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IN VITRO EFFECTS OF QUINOLINE DERIVATIVES ON CYTOCHROME P-450 AND AMINOPYRINE *N*-DEMETHYLASE ACTIVITY IN RAT HEPATIC MICROSOMES

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Abstract—A series of quinoline drugs was evaluated for the ability to inhibit rat liver microsomal aminopyrine *N*-demethylase (APDM) activity *in vitro*. Quinine was found to be a quite potent inhibitor of APDM from control rat liver (I_{50} = 0.061 mM) but was only approximately half as potent against APDM from phenobarbitone-induced rat liver (I_{50} = 0.14 mM). Primaquine and amodiaquine were also relatively potent inhibitors of these activities, but quinidine and chloroquine were essentially non-inhibitory, especially against control-type APDM. Primaquine and quinine elicited characteristic type II optical difference spectra with oxidised cytochrome P-450 from both types of microsomes whereas chloroquine and quinidine were type IIb ligands for cytochrome P-450 in phenobarbitone-induced microsomal fractions. Good correlations were obtained for the logarithmic relationship between binding affinity (K_s) and inhibition potency (I_{50}), as well as the logarithmic relationship between efficiency of binding ($\Delta A_{max}/K_s$) and inhibition. These findings suggest that the capacity of quinoline antimalarials, and similar drugs, to inhibit microsomal APDM activity is related to the affinity of the type II spectral binding interaction between the drug and oxidised cytochrome P-450.

A variety of compounds representing several classes of nitrogen heterocycles including imidazoles [1–3], benzimidazoles [4, 5] and quinolines [6, 7] are established inhibitors of microsomal drug oxidation. Numerous drugs have also been documented for their capacity to inhibit drug metabolism. In particular the H_2-receptor antagonist cimetidine [8–10], and antifungal imidazoles such as clotrimazole and ketoconazole [11–13] have been identified as potent inhibitors of mixed-function oxidase (MFO) activity in man and in experimental animals.

A recent study by Back *et al.* [14] demonstrated that the antimalarial quinoline drugs chloroquine and primaquine are inhibitors of microsomal drug oxidation *in vitro* and *in vivo* in the rat. Others have reported that chloroquine treatment alters the sensitivity of rats to aflatoxin and benzo[*a*]pyrene toxicity [15]. It has also been suggested that chloroquine may affect drug oxidation by direct interaction with a component of the MFO system [16]. In view of these observations the present study was undertaken to evaluate several therapeutic drugs based on the quinoline ring system (Fig. 1) as inhibitors of oxidative drug metabolism. In addition the capacity of these compounds to interact with oxidised cytochrome P-450 was evaluated in relation to the MFO inhibition data.

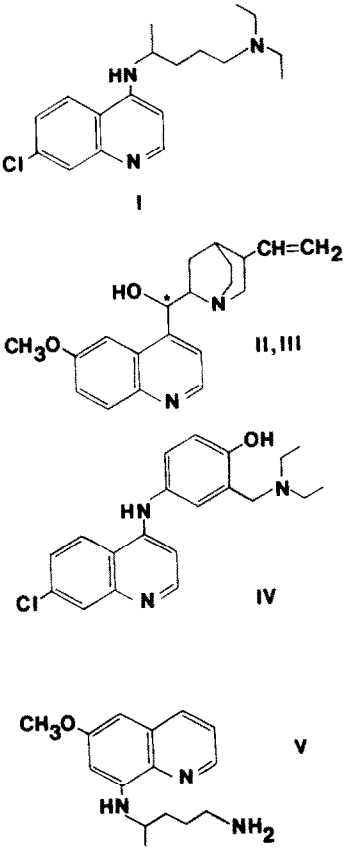

Fig. 1. Structural formulae of drugs used in this study (**I**) chloroquine, (**II** and **III**) quinine and quinidine, (**IV**) amodiaquine, and (**V**) primaquine. The asterisk represents the centre of asymmetry in **II** and **III**.

MATERIALS AND METHODS

Animals. Male Wistar rats (250–300 g) were obtained from the Institute of Clinical Pathology and Medical Research at the Westmead Centre. Phenobarbitone (PB) induction was achieved when appropriate by the intraperitoneal injection of PB (100 mg/kg) on three consecutive days. Induced animals were

sacrificed by cervical dislocation 48 hr after the final exposure to inducer. Livers were immediately removed, perfused with ice cold saline, homogenised, and hepatic microsomes were prepared as described previously [17]. All animals were fasted for at least 12 hr prior to sacrifice. Microsomes were stored as frozen pellets until required for use.

Chemicals. Primaquine diphosphate, chloroquine diphosphate, quinine sulphate, quinidine sulphate and biochemicals were obtained from Sigma Chemical Co., St. Louis, MO. Amodiaquine, originally supplied by Warner-Lambert, was the gift of Dr. D. E. Moore, Pharmacy Department, University of Sydney. All other chemicals and reagents were of analytical reagent grade.

Aminopyrine N-demethylase. Aminopyrine *N*-demethylase (APDM) activity in control and PB-induced rat hepatic microsomal fractions was determined essentially as described previously [4]. However, isocitrate dehydrogenase (1 unit), isocitric acid (5 mM final concentration) and NADP (0.4 mM final concentration) was used as the NADPH-generating system in place of glucose 6-phosphate, glucose 6-phosphate dehydrogenase and NADP.

In both types of microsomal fractions formaldehyde formation was found to be linear for up to at least 15 min and to a substrate concentration of 0.53 mM. Linearity conditions were observed up to 5.6 mg (PB-induced) and 5 mg (control) of microsomal protein.

Binding studies. Microsomal pellets were resuspended in 0.1 M potassium phosphate buffer containing 1 mM EDTA (pH 7.4) to a protein concentration of about 2 mg/ml. The suspension was then divided equally between two cuvettes and a baseline of zero light absorbance was established in an Aminco DW-2a spectrophotometer operating in the split beam mode. Optical difference spectra were obtained as described before [5]. Spectral dissociation constants (K_s) and maximal absorbance changes (ΔA_{max}) were determined from the x-axis and y-axis intercepts, respectively, of double reciprocal plots of ΔOD (peak to trough) vs compound concentration (four to six different concentrations).

Cytochrome P-450 was measured according to the method of Omura and Sato [18] and protein was determined by the method of Lowry *et al.* [19].

RESULTS

The five drugs included in the present study inhibited aminopyrine *N*-demethylase activity from phenobarbitone-induced (PB/APDM) and control (C/APDM) rat liver microsomes (Table 1). Primaquine, amodiaquine and quinine were relatively potent inhibitors of PB/APDM, but only primaquine and quinine were strongly inhibitory towards C/APDM. Quinidine was surprisingly inactive as an inhibitor of either APDM activity when the potency of its optical isomer (quinine) is considered. In fact quinine was approximately three times more potent than its stereoisomer against PB/APDM and about twenty-three times more potent against C/APDM (Table 1). Chloroquine was a feeble inhibitor of both types of APDM activity.

To account for the observed inhibitory potencies of the quinoline drugs a series of spectral binding studies was undertaken. Two of the most potent inhibitors, primaquine and quinine, elicited characteristic type II optical difference spectra (absorption maximum near 430 nm and a broad minimum between 390 and 410 nm) in both control and PB-induced microsomal fractions. In contrast, the less potent inhibitors quinidine and chloroquine elicited type IIb difference spectra (maximum near 430 nm and minimum near 410 nm) in PB-induced microsomes. Chloroquine produced a feeble reverse type I spectral change in control liver microsomes but the change was too small to be accurately quantified. Quinidine produced an atypical spectral change in which a trough was observed near 426 nm, but no absorption maximum was apparent. Optical difference spectra were not obtained for amodiaquine due to the intense absorption of that compound in the 380–450 nm region of the spectrum.

Figure 2 represents the relationship between the negative logarithm of the I_{50} value (pI_{50}) and the negative logarithm of the spectral dissociation constant (pK_s) for those compounds that elicited measurable spectra. A strong relationship between pI_{50} and pK_s is suggested from the linear regression of these parameters in PB-induced microsomes ($r = 0.96$, $N = 4$) and in both microsomal types ($r = 0.93$, $N = 6$).

An alternate linear relationship was found to exist

Table 1. Inhibition of aminopyrine *N*-demethylase activity (APDM) from phenobarbitone-induced (PB) and control (C) rat liver microsomes by quinoline drugs

Compound	PB/APDM* I_{50} (mM)	C/APDM I_{50} (mM)	I_{50} ratio†
Chloroquine (I)	5.3	20	0.27
Quinine (II)	0.14	0.061	2.3
Quinidine (III)	0.41	1.4	0.29
Amodiaquine (IV)	0.13	0.36	0.36
Primaquine (V)	0.13	0.12	1.1

* I_{50} values were determined in duplicate from plots of log (inhibitor) concentration versus percent inhibition, using at least four concentrations of drug. Control activities were: 3.8 ± 0.2 and 2.5 ± 0.1 nmoles formaldehyde produced/mg protein/min for PB-induced and control APDM, respectively.

† Ratio of I_{50}s against PB/APDM and C/APDM.

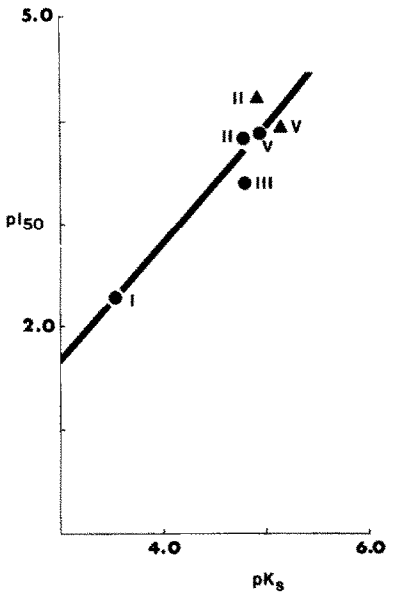

Fig. 2. Relationship between negative logarithm of the spectral dissociation constant (pK_s) and the negative logarithm of the I_{50} value (pI_{50}) for a series of quinoline derivatives. Regression line includes 6 points derived in control (▲) and PB-induced (●) microsomal fractions. Data taken from Tables 1 and 2.

between pI_{50} and the logarithm of the $\Delta A_{max}:K_s$ ratio (Fig. 3). At this stage the significance of this relationship is not certain because, although a good correlation was obtained for the linear regression in PB-induced microsomes ($r = 0.98$, $N = 4$), a poorer correlation was observed when data from both microsomal types was combined ($r = 0.85$, $N = 6$). Very

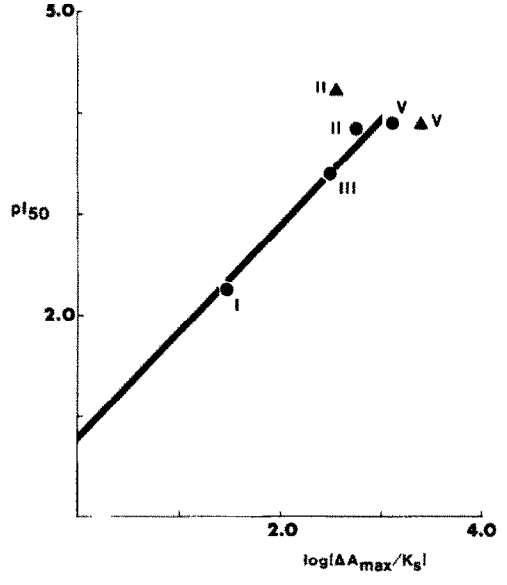

Fig. 3. Relationship between the logarithm of the binding efficiency [$\log(\Delta A_{max}/K_s)$] and pI_{50} for a series of quinoline derivatives. Regression line includes 4 points derived in PB-induced (●) microsomal fractions. (▲) Represents data obtained using control liver microsomes. Data taken from Tables 1 and 2.

poor correlations were obtained for linear relationships between pI_{50} and ΔA_{max} (or $-\log \Delta A_{max}$). Thus it would appear that the binding affinity of the type II interaction between a quinoline derivative and ferric cytochrome P-450 is an appropriate spectral parameter to account for APDM inhibition potency.

DISCUSSION

The present paper demonstrates that several quinoline drugs are inhibitors of oxidative metabolism in rat liver *in vitro* and that their effects may be mediated, at least in part, by an interaction with the ferric form of cytochrome P-450. Other quinolines have been found to inhibit hepatic drug oxidations both *in vitro* and *in vivo*. For example, the antioxidant ethoxyquin has been described as a potent competitive inhibitor of certain MFO activities *in vitro* [6, 20], and the structurally similar 8-hydroxyquinoline-5-sulphonic acid (OHSA) was weakly inhibitory towards APDM activity *in vitro* [7]. A recent report established that the antimalarial agents primaquine and chloroquine are inhibitors of control APDM activity from rat liver [14]. The data in the present paper confirm these observations and extend them to include the related compounds quinine, quinidine and amodiaquine. It was noted that quinine and primaquine were more potent inhibitors of PB/APDM activity whereas chloroquine, quinidine and amodiaquine were more active against APDM activity from control rat liver (compare I_{50} ratios, Table 1). In order to account for the different susceptibilities of PB/APDM and C/APDM to inhibition by quinolines it is important to recognise that the latter activity appears to be catalysed by two enzymes with different affinities for aminopyrine [21]. In contrast, a single enzyme appears to catalyse the metabolism of aminopyrine in PB-type rat liver. Consequently the I_{50}s determined in PB-induced and control liver microsomes probably represent the capacity of the quinoline derivatives to interact with different oxidative enzymes that are involved in the turnover of a common substrate.

Spectral binding interactions between four of the quinoline drugs and ferricytochrome P-450 were studied in control and PB-induced rat liver microsomes. Type II optical difference spectra were generated by quinine (Fig. 4) and primaquine in control and PB-induced liver microsomal fractions. Type IIb difference spectra were generated in PB-induced microsomes by quinidine (Fig. 4) and chloroquine. However, neither compound generated reproducible spectra in control microsomes that were quantifiable. It is well established that the type II spectral change reflects a change in the low spin–high spin equilibrium toward the low spin component as a consequence of ligand co-ordination at the sixth axial position of the haem iron [22–24]. The type IIb change represents a change in the low spin configuration of ferric cytochrome P-450 in which the endogenous sixth ligand is replaced by an exogenous type IIb ligand (in this case a nitrogen atom in quinidine or chloroquine) [25]. Type IIb ligands are not considered to interact significantly with the high spin form of the oxidised cytochrome. Primaquine and quinine probably interact with the haem iron of

Table 2. Spectral binding parameters for the interaction of quinoline drugs with microsomal ferricytochrome P-450*

	Microsmal type					
	Phenobarbitone-induced			Control		
Compound	(K_s)	($\Delta A_{max} \times 10^3$)	($\Delta A_{max}/K_s$)	(K_s)	($\Delta A_{max} \times 10^3$)	($\Delta A_{max}/K_s$)
Chloroquine	290 ± 50	8.1 ± 0.6	28		ND†	
Quinine	16	8.9	556	13 ± 2	4.9 ± 0.5	369
Quinidine	16 ± 0.3	4.6 ± 0.3	293		ND	
Primaquine	12 ± 2	15 ± 4	1250	7.2 ± 0.9	17 ± 1	2360

* Values are mean ± S.D. of 3 or 4 individual spectral titrations with at least five different ligand concentrations. In the case of the quinine interaction in phenobarbitone-induced microsomes the data are means of duplicate determinations that differed by less than 9%.

Units are: K_s, μM; ΔA_{max}, absorbance units/nmole cytochrome P-450; $\Delta A_{max}/K_s$, absorbance units/nmole cytochrome P-450/M.

† ND, not determined due to small spectral change of inconsistent magnitude.

cytochrome P-450 via the side chain amino nitrogen and the quinuclidine nitrogen, respectively, as these provide lone electron pairs that are sterically accessible to the haem iron. In contrast, chloroquine has only relatively hindered nitrogen atoms in its alkylaminoalkyl side chain and in the quinoline ring system through which it may interact in type IIb fashion with the ferric cytochrome. Unlike the situation that exists in quinine, the quinuclidine nitrogen in quinidine is sterically inaccessible (Fig. 5) and may not be available for a ligand interaction with high spin ferric cytochrome P-450. It appears likely that this change in the stereochemistry about the carbon atom between the quinuclidine and quinoline ring systems is responsible for the apparent difference in the interaction of quinine and quinidine with cytochrome P-450.

It is of major interest that the more potent inhibitors of APDM activity also elicited type II difference

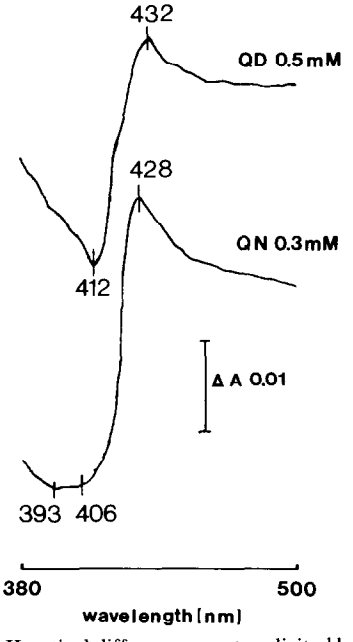

Fig. 4. Type II optical difference spectra elicited by 0.5 mM quinidine (upper trace) and 0.3 mM quinine (lower trace) in phenobarbitone-induced microsomal fractions.

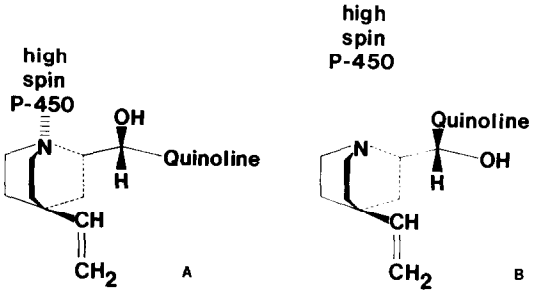

Fig. 5. The effect of the α-carbon stereochemistry on binding to high spin ferric cytochrome P-450. (A) represents the interaction between the quinuclidine nitrogen of quinine and the high spin ferric cytochrome. (B) represents the absence of an interaction between quinidine and high spin ferric cytochrome P-450.

spectra and that less potent inhibitors produced the type IIb change. Non-potent inhibitors elicited extremely feeble difference spectra. Therefore the present results demonstrate that a good relationship appears to exist between anti-APDM potency and type II spectral binding affinity (Fig. 2). It is also possible that the efficiency of binding ($\Delta A_{max}/K_s$) may be related to inhibition potency (Fig. 3), although this possibility must await the results of further studies with quinoline derivatives. There appears to be no relationship between the extent of type II binding to microsomal cytochrome P-450 (ΔA_{max}) and APDM inhibition.

The results of the present study clearly suggest that some antimalarial quinolines may affect the biotransformation of other drugs in the liver. In particular, significant inhibition of MFO activity may result from the administration of quinine and primaquine. As proposed by others [14], pharmacokinetic interactions may be a consequence of the co-administration of an antimalarial quinoline and another drug that is extensively metabolised by the liver MFO system.

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