Biochemical Pharmacology, Vol. 36, No. 13, pp. 2061–2062, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

RAPID COMMUNICATION

AUTOXIDATIVE FORMATION OF A CHEMICALLY REACTIVE INTERMEDIATE FROM AMODIAQUINE, A MYELOTOXIN AND HEPATOTOXIN IN MAN

J. L. MAGGS, N. R. KITTERINGHAM, A. M. BRECKENRIDGE and B. K. PARK*
Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3BX, U.K.

(*Received* 5 *January* 1987; *accepted* 10 *February* 1987)

Amodiaquine [AQ, 7-chloro-4-(3′-diethylamino-4′-hydroxyanilino)quinoline], a 4-aminoquinoline antimalarial [1], has been associated with agranulocytosis and liver damage in man [2–4]. Since the hepatotoxicity of paracetamol (4-hydroxyacetanilide) is considered to be a consequence of metabolism to a reactive semiquinone/quinone imine [5, 6], it seemed possible that AQ's toxicity might stem from an analogous cytochrome P-450-mediated oxidation of the 4-hydroxyanilino moiety (Fig. 1). However, although AQ was extensively de-ethylated by human liver microsomes and formed a reactive derivative which irreversibly bound to protein [7], oxidation by cytochrome P-450 did not appear to be responsible for its metabolic activation. In this communication, we report investigations of the autoxidative formation of reactive intermediates from AQ and indentifications of thioether adducts similar to those derived from paracetamol's reactive metabolite.

Materials and methods

[Quinoline-2-^{14}C]AQ (3.2 mCi/mmol) was synthesized by Amersham International. It was purified to ≥98% radiochemical purity (determined by HPLC [8]) by TLC. Human serum albumin (HSA, Fraction V, 96–99% pure), bovine serum albumin (BSA, Fraction V) and bovine α-casein (Bα-C, *ca.* 70% pure, balance primarily β-casein) were from Sigma Chemical Co. Proteins (2 mg/ml) were incubated with ^{14}C-AQ (10 μM; 0.13 μCi) in 4 ml 0.1 M sodium phosphate, pH 7.4, at 37° for up to 24 hr. Reactions were performed in 25 ml Ehrlenmeyer flasks open to the air. They were stopped by cooling on ice, and unreacted ^{14}C-AQ extracted into Aristar (peroxide-free) ether (5 ml × 2). The protein was precipitated with acetone (1:1, v/v) at 5° over *ca.* 15 hr. Irreversibly-bound radiolabelled material was determined by exhaustive extraction of the precipitate with methanol (up to 5 ml × 6). Extracted protein was dissolved in 1 M NaOH and aliquots removed for protein assay [9] and liquid scintillation counting. The putative quinone imine of AQ (AQQI) was synthesized by oxidation with silver oxide, a method used to prepare the quinone imine of paracetamol (NAPQI) [5]. An orange product was isolated: UV λ_{max} (*n*-hexane) 271 nm (ε 0.23×10^4 M^{-1} cm^{-1}); IR (Nujol) 3,400 (m, OH) and 3,140 (w, NH) of AQ absent, 1,615 (s, quinone imine). *N*-Acetylcysteine was reacted with ^{14}C-AQQI to obtain thioether adducts. They were purified by ion-pair HPLC and characterized by fast atom bombardment (FAB) mass spectrometry and u.v. spectroscopy. Water-soluble compounds formed when ^{14}C-AQ was incubated with *N*-acetylcysteine and HSA in phosphate buffer at 37° for 24 hr were separated on a 10 μm C_{18} column. Gradients of acetonitrile in $NH_4H_2PO_4$ (10 mM, pH 4.6) containing octane sulphonate (5 mM) were used. Eluate was monitored at 254 nm and

* Wellcome Senior Lecturer and author for correspondence.

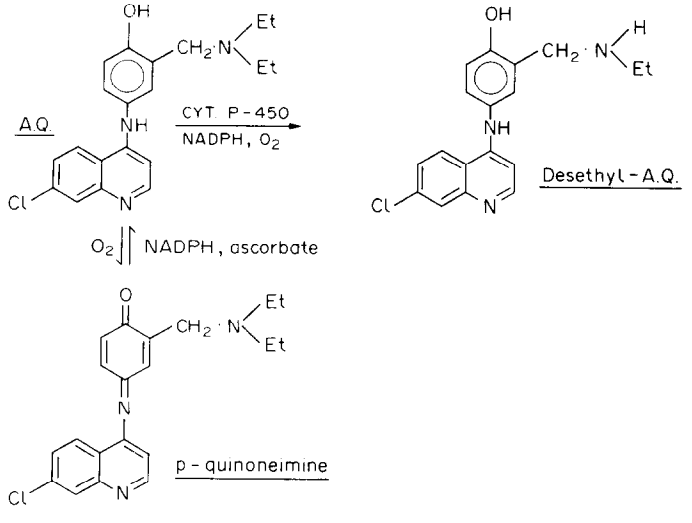

Fig. 1. Metabolism and autoxidation of amodiaquine (AQ) *in vitro*.

Table 1. Inhibition of the irreversible binding of ^{14}C-AQ to HSA during 2 hr incubations. Data derived from means of triplicate determinations of binding in presence and absence of inhibitor

Inhibitor	Irreversible binding (% Control)
N-Acetylcysteine (1 mM)	3
Ascorbate (1 mM)	3
NADPH (1 mM)	13
Reduced glutathione (1 mM)	14
BHT (0.5 mM)	44
N^2-Acetyl-lysine (1 mM)	101

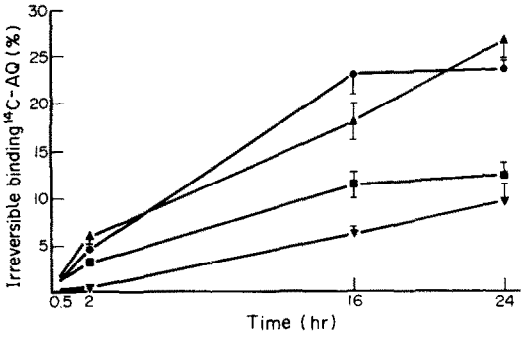

Fig. 2. Irreversible binding of ^{14}C-AQ to HSA (▲), HSA in presence of reduced glutathione (▼), BSA (●) and Bα-C (■). Points are means of triplicate determinations; bars are SD (omitted when <1.0).

collected for radioactivity determination. The compounds' R_f were compared with those of the synthetic compounds.

Results and discussion

^{14}C-AQ in phosphate buffer reacted irreversibly with soluble proteins (Fig. 2). Over 24 hr, 26.5 ± 1.8%, 23.6 ± 0.9% and 12.3 ± 1.5% (means ±SD, N = 3) of incubated radioactivity became irreversibly bound to HSA, BSA and Bα-C, respectively. Material bound to HSA was equivalent to 1.30 ± 0.09 nmol/mg; for a molecular weight of 68,000, this equalled 88 nmol/μmol. The binding was decreased by ascorbate, thiols, butylated hydroxytoluene (BHT) and NADPH but not N^2-acetyl-lysine (Table 1).

Inhibition of binding by glutathione and *N*-acetylcysteine was associated with considerable formation of water-soluble products; in HSA incubations after 24 hr, they represented 18.6 ± 0.7% and 17.3 ± 0.3% of incubated radioactivity, respectively. The corresponding reductions in binding equalled 17% and 21% of incubated radioactivity.

Oxidation of AQ with silver oxide yielded a product having u.v. and i.r. spectra consistent with a quinone imine. Characteristically [10], as revealed by rapid discharge of its dark red colour in chloroform, the product was reduced by ascorbate and reacted with thiols to form water-soluble compounds. Three putative *N*-acetylcysteine adducts, possibly regio-isomers, were obtained. Their u.v. spectra (λ_{max} in methanol, 254–255 nm and 338–344 nm) indicated intact phenyl and aminoquinoline moieties [11]. FAB spectra contained peaks at m/z 517 ($[M + 1]^+$, relative intensity 37–61%), m/z 444 ($[M - N \cdot (C_2H_5)_2]^{+\cdot}$) and m/z 315 (m/z 444–129, loss of *N*-acetylcysteine fragment).

The data indicated extensive autoxidation of AQ to a derivative possessing the characteristics of a quinone imine which selectively reacted with SH groups of proteins. Lower binding to Bα-C, which lacks cysteine residues but otherwise has an amino acid composition similar to that of BSA [12], and deactivation by thiols (nucleophiles), NADPH and ascorbate (reductants) but not amines are seen with paracetamol, NAPQI and other quinone imines [10, 12–14]. Additionally, the major radiolabelled water soluble compound formed in incubations containing ^{14}C-AQ and *N*-acetylcysteine co-chromatographed with the principal thioether adduct of synthetic AQQI. However, inhibition by the radical scavanger BHT suggests involvement of AQ semiquinone imine [5, 12].

AQ readily forms a reactive derivative(s) in aqueous solution. If it were generated in liver and bone marrow cells, its reaction might lead to the cellular damage associated with AQ.

Acknowledgements—We are indebted to Dr P. A. Winstanley for providing purified ^{14}C-AQ. Mass spectra were obtained by Dr D. A. Smith and Mr Z. R. Cybulski, Pharmaceutical Division, Fisons (Loughborough, Leicestershire).

REFERENCES

1. S. Looareesuwan, R. Philips, N. White, J. Karbwang, Y. Banjasurat, P. Attanath and D. Warrell, *Lancet* **ii**, 805 (1985).
2. C. Hatton, T. Peto, C. Bunch, G. Pasvol, S. Russell, C. Singer, G. Edwards and P. Winstanley, *Lancet* **i**, 411 (1986).
3. K. A. Neftel, W. Woodtly, M. Schmid, P. G. Frick and J. Fehr, *Br. med. J.* **292**, 721 (1986).
4. E. G. H. Rhodes, J. Ball and I. M. Franklin, *Br. med. J.* **292**, 717 (1986).
5. D. C. Dahlin and S. D. Nelson, *J. med. Chem.* **25**, 885 (1982).
6. D. C. Dahlin, G. T. Miwa, A. Y. H. U. Lu and S. D. Nelson, *Proc. natn. Acad. Sci. U.S.A.* **81**, 1327 (1984).
7. J. L. Maggs, J. Colbert, P. A. Winstanley, M. L'E. Orme and B. K. Park, *Br. J. clin. Pharmac*, (in press).
8. G. W. Mihaly, D. D. Nicholl, G. Edwards, S. A. Ward, M. L'E. Orme, D. A. Warrell and A. M. Breckenridge, *J. Chromat.* **337**, 166 (1985).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. R. Larsson, D. Ross, M. Nordenskjöld, B. Lindeke, L-I. Olsson and P. Moldéus. *Chem.-Biol. Interact.* **52**, 1 (1986).
11. A. Barrow, *Xenobiotica* **4**, 669 (1974).
12. A. J. Streeter, D. C. Dahlin, S. D. Nelson and T. A. Baillie, *Chem.-Biol. Interact.* **48**, 349 (1984).
13. J. A. Hinson, L. R. Pohl, T. J. Monks, J. R. Gillette and F. P. Guengerich, *Drug. Metab. Disp.* **8**, 289 (1980).
14. D. W. Potter and J. A. Hinson, *Molec. Pharmac.* **30**, 33 (1986).