclusion (35). Isolated PMN (10^6 cells ml^{-1}) were incubated in Dulbecco's PBS (Sigma) (37°C; 1 h) in the presence and absence of drug. After incubation, the cells were sedimented and resuspended in drug-free Dulbecco's PBS. Cell viability was then assessed microscopically by comparing the percentage of test cells able to exclude trypan blue (0.2% [wt/vol]) with those of control incubations.

Determination of depletion of intracellular glutathione. Isolated PMN were suspended in Dulbecco's PBS, pH 7.4 (37°C; 1 h), at a density of 0.5×10^6 cells ml^{-1}, in the presence and absence of both phorbol-12′-myristate-13′-acetate (10 ng ml^{-1}) and various concentrations of the drug under study. Glutathione (GSH) levels were determined by the method of Cotgreave and Moldeus (10) as described previously. Bromobimane (3 mM) in *N*-ethyl morpholine (3 mM; pH 8.0) was added to each incubation mixture, and these incubation mixtures were then left in the dark for 5 min. Protein was precipitated with trichloroacetic acid (100%; 10 μl) and sedimented by centrifugation (1,000 × *g*; 5 min). GSH adducts were separated by gradient HPLC as described previously (37) and were detected with a Hitachi 1080 fluorescence detector (excitation wavelength = 304 nm; emission wavelength = 480 nm). The amount of GSH adduct formed was calculated by comparison with known standards. Eugenol (4-allyl-2-methoxyphenol), which has been shown to deplete intracellular GSH (36), was used (at concentrations ranging from 1 to 300 μM) as a positive control.

RESULTS

As predicted, both AMOPYRO and NTBAQ were more lipophilic than DesAQ at pH 7.2 but were less lipophilic than AQ (Table 1). In agreement with the original hypothesis, the ability of VP to improve antiparasitic potency against CQ-resistant parasites (as shown in Table 1) could be predicted on the basis of this measure of lipophilicity. These data are presented as an activity enhancement index (AEI). This is the ratio of the IC_{50} of the drug in the absence of VP to the IC_{50} of the drug in the presence of VP (5 μM). It can be seen that the ability of VP to enhance activity is inversely related to log *D* at pH 7.2 (Fig. 2). VP can substantially improve the activity of DesAQ against resistant isolates (AEI = 2.40 and 2.63 for the K1 and PH3 isolates, respectively). This effect is markedly reduced with the more lipophilic NTBAQ isolates (AEI = 1.16 and 1.24 for the K1 and PH3 isolates, respectively) and is absent with AMOPYRO and AQ (for which AEI is <1 for both drugs against both the K1 and PH3 isolates [Table 1]).

TABLE 1. Summary of data for the five antimalarial agents and four *P. falciparum* isolates used in this study

Antimalarial agent	Absolute IC_{50} (nM)				Experimentally derived CAR[a]				Log *D*	AEI with VP (5 μM)	
	3D7	HB3	K1	PH3	3D7	HB3	K1	PH3		K1	PH3
AQ	2.40	3.70	16.8	10.5	8,955	12,613	3,653	4,824	2.60	0.89	0.99
DesAQ	3.26	2.93	36.9	34.8	19,250	17,443	3,758	4,543	1.18	2.40	2.63
NTBAQ	1.67	2.73	7.21	6.85	47,171	21,081	14,694	13,948	1.78	1.16	1.24
AMOPYRO	0.84	1.74	4.57	4.07	48,173	39,560	12,994	19,556	1.89	0.96	0.97
CQ	12.1	15.4	189	152							

[a] Derived from inoculum effect analysis.

The absolute IC_{50}s of each drug against each of the four isolates used are displayed in Table 1. Each of these values was calculated from a single inoculum effect experiment involving triplicate drug susceptibility assays at five separate inoculum sizes. In each case, the standard error associated with the slope of the linear fit of the IC_{50} of the drug versus the inoculum size was always less than 5% and *r* was greater than 0.96. The absolute drug potency of each of the four drugs against four isolates of *P. falciparum* was found to correlate with the CAR, derived from inoculum effect analysis (Table 1). This observation confirms earlier studies (19). Clearly DesAQ shows the greatest degree of cross-resistance with CQ susceptibility status. Despite having activity comparable to that of AQ against CQ-susceptible isolates, DesAQ is approximately two- to threefold less active than AQ against resistant isolates. In comparison with AQ, AMOPYRO and NTBAQ have superior antimalarial activities against both CQ-resistant and -susceptible isolates, although the degrees of in vitro cross-resistance for all three drugs are similar (Table 1). Importantly, NTBAQ and AMOPYRO, which are less likely than AQ to undergo side chain modification in vivo, show five- to eightfold improvements in activity compared with the in vivo metabolite of AQ, DesAQ, against resistant parasite isolates (Table 1).

Previous studies designed to assess the toxicity of AQ have used PMN in vitro as a marker for toxicity in vivo (37). Figure 3A shows the effects of the compounds studied on the viability of PMN. It can be seen that all four of the compounds studied show little direct toxicity to PMN at concentrations of less than 50 μM. Both NTBAQ and AMOPYRO displayed greater toxicities at concentrations of greater than 50 μM than did AQ and DesAQ. Furthermore, none of the four drugs tested exerted any significant effect on cell recovery at concentrations of less than 50 μM (Fig. 3B).

All four of the drugs studied exhibited similar effects on the level of intracellular GSH in activated PMN (Fig. 4). Indeed, all four compounds depleted intracellular GSH levels at concentrations in excess of 1 μM, with maximal GSH depletion occurring at approximately 100 μM.

DISCUSSION

The growing problem of parasite resistance to CQ emphasizes the need to develop novel compounds of this class for the treatment of *P. falciparum*. The 4-aminoquinoline antimalarial agent AQ is highly effective against both CQ-susceptible and -resistant parasites in vitro, although its potency against resistant parasites in vivo is reduced (24, 28, 32). The reduction in AQ's potency against resistant isolates in vivo is due to the fact that the drug rapidly undergoes first-pass metabolism to DesAQ. This metabolite is less active against CQ-resistant isolates than against the parent drug (4, 6), because DesAQ is a better substrate for the VP-sensitive component of CQ resistance as a result of decreased lipophilicity at pH 7.2 (4).

We have therefore reasoned that a 4-aminoquinoline analog

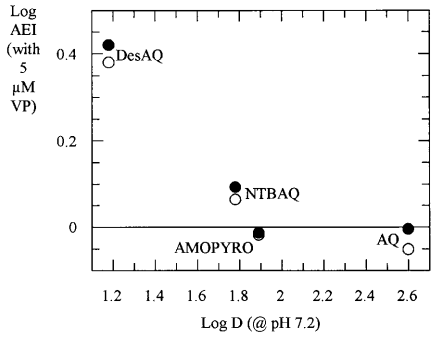

FIG. 2. The relationship between log *D* (at pH 7.2) and log VP AEI for AQ, DesAQ, AMOPYRO, and NTBAQ against the K1 (○) (r^2 = 0.77) and PH3 (●) (r^2 = 0.70) CQ-resistant isolates.

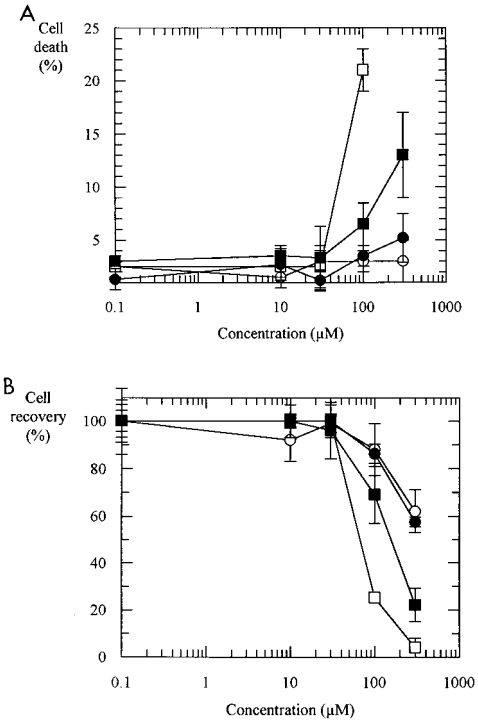

FIG. 3. Effects of AQ (○), DesAQ (●), AMOPYRO (■), and NTBAQ (□) on the viability (A) and cell recovery (B) of human PMN. Data are means ± standard deviations (error bars) from at least three separate observations.

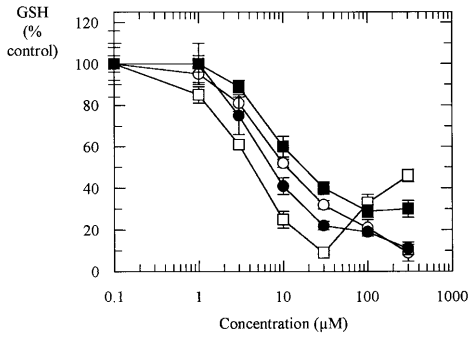

FIG. 4. Effects of AQ (○), DesAQ (●), AMOPYRO (■), and NTBAQ (□) on the level of intracellular GSH in activated human PMN. Data are means ± standard deviations (error bars) from at least three separate observations.

which has physicochemical properties similar to those of AQ but is metabolically less labile and so cannot be metabolized in vivo to a more water-soluble metabolite would be superior to both CQ and AQ in the treatment of *P. falciparum* malaria in vivo. AMOPYRO and NTBAQ possess these superior structural and physicochemical properties. Both AMOPYRO and NTBAQ show greater levels of lipophilicity than does DesAQ at pH 7.2.

Consistent with previous studies (4, 6), it was found that DesAQ is less active against resistant isolates than is AQ and that VP can selectively increase the activity of DesAQ against these parasites (Table 1). In contrast, the effects of VP on the activity of AMOPYRO and NTBAQ are less pronounced than with the more hydrophilic DesAQ. The activity of AMOPYRO was not influenced by VP, and NTBAQ activity was only slightly increased, by 14 and 19%, against the K1 and PH3 isolates, respectively. These data are consistent with previous findings from this laboratory showing an inverse relationship between lipid solubility characteristics and the VP-sensitive component of resistance (4).

AMOPYRO and NTBAQ show greater levels of activity than both AQ and DesAQ in vitro, although the differences seen with the CQ-susceptible parasite would be of limited importance in a clinical setting. However, against resistant isolates the activities of both AMOPYRO and NTBAQ are clearly superior to that of DesAQ. The observation that the activity of AMOPYRO is similar to that of DesAQ against CQ-susceptible isolates but greatly increased compared with that of DesAQ against CQ-resistant isolates in vitro is consistent with earlier reports (1). AQ exhibits an approximately fivefold reduction in activity against CQ-resistant isolates compared with that against CQ-susceptible isolates. This compares to an approximately 10-fold reduction in the potency seen with DesAQ. AQ's activity, which is enhanced compared with that of DesAQ, is almost certainly due to its reduced affinity for the VP-sensitive resistance mechanism; however, the fact that there is still a fivefold reduction in the activity of AQ against resistant isolates indicates that there could be additional factors involved in the CQ resistance phenotype. Similarly, both NTBAQ and AMOPYRO show an approximately fivefold reduction in activity against resistant isolates; however, these compounds are still between five- and eightfold more potent than DesAQ against these isolates. The differences in activity reported here can be explained in terms of parasite drug accumulation as has been seen with other aminoquinoline drugs (19).

All four drugs in this study exhibited limited toxicity against PMN, which was observed only at high concentrations, i.e., in excess of 50 μM. Several studies have supported the role of oxidative bioactivation to a quinoneimine metabolite as the basis for the side effects, such as hepatotoxicity and agranulocytosis, associated with the prophylactic use of AQ (7, 9, 18). Drug bioactivation to a chemically reactive metabolite can be assessed by monitoring intracellular GSH depletion from activated PMN in vitro (37). Both AMOPYRO and NTBAQ depleted GSH, and therefore it is possible that these compounds also have the potential to form toxic metabolites in vivo. However, previous work from this laboratory has shown that by substitution of fluorine for the hydroxyl group of AQ, it is possible to block the metabolic oxidation of AQ to a quinoneimine, while retaining antimalarial drug potency (26). Such an approach could be of value if a similar toxicity were indeed associated with AMOPYRO and/or NTBAQ. Thus, by the appropriate substitution of both the diethylamine side chain and the hydroxyl moiety, it might be possible to eliminate the problems associated with resistance to and toxicity of AQ in vivo.

In summary, on the basis of an understanding of the pharmacology of both CQ and AQ we have identified two compounds, AMOPYRO and NTBAQ, which have potent antimalarial activity and reduced cross-resistance with CQ. Furthermore, previous studies have indicated that both *N*-tertbutyl (31)- and pyrrolidine (29, 39)-substituted 4-aminoquinolines are metabolically less labile than AQ and are therefore less likely to undergo bioactivation to an *N*-dealkylated metabolite. These two studies found that the major metabolite of AMOPYRO in humans, namely, the primary amine, accounts for less than 2% of the total dose and that AMOPYRO is excreted essentially unchanged (29, 39). This means that both AMOPYRO and NTBAQ are unlikely to be metabolized to a species that is a substrate for the VP-sensitive component of resistance. Therefore, these pyrrolidine- and *N*-tertbutyl-substituted compounds should retain antimalarial activity against resistant parasites in vivo.

ACKNOWLEDGMENTS

S.R.H. is in receipt of an MRC studentship. S.A.W. is in receipt of a Wellcome Trust Study Leave Fellowship. B.K.P. is a Wellcome Trust Principal Research Fellow. This work was supported by a Wellcome Trust Programme Grant.

REFERENCES

1. **Basco, L. K., and J. Le Bras.** 1993. *In vitro* activity of monodesethylamodiaquine and amopyroquine against African isolates and clones of *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. **48:**120–125.
2. **Bitonti, A. J., A. Sjoerdsma, P. P. McCann, D. E. Kyle, A. M. J. Oduola, R. N. Rossan, W. K. Milhous, and D. E. Davidson.** 1988. Reversal of chloroquine resistance in malaria parasite *Plasmodium falciparum* by desipramine. Science **242:**1301–1302.
3. **Bray, P. G., M. K. Boulter, G. Y. Ritchie, and S. A. Ward.** 1994. Relationship of global chloroquine transport and reversal of chloroquine resistance in *P. falciparum*. Mol. Biochem. Parasitol. **63:**87–94.
4. **Bray, P. G., S. R. Hawley, M. Mungthin, and S. A. Ward.** Physico-chemical properties correlated with drug resistance and the reversal of drug resistance in *Plasmodium falciparum*. Mol. Pharmacol., in press.
5. **Bray, P. G., S. R. Hawley, and S. A. Ward.** 4-Aminoquinoline resistance of *Plasmodium falciparum*: insights from the study of amodiaquine uptake. Mol. Pharmacol., in press.
6. **Childs, G. E., E. F. Boudreau, W. K. Milhous, T. Wimonwattratee, N. Pooyindee, L. Pang, and D. E. Davidson, Jr.** 1989. A comparison of the *in vitro* activities of amodiaquine and desethylamodiaquine against isolates of *Plasmodium falciparum*. Am. J. Trop. Med. Hyg. **40:**7–11.
7. **Christie, G., A. M. Breckenridge, and B. K. Park.** 1989. Production of antibodies towards amodiaquine and amodiaquine quinoneimine in man and in the rat. Biochem. Pharmacol. **38:**1451–1458.
8. **Churchill, F. C., L. C. Patchen, C. C. Campbell, I. K. Schwartz, P. Nguyen-Dinh, and C. M. Dickinson.** 1984. Amodiaquine as a prodrug: importance of metabolite(s) in the antimalarial effect of amodiaquine in humans. Life Sci. **36:**53–62.

9. **Clarke, J. B., J. L. Maggs, N. K. Kitteringham, and B. K. Park.** 1990. Immunogenicity of amodiaquine in the rat. Int. Arch. Allergy Appl. Immunol. **91:**335–342.
10. **Cotgreave, I. A., and P. Moldeus.** 1986. Methodologies for the application of monobromobimane to the simultaneous analysis of soluble and protein thiol components of biological systems. J. Biochem. Biophys. Lett. **13:**231–249.
11. **Desjardins, R. E., J. Canfield, D. Haynes, and J. D. Chulay.** 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob. Agents Chemother. **16:**710–718.
12. **Ekweozor, C., A. A. Fadeke, and O. Sodeinde.** 1985. Comparison of the relative *in vitro* activity of chloroquine and amodiaquine against chloroquine-sensitive strains of *P. falciparum*. Ann. Trop. Med. Parasitol. **81:**95–99.
13. **Fitch, C. D., N. G. Yunis, R. Chevli, and Y. Gonzalez.** 1974. High-affinity accumulation of chloroquine by mouse erythrocytes infected with *Plasmodium berghei*. J. Clin. Invest. **54:**24–33.
14. **Fojo, A. T., S. Aikiyama, M. M. Gottesman, and I. Pastan.** 1985. Reduced drug accumulation in multiple drug-resistant human KB carcinoma cell lines. Cancer Res. **45:**3002–3007.
15. **Geary, T. G., A. A. Divo, and J. B. Jensen.** 1987. Activity of quinoline-containing antimalarials against chloroquine sensitive and resistant strains of *Plasmodium falciparum in vitro*. Trans. R. Soc. Trop. Med. Hyg. **81:**499–503.
16. **Geary, T. G., A. D. Divo, J. B. Jensen, M. Zangwill, and H. Ginsburg.** 1990. Kinetic modelling of the response of *Plasmodium falciparum* to chloroquine and its experimental testing *in vitro*. Implications for mechanism of action and resistance to the drug. Biochem. Pharmacol. **40:**685–691.
17. **Geary, T. G., and J. B. Jensen.** 1983. Lack of cross resistance to 4-aminoquinolines in chloroquine-resistant *Plasmodium falciparum*. J. Parasitol. **69:** 97–105.
18. **Harrison, A. C., N. R. Kitteringham, J. B. Clarke, and B. K. Park.** 1992. The mechanism of bioactivation and antigen formation of amodiaquine in the rat. Biochem. Pharmacol. **43:**1421–1430.
19. **Hawley, S. R., P. G. Bray, P. M. O'Neill, B. K. Park, and S. A. Ward.** The role of drug accumulation in 4-aminoquinoline antimalarial potency: the influence of structural substitution and physicochemical properties. Biochem. Pharmacol., in press.
20. **Hawley, S. R., P. G. Bray, B. K. Park, and S. A. Ward.** Amodiaquine accumulation in *Plasmodium falciparum* as a possible explanation for its superior antimalarial activity over chloroquine. Mol. Biochem. Parasitol., in press.
21. **Jensen, J. B., and W. Trager.** 1977. *Plasmodium falciparum* in culture: use of outdated erythrocytes and description of the candle-jar method. J. Parasitol. **63:**883–886.
22. **Krogstad, D. J., I. Y. Gluzman, D. E. Kyle, A. M. J. Oduola, S. K. Martin, W. K. Milhous, and P. H. Schlesinger.** 1987. Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. Science **235:** 1283–1285.
23. **Lambros, C., and J. P. Vandenburg.** 1979. Synchronisation of *Plasmodium falciparum* erythrocytic stages in culture. J. Parasitol. **65:**418–420.
24. **Le Bras, J., and J. P. Coulaud.** 1986. Evolution de la chimiorésistance de *Plasmodium falciparum* en Afrique entre 1983 et 1985. Med. Hyg. **44:**618–625.
25. **Martin, S. K., A. M. J. Oduola, and W. K. Milhous.** 1987. Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. Science **235:** 899–901.
26. **O'Neill, P. M., A. C. Harrison, R. C. Storr, S. R. Hawley, S. A. Ward, and B. K. Park.** 1994. The effect of fluorine substitution on the metabolism and antimalarial activity of amodiaquine. J. Med. Chem. **37:**1362–1370.
27. **Peters, W., R. Ekong, B. L. Robinson, D. C. Warhurst, and X.-Q. Pan.** 1989. Antihistaminic drugs that reverse chloroquine resistance in *Plasmodium falciparum*. Lancet **ii:**334–335.
28. **Pinichpongse, S., E. B. Doberstyn, J. R. Cullen, L. Yisunsri, Y. Thongsombum, and K. Thimsaran.** 1982. An evaluation of five regimens for the outpatient therapy of malaria in Thailand 1980–81. Bull. W. H. O. **60:**907–912.
29. **Pussard, E., D. Chassard, F. Clavier, P. Bry, and F. Verdier.** 1994. Pharmacokinetics and metabolism of amopyroquine after administration of two doses of 6 mg/kg i.m. 24 h apart to healthy volunteers. J. Antimicrob. Chemother. **34:**803–808.
30. **Rogan, A. M., T. C. Hamilton, R. C. Young, R. W. Klecker, Jr., and R. F. Ozols.** 1984. Reversal of adriamycin resistance by verapamil in human ovarian cancer. Science **244:**994–996.
31. **Ruscoe, J. E.** 1996. Ph.D. thesis. University of Liverpool, Liverpool, United Kingdom.
32. **Schmidt, L. H., D. Vaughan, D. Mueller, R. Crosby, and R. Hamilton.** 1977. Activities of various 4-aminoquinolines against infections with chloroquine-resistant strains of *Plasmodium falciparum*. Antimicrob. Agents Chemother. **11:**826–843.
33. **Scott, H. V., W. L. Tan, and G. B. Barlin.** 1987. Antimalarial activity of mannich bases derived from 4-(7′-bromo-1′,5′-napthyridin-4′-ylamino) phenol and 4-(7′-trifluoromethylquinolin-4′-ylamino) phenol against *Plasmodium falciparum in vitro*. Ann. Trop. Med. Parasitol. **81:**85–93.
34. **Slater, L. M., S. L. Murray, M. W. Wetzel, R. M. Wisdom, and E. M. Duvall.** 1982. Verapamil restoration of daunorubicin-resistant Ehrlich ascites carcinoma. J. Clin. Invest. **70:**1131–1134.
35. **Spielberg, S. P.** 1980. Acetaminophen toxicity in human lymphocytes *in vitro*. J. Pharmacol. Exp. Ther. **213:**395–398.
36. **Thompson, D., D. Constantin-Teodosiu, K. Norbeck, B. Svenssonn, and P. Moldeus.** 1986. Metabolic activation of eugenol by myeloperoxidase and polymorphonuclear leukocytes. Chem. Res. Toxicol. **2:**186–192.
37. **Tingle, M. D., H. Jewell, P. O'Neill, and B. K. Park.** 1995. The effect of fluorine substitution on the bioactivation of amodiaquine by human polymorphonuclear leukocytes *in vitro*. Biochem. Pharmacol. **50:**1113–1119.
38. **Verdier, F., J. Le Bras, F. Clavier, I. Hatin, and M.-C. Blayo.** 1985. Chloroquine uptake by *Plasmodium falciparum*-infected human erythrocytes during in vitro culture and its relationship to chloroquine resistance. Antimicrob. Agents Chemother. **27:**561–564.
39. **Verdier, F., E. Pussard, F. Clavier, J. Le Bras, and C. Gaudebout.** 1989. Pharmacokinetics of intramuscular amopyroquine in healthy subjects and determination of a therapeutic regimen for *Plasmodium falciparum* malaria. Antimicrob. Agents Chemother. **33:**316–321.
40. **Wernsdorfer, W. H., and D. Payne.** 1991. The dynamics of drug resistance in *Plasmodium falciparum*. Pharmacol. Ther. **50:**95–121.
41. **Zamora, J. M., H. L. Pearce, and W. T. Beck.** 1988. Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukaemic cells. Mol. Pharmacol. **33:**454–462.