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Inhibition of Metoprolol Metabolism by Chloroquine and other Antimalarial Drugs*

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Abstract—The ability of a series of antimalarial drugs to impair the metabolism of metoprolol in rat and man has been examined. Chloroquine was a potent inhibitor in rat liver microsomes (K_i value for metoprolol α-hydroxylation = 0·18 μM and for *O*-demethylation = 0·36 μM). The other antimalarial drugs also inhibited metoprolol oxidation. Quinine was similar to chloroquine in potency, while quinidine, primaquine and mefloquine were slightly less potent. Chloroquine also inhibited metoprolol oxidation in human liver microsomes, although it was about two orders of magnitude less potent than in the rat and the extent of impairment varied greatly between individual livers. Intraperitoneal administration of chloroquine to anaesthetized rats decreased the clearance of metoprolol (40 mg tartrate salt kg^{-1} i.p.) to 54, 34, 20 and 26% of the control value at doses of 2·5, 4·0, 25 and 40 mg kg^{-1}, respectively. We conclude that antimalarial treatment might have contributed to a previously reported difference in the metabolic pattern of metoprolol between Caucasians and Nigerians.

Polymorphic control of the metabolism of the β-adrenoceptor antagonist metoprolol, has been demonstrated in Caucasians and is of the 'debrisoquine-type' (McGourty et al 1985). The role of genetic polymorphism in the metabolism of metoprolol and debrisoquine has also been investigated in Nigerians but, in contrast to the Caucasian data (Iyun et al 1986), bimodality in the $\log_{10}$ urinary metoprolol/α-hydroxymetoprolol (M/HM) and $\log_{10}$ urinary debrisoquine/4-hydroxydebrisoquine (D/HD) ratios was not apparent. In addition, Iyun et al (1986) found that the median M/HM and D/HD ratios were significantly higher in Nigerians compared with Caucasians.

One explanation for these observations is that non-genetic factors may have altered the distribution of the drug/metabolite ratios. Thus, Iyun et al (1986) suggested that antimalarial therapy may have caused a systematic increase in the drug/metabolite ratio in Nigerians by inhibition of metoprolol metabolism. Antimalarial aminoquinoline drugs such as chloroquine have been shown to inhibit drug metabolism in-vitro and in-vivo (Ayitey-Smith 1980; Riviere & Back 1986) and chloroquine has a terminal half-life of up to two months in man (White 1985). None of the Nigerians studied by Iyun et al (1986) were taking the drug at the time of drug administration. However, most subjects are likely to have taken chloroquine to treat malarial attacks in the months before the study.

Two of the major routes of metoprolol metabolism involve benzylic hydroxylation and *O*-demethylation (Borg et al 1975). We have examined the ability of chloroquine and other antimalarial drugs to inhibit these pathways in rat and human liver microsomes. The effect of chloroquine on metoprolol kinetics in the intact rat has also been studied.

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*This paper is dedicated to the memory of our friend and valued colleague Dr Ayo Iyun, who died on December 13th, 1988 in Ibadan, Nigeria.

Materials and Methods

Chemicals and Drugs

Metoprolol tartrate, α-hydroxymetoprolol *p*-hydroxybenzoate, *O*-demethylmetoprolol base and pamatolol hydrochloride were gifts from AB Hassle (Molndal, Sweden) and nadolol base was a gift from Squibb and Sons Ltd (Hounslow, Middlesex). Chloroquine diphosphate, primaquine diphosphate and mefloquine hydrochloride were kindly donated by Dr D. J. Back, Department of Pharmacology and Therapeutics, University of Liverpool and (+)- and (−)-chloroquine were provided by Professor F. Sjoqvist, Department of Clinical Pharmacology, Karolinska Institute, Stockholm, Sweden. Quinidine and quinine hydrochlorides were purchased from the Sigma Chemical Company, Poole, UK. Except where stated otherwise all drug names refer to the racemic form of the base.

Animals

An outbred strain of Wistar rats from the University of Sheffield Breeding Colony was used. Male rats (200–220 g for microsome studies; 250–350 g for whole animal studies) maintained on a standard laboratory small animal diet (Labsure Foods, Poole UK) and allowed free access to tap water were used. For the preparation of microsomes the rats were stunned and killed by cervical dislocation and the livers were removed immediately. The tissue was then frozen in liquid nitrogen and stored at −80°C until use.

Source of human liver

Small samples of seven human livers were obtained from male Caucasian renal transplant donors with the approval of the Royal Hallamshire Hospital ethics committee and the local coroner. The samples were taken as soon as possible after the kidneys were removed, cut into 2 cm cubes, frozen in liquid nitrogen and stored at −80°C until use. The period between cessation of organ perfusion and cooling of the tissue was about 30 min. The drugs given to the donors before and during organ removal are described elsewhere (Shaw et al 1987; Otton et al 1988).

Studies with liver microsomes

Liver microsomes were prepared as described previously (Otton et al 1988). Microsomal protein concentration was measured by the method of Lowry et al (1951) using bovine serum albumin as the standard.

Liver microsomal suspensions (0·2 mL containing about 4 mg protein, equivalent to 200 mg tissue) were incubated at 37°C and pH 7·4 with metoprolol dissolved in 1·15% (w/v) KCl solution (0·2 mL), the appropriate antimalarial drug in 1·15% (w/v) KCl solution (0·2 mL) and an NADPH generating system containing 4 μmol G6P, 0·4 μmol NADP, 0·4 units G6PD and 2 μmol $MgCl_2$ in 0·2 M phosphate buffer (0·4 mL). Solutions of the antimalarial drugs were prepared each week and stored at −20°C. The reaction was stopped by transferring 0·4 mL duplicates of the incubate to capped plastic vials containing 6% (v/v) perchloric acid solution (50 μL). Pamatolol hydrochloride (20 μL of a 10 μg mL^{-1} solution) was added as the internal standard. The acid precipitated samples could then be stored at 4°C for at least 6 months without detectable degradation of metoprolol metabolites.

Whole rat studies

Rats were anaesthetized using an intraperitoneal injection of urethane (1·25 g kg^{-1}). The trachea, carotid artery and jugular vein were cannulated with polyethylene catheters. Drugs were administered either intravenously or intraperitoneally. Serial blood samples (0·05 or 0·1 mL) were taken from the carotid artery and each sample was replaced by the same volume of heparinised saline.

Drug analysis

All analyses were performed using high-performance liquid chromatography (HPLC) with fluorescence detection.

Rat whole blood samples were assayed for metoprolol as described by Lennard & Silas (1983).

A modification of the method of Lennard (1985) was used to assay α-hydroxymetoprolol and *O*-demethylmetoprolol in microsomal incubates. Sample, NaOH (1·2 M, 50 μL) and water (50 μL) were mixed gently with dichloromethane (5 mL) for 10 min. After centrifugation (3000 rev min^{-1}, 5 min) the organic layer was transferred to a conical glass tube and evaporated to dryness using a Buchler vortex evaporator (Baird and Tatlock, Romford, UK). The residue was reconstituted in mobile phase and an aliquot was injected onto the HPLC column. HPLC was performed using a Model 6000A pump (Waters Associates, Northwich, UK), a Model 7125 Rheodyne injector (HPLC Technology, Macclesfield, UK), a Z-Module column system containing a cartridge packed with Nova-Pak C_{18} reversed phase packing material (Waters Associates) and Model 970FS Kratos Fluorescence Detector (Applied Biosystems, Warrington, UK). A water-acetonitrile mixture (90:10) containing 1% (w/v) triethylamine and adjusted to pH 3 with orthophosphoric acid was used as the mobile phase. Chromatography was performed isocratically at a flow rate of 3 mL min^{-1} and at ambient temperature (20°C). The detector was operated at an excitation wavelength of 193 nm and no emission filter was used.

Intra-assay coefficients of variation were less than 5% for all compounds.

Analysis of data from intact rat experiments

The area under the blood metoprolol concentration vs time curve (AUC) was calculated using the linear trapezoidal rule with extrapolation to time infinity ($C_{(last)}$/elimination rate constant). The elimination rate constant (k) was calculated from the slope of the terminal phase of the $\log_{10}$ blood drug concentration vs time curve. Clearance (CL) was calculated from dose/AUC and terminal elimination half-life from 0·693/k. Mean clearance data were compared using a one-way analysis of variance. Data from individual treatment groups were then compared to control data using Student's *t*-test.

Results

Rat liver studies

Chloroquine was a potent inhibitor of the formation of α-hydroxymetoprolol and *O*-demethylmetoprolol from metoprolol when incubated with rat liver microsomes (Table 1). Values of K_m for both reactions increased with increasing inhibitor concentration whereas those for V_{max} remained essentially unchanged (data not shown). This finding together with the approximately parallel nature of plots of substrate concentration/velocity vs inhibitor concentration suggested that inhibition was competitive over the concentration range studied.

Table 1. The effect of chloroquine and other antimalarial drugs on metoprolol metabolism in rat liver microsomes.

	α-Hydroxylation		*O*-Demethylation	
Drug	K_i (μM)	IC50 (μM)	K_i (μM)	IC50 (μM)
Chloroquine	0·18 ± 0·005	0·30 ± 0·008	0·36 ± 0·11	0·52 ± 0·21
Quinine	0·11 ± 0·06*	0·37**	0·38 ± 0·04*	0·66 ± 0·11
Quinidine	0·50 ± 0·33	1·84 ± 0·23*	0·94 ± 0·23*	0·91 ± 0·72*
Primaquine	0·89 ± 0·21	1·15 ± 0·64*	0·83 ± 0·17	1·52 ± 0·65
Mefloquine	1·53 ± 1·32	3·79 ± 0·48	3·20 ± 0·69	7·65 ± 2·12*

*n = 2; **n = 1.

Incubations were performed over 5 min. Apparent K_i (inhibitor constant) values were estimated using the method of Dixon (Cornish-Bowden 1977). IC50 is concentration of inhibitor required to impair metabolite appearance by 50% at a substrate concentration of 20 μM. Each value represents the mean (± s.d.) of three experiments, except where stated otherwise.

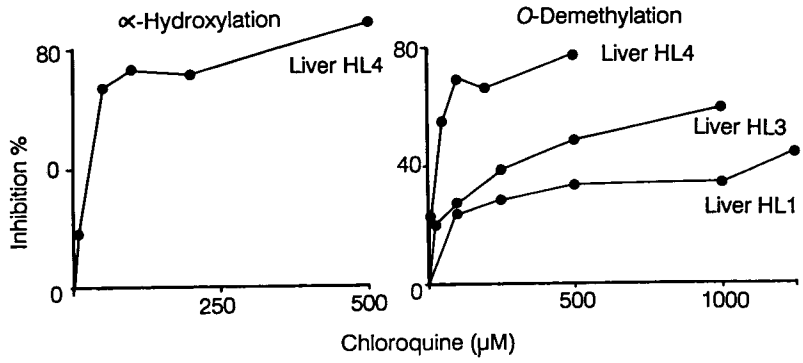

FIG. 1. Effect of chloroquine (10–500 μM) on metoprolol (25 μM) oxidation in human liver microsomes. The incubations were performed over 20 min. Each datum point represents the mean value of duplicate analyses. Apparent K_m and V_{max} values for α-hydroxylation and *O*-demethylation are given by Otton et al (1988). Data are from HL1 and HL3, putative 'poor metabolizer' livers and from HL4, a representative 'extensive metabolizer' liver.

Table 2. Effect of racemic (±)-chloroquine and its enantiomers on metoprolol oxidation in human liver microsomes.

		IC50 (mM)	
Inhibitor	Liver	α-Hydroxylation	*O*-Demethylation
(±)-Chloroquine	HL4	24	39
	LP9	42	68
	W21	37	73
	F45	36	73
	M1	37	47
	HL3	—	615
	HL1	—	>1250
(+)-Chloroquine	HL4	22	130
(−)-Chloroquine	HL4	15	42

The values represent mean data from one or two experiments. Limited supplies of liver did not permit additional experiments to be performed. The substrate concentration was 25 μM and the incubation time was 30 min. Apparent K_m and V_{max} values for α-hydroxylation and *O*-demethylation enzymes from individual livers are given by Otton et al (1988).

The other antimalarial drugs tested also inhibited metoprolol oxidation. Quinine was similar to chloroquinine in potency, while quinidine, primaquine and mefloquine were slightly less potent (Table 1).

Human liver studies

Chloroquine also inhibited the α-hydroxylation and *O*-demethylation of metoprolol in incubations of human liver microsomes (Fig. 1; Table 2). It was much less potent an inhibitor of *O*-demethylation in the two livers that did not α-hydroxylate metoprolol (HL1 and HL3) than in the other five livers. (−)-Chloroquine was more inhibitory than its (+)-antipode.

Intact rat studies

The intraperitoneal injection of chloroquine decreased significantly the clearance of metoprolol (40 mg kg^{-1} i.p.) in a dose-dependent manner ($P < 0.01$, one-way analysis of vari-

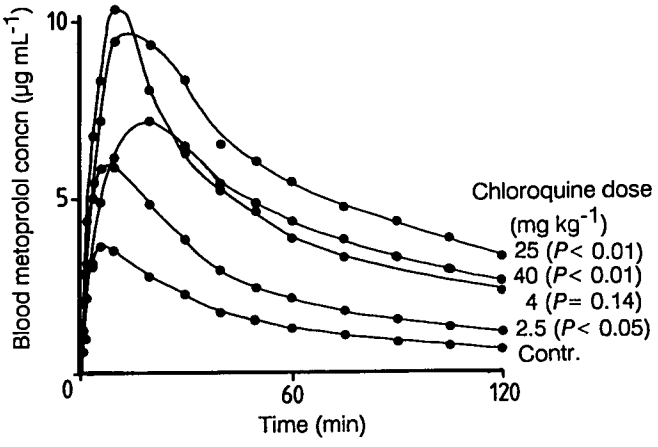

FIG. 2. The effect of chloroquine on blood metoprolol concentration-time profiles after i.p. injection of 40 mg kg^{-1} metoprolol tartrate in the anaesthetized rat. Each point is the mean value of two to four experiments. Ranges of clearance (mL min^{-1} kg^{-1}) values: control = 131–167, n = 4; 2·5 mg kg^{-1} chloroquine = 62–114, n = 3; 4·0 mg kg^{-1} = 34–75, n = 2; 25 mg kg^{-1} = 29–35, n = 4; 40 mg kg^{-1} = 20–65, n = 3. *P* values are from comparisons of treatment vs control groups.

ance) (Fig. 2). Thus, mean clearance fell to 54, 34, 20 and 26% of the control value after doses of 2·5, 4·0, 25 and 40 mg kg^{-1} chloroquine, respectively. Mean ± s.d. values of elimination half-life, t_{max} and C_{max} in the control group were 66 ± 16 min, 8 ± 2 min and 3727 ± 1080 ng mL^{-1}, respectively. No significant differences between treatment and control groups were observed for any of these measurements. Chloroquine (50 mg kg^{-1} i.p.) also impaired the elimination of intravenously administered metoprolol (10 mg kg^{-1}). Thus, mean (± s.d.) clearance (i.v.) fell from 123 ± 56 (n = 5) to 52 ± 26 mL min^{-1} kg^{-1} (n = 5) ($P = 0.051$).

Discussion

The mixed-function oxidation of metoprolol by rat liver microsomes was found to be sensitive to inhibition by chloroquine and other antimalarial agents. Chloroquine was much more potent than some other drugs shown to inhibit metoprolol oxidation by rat liver microsomes. For example, K_i values obtained for debrisoquine, guanoxan, cimetidine and ranitidine (Lennard et al 1986) are at least one order of magnitude higher than those for chloroquine (Table 1).

Studies of the effect of chloroquine on the metabolism of other drugs have produced conflicting results. Ayitey-Smith (1980) reported that chronic administration of chloroquine (20 mg kg^{-1} for 21 days) prolonged pentobarbitone sleeping time in the rat by 16–105%. In contrast, the metabolism of ethinyloestradiol, tolbutamide (Riviere & Back 1986) and aminopyrine (Murray 1984) by rat liver microsomes was virtually unaffected by co-incubation with chloroquine. However, primaquine, mefloquine, quinine, quinidine and amodiaquine all caused significant inhibition of these pathways. In another study, a single dose of chloroquine (10 mg kg^{-1} i.p.) to intact rats was associated with a significant decrease in the hepatic microsomal *N*-demethylation of benzphetamine but not in the *O*-deethylation of ethoxyresorufin (Thabrew & Ioannides 1984).

There is evidence that these substrates are catalysed by different forms of the cytochrome P450 family of enzymes. Thus, using the most recent nomenclature (Nebert et al 1989) P450IID1 (rat) and P450IID6 (human) are probably involved in metoprolol oxidation (Larry et al 1984), whereas ethinyloestradiol is metabolized by P450s IIC6, IIC12 and possibly IIIA (D. J. Back, personal communication). In addition there is evidence that tolbutamide is metabolized by the IIB1 (rat) (D. J. Back) and IIC10 (human) (Brian et al 1989). Therefore, chloroquine appears to be a selective inhibitor of cytochrome P450.

Chloroquine was at least two orders of magnitude less potent in its ability to inhibit metoprolol oxidation in human compared with rat liver microsomes. If chloroquine and metoprolol are substrates for a common enzyme, this species difference may reflect a lower affinity and, therefore, a higher K_m of chloroquine for the human than for the rat enzyme.

The extent of inhibition of metoprolol metabolism by chloroquine varied markedly between human livers. The polymorphic oxidation of metoprolol in man may be an important contributory factor to such variability. Impaired metabolism of metoprolol occurs in about 8% of Caucasians and is of the debrisoquine-type (McGourty et al 1985). These individuals, designated poor metabolizers (PMs), possess anomalous form(s) of P450IID6 (Gonzales et al 1988). The α-hydroxylation of metoprolol is absent in PMs, whereas its *O*-demethylation is only partially impaired (McGourty et al 1985). Furthermore, recent in-vitro evidence indicates that P450IID6 is only partially responsible for the latter reaction (Otton et al 1988). There is good evidence that, of the seven livers used in the present work, HL1 and HL3 were from PM subjects (Otton et al 1988) and do not α-hydroxylate metoprolol. From the data shown in Table 2 it is clear that metoprolol *O*-demethylation is much less sensitive to inhibition by chloroquine in livers HL1 and HL3 than in the other five livers. This suggests that P450IID6 is relatively sensitive to chloroquine inhibition while other P450s are insensitive. Detailed kinetic studies are required to test this hypothesis and to determine whether chloroquine is a substrate for P450IID6.

Whether treatment with chloroquine impairs the clearance of metoprolol in man is not known. However, there is circumstantial evidence to suggest that this may occur. First, our findings have shown that chloroquine decreases the clearance of metoprolol in intact rats at doses which are comparable on a weight for weight basis to those used in the treatment of malaria. Second, although few data are available on the human hepatic disposition of chloroquine, concentrations high enough to cause the observed in-vitro inhibition of metoprolol seem to be attained during prophylactic treatment for malaria. Thus, analysis of liver samples from Korean military personnel taking 500 mg of chloroquine phosphate per week indicated drug concentrations of up to 48 μM (Prouty & Kuroda 1958), a value which is in excess of the IC50 for inhibition of metoprolol oxidation in liver HL4.

In summary, our findings suggest that chloroquine treatment could contribute to the differences in metoprolol metabolism between Nigerians and Caucasians reported by Iyun et al (1986). However, this hypothesis must be considered in the light of a second, more recent study in which a small but statistically significant decrease in the median metoprolol/α-hydroxymetoprolol ratio was observed in a further sample of Nigerian subjects when compared with the original Caucasian population (Lennard et al 1989). Unfortunately, any differences in antimalarial therapy between the two Nigerian groups could not be evaluated.

Our findings emphasise the importance of considering the contribution of environmental as well as genetic factors when interpreting ethnic differences in drug metabolism.

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