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The effect of pyrogen on the in vivo metabolism and initial kinetics of chloroquine in rats

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The possible contribution of hepatic metabolism impairment, caused by pyrogen, to the increased toxicity and altered pharmacokinetics of chloroquine in rats was investigated. The ratio of unchanged chloroquine (CQ) to total quinolines (TQ) was determined in the 24-h urine collection, the plasma and the liver. Pretreatment with massive doses of *E. coli* lipopolysaccharide pyrogen did not produce any significant change in the ratio of CQ/TQ in the urine, liver or plasma between control and pyrogen-treated rats. This finding occurred in spite of a pronounced initial behavioural effect of the pyrogen on the rats. The ratio of CQ/TQ was also preserved in the 7th day 24-h urine collection from a control and a pyrogen-treated rat although the total urinary excretion of TQ had decreased by about 99·5% of the first 24-h collection. Analysis of the pharmacokinetic parameters from plasma levels in the first 24 h showed significant alterations in the apparent volume of distribution (AVD), the half-life (T 1/2) and the rate of decay constant (K) of the drug in pyrogen-treated rats. These findings were interpreted to indicate that chloroquine metabolism in vivo, which occurs mainly via a dealkylation process, may not be readily deranged by pyrogen. The changes in the pharmacokinetic parameters are discussed in relation to the known haemodynamic changes that occur in the febrile state.

Attempts to explain the altered toxicity and disposition of the antimalarials in fever have so far remained speculative. Hepatic metabolic dysfunction was mentioned as a possible explanation by Trenholme et al (1976) because of the associated finding of serum transaminase elevations in their subjects.

It is known that hepatic disturbance occurs during fever and malarial infection as indicated by serum transaminase elevations (Deller et al 1967), altered bromosulphalein clearance (Blaschke et al 1973) and liver biopsies (White & Doermer 1954), but the extent to which this contributes to the altered pharmacokinetics and toxicity of antimalarials is not known.

Direct inhibition of tricarboxylic acid cycle enzymes by pyrogen has been reported in the heart (Miyanishi et al 1969) and in the liver (Plaut & Goldman 1970) as well as indirect inhibition by pyrogen of steroid-induction of several enzymes (Berry et al 1968). Song et al (1972) found that whereas glucuronidation was impaired by pyrogen pretreatment, sulphation and hydroxylation were increased, in the metabolism of salicylamide in volunteers. Furthermore, they found that the alterations in some of the pharmacokinetic parameters of salicylamide could be correlated well with haemodynamic changes. Thus, peripheral vasoconstriction and increased visceral perfusion during pyrogen-induced fever (Bradley et al 1945) were associated with increased clearance, whereas increased peripheral perfusion and decreased visceral blood flow during artificial heating of subjects (Rowell et al 1970) was associated with a decreased clearance of salicylamide.

Investigations in man, aimed at elucidating the mechanism of the enhanced toxicity and altered pharmacokinetics of antimalarials, including a recent study on the binding of chloroquine to plasma proteins (Buchanan & Van der Walt 1977) have been limited.

I have attempted to elucidate the effect of a pyrogen on the hepatic metabolism of chloroquine in the rat. It has been demonstrated through monitoring of radioactive labelled bacterial lipopolysaccharide in animals (Rowley et al 1956; Cooper & Cranston 1963) that most of the bacterial pyrogen is cleared rapidly from the circulation during an initial phase lasting a few minutes; the remainder is cleared during a longer phase such that within 30 min of injection of radioactive labelled pyrogen, about 50% of the dose is found in the liver with less than 5% each in the spleen, lungs and kidneys. The pyrogen in the liver begins to decrease only after 48 h and a third of the original amount is still present at the end of one week (Rowley et al 1956).

MATERIALS AND METHODS

Male albino Sprague-Dawley rats, 200–250 g, housed individually in metabolic cages, with free

access to food and water were allowed to become habituated to their cages for 2 days.

Escherichia coli lipopolysaccharide was obtained in crystalline form from Sigma Chemical Co., U.S.A., and administered as an aqueous solution (1 mg ml^{-1} kg^{-1}) i.p. This dose amounts to 10 times the dose that produced a regular febrile response in a previous study (Osifo & DiStefano 1978).

This initial massive dose of pyrogen had a pronounced effect on the rats; they did not eat or drink for the next 24 h. This prompted omitting one of the 3 daily doses of pyrogen initially planned; the second dose of pyrogen was administered 48 h after the first dose. Each rat was monitored for a febrile response with a flexible vinyl thermistor probe inserted at least 8 cm into the rectum. The febrile response resembled the response found to smaller doses of pyrogen in an earlier study (Osifo & DiStefano 1978). From a basal temperature of about 37·7 °C, there was an increase of the rectal temperature by 0·9 (0·26 °C) (mean with s.d.) about 1 h after pyrogen administration. This febrile response did not occur in control rats injected with sterile water 1 ml kg^{-1} i.p.

One h after the second pyrogen dose, 14 mg ml^{-1} kg^{-1} of an aqueous solution of chloroquine diphosphate was administered intraperitoneally. This dose amounts to one-tenth the i.p. LD50 of chloroquine diphosphate established in rats in the previous study. Control rats, similarly caged, only received the standard dose of chloroquine diphosphate.

Twenty-four h after chloroquine challenge, samples were obtained as follows.

(a) The collected 24 h urine was measured, centrifuged (1000 rev min^{-1}) and 3 ml of supernatant used.

(b) Each rat was anaesthetized with ether and 7–9 ml of blood was obtained by open cardiac puncture with a syringe and a 19-gauge needle and transferred promptly into a heparinized Vacutainer glass tube, centrifuged at 1500 rev min^{-1} for 30 min and 3 ml of plasma assayed.

(c) From a piece of the right lobe of the liver, 1 g was weighed wet and put in 5 ml of 0·1 M HCl for homogenization with a Polytron homogenizer. The suspension was centrifuged at 1000 rev min^{-1} for 10 min and 3 ml of the top layer of the suspension used for assay.

The urine, plasma and liver homogenate from unmedicated rats were similarly processed and served as blanks.

The samples were assayed for total quinoline (unwashed aliquot) and unchanged chloroquine (washed aliquot) according to McChesney et al (1962). The fluorescence of the extracted quinolines was determined in an Aminco-Bowman spectrophotofluorometer at 340 nm and 390 nm for activation and emission respectively.

Parallel assays of total quinoline and unchanged chloroquine were thus performed on liver and urine (5 times) and plasma (4 times) of control and pyrogen-treated rats.

Two chloroquine-medicated rats (one control and one pyrogen-treated) were kept in the metabolic cages for a further 7 days after chloroquine administration. The 7th day 24-h urine collection was prepared and assayed in the usual way for chloroquine and total quinolines.

Pharmacokinetic parameters

The elimination profiles of chloroquine after single or repeated oral administration (McChesney et al 1962, 1967) show a multiexponential decay of the post-absorption plasma concentrations, with an initial rapid phase of decay spanning several days. The initial rapid phase of decline in those studies appeared to be governed by a long distributive phase into tissues from which a greatly prolonged elimination then occurred.

The pharmacokinetic parameters presented in Table 4 were computed from the 1 h and 24 h plasma concentrations of chloroquine assuming a monoexponential decay of the initial phase of rapid decline. The regression lines were plotted for four each of control and pyrogen-treated rat groups killed at 1 and 24 h and assayed for total quinoline in the plasma. The semi-logarithmic linear functions allowed the calculation of the half-lives ($T\frac{1}{2}$), the apparent volume of distribution (AVD) by extrapolation of the line to time zero, and the constant (K) of the rate of decay. The following equations were used to calculate the parameters:

(a) $K = 0{\cdot}693/T\frac{1}{2}$

(b) $AVD = \dfrac{\text{Dose (chloroquine base)}}{CP_0}$ (where CP_0 = plasma concentration of quinoline at time zero)

(c) $T\frac{1}{2}$ = time required to reduce the plasma concentration by 50%.

The results of the metabolic studies and the pharmacokinetic parameters were analysed by the paired Student's *t*-test for 2 samples (Goldstein 1964).

RESULTS

The metabolism of chloroquine, as measured by the ratios of unchanged chloroquine to total quinoline in the urine, plasma and liver of control and pyrogen-treated rats, was not significantly different (Table 1).

Table 1. Total quinolines (TQ) and unchanged chloroquine (CQ) content of plasma (μg ml^{-1}), liver (μg g^{-1}) and urine (μg 24 h collection) of control and pyrogen-treated rats 24 h after CQ diphosphate administration (14 mg ml^{-1} kg^{-1} i.p.).

	Plasma						Liver						Urine					
	Control			Pyrogen-treated			Control			Pyrogen-treated			Control			Pyrogen-treated		
	TQ	CQ	CQ/TQ	TQ	CQ	CQ/TQ	TQ	CQ	CQ/TQ	TQ	CQ	CQ/TQ	TQ	CQ	CQ/TQ	TQ	CQ	CQ/TQ
Mean	0·015	0·012	0·76	0·018	0·015	0·80	21·26	12·37	0·58	25·51	15·2	0·60	388·4	280·4	0·72	363·6	250·9	0·70
s.e.m.	0·0003	0·0005		0·0005	0·0007		1·86	1·44		2·60	1·17		43·7	35·45		32·4	15·99	
N	4	4		4	4		5	5		5	5		5	5		5	5	
P (TQ control vs TQ pyrogen-treated)			<0·01						N.S.						N.S.			
P (CQ/TQ control vs CQ/TQ pyrogen-treated)			N.S.						N.S.						N.S.			

s.e.m. ± = standard error of means.
N = number of samples assayed.
N.S. = Not statistically significant ($P > 0·05$).

The concentration of total quinolines in the plasma of pyrogen-treated rats, 24 h following chloroquine administration is significantly greater than that in controls ($P < 0·01$). This finding is reminiscent of significant differences in the plasma concentrations of quinolines in pyrogen-treated rats 1 h after chloroquine challenge at the same dosage in a previous study (Osifo & DiStefano 1978).

The unchanged chloroquine and total quinolines in the total 24 h urines of both control and pyrogen-treated rats were identical. Whereas the ratio of unchanged chloroquine to total quinolines in the plasma and urine was 0·77 and 0·71, respectively, the ratio in the liver was 0·58. The latter differs significantly ($P < 0·05$) from the ratios in plasma and urine. Thus it appears that the intrahepatic metabolism of chloroquine proceeds faster than the metabolites are washed into the blood and subsequently excreted. Furthermore, the liver loses about half its content of quinolines within 24 h following chloroquine administration; the plasma concentration over a similar period falls to about one-eighth the 1 h value (Table 2).

Seven days after exposure to chloroquine, detectable quantities of quinolines were still being excreted in the urine (Table 3).

Table 3. Total quinolines (TQ) and unchanged chloroquine (CQ) excreted in 24-h urine by a control and a pyrogen-treated rat 7 days after CQ diphosphate (14 mg ml^{-1} kg^{-1} i.p.).

Control μg/24 h			Pyrogen-treated μg/24 h		
TQ	CQ	CQ/TQ	TQ	CQ	CQ/TQ
1·98	1·287	0·722	2·72	1·92	0·714

The calculated T ½ and the AVD of control rats are significantly increased over pyrogen-treated rats. The rate of decay constant (K) is significantly greater in pyrogen-treated rats over controls and appears to correlate with the decrease in T ½ in the pyrogen-treated rats.

DISCUSSION

The ability of the rat liver to metabolize chloroquine appears unchanged by massive doses fo *E. coli* lipopolysaccharide, despite the pronounced initial adverse effect of the pyrogen on the feeding and fluid intake of the rats exposed to pyrogen. Fletcher et al (1975) using a gas chromatographic/mass spectrometric technique found similar profiles of chloroquine and metabolites in urine, liver and kidney of both normal and *Plasmodium knowlesi*

Table 2. Comparison of the mean total quinolines in the plasma and liver of control and pyrogen-treated rats at 1 h* and 24 h after CQ diphosphate 14 mg ml^{-1} kg^{-1} i.p. (in N rats).

	Control				Pyrogen-treated			
	1 h		24 h		1 h		24 h	
	Plasma (μg ml^{-1})	Liver (μg g^{-1})	Plasma (μg ml^{-1})	Liver (μg g^{-1})	Plasma (μg ml^{-1})	Liver (μg g^{-1})	Plasma (μg ml^{-1})	Liver (μg g^{-1})
Mean	0·115	48·31	0·015	21·26	0·182	49·31	0·018	25·51
N =	5	5	4	5	5	5	4	5

* From Osifo & DiStefano (1978).

infected rhesus monkeys. Failure to demonstrate a significant difference in the metabolic ratios of chloroquine in the plasma and urine samples in the present study, could be interpreted as reflecting a low discriminating ability of the washing procedure for removing the metabolites from the organic solvent phase; but, this is unlikely because the same procedures yielded a different ratio for the liver samples.

Furthermore, in the original studies by McChesney et al (1956, 1962, 1966) the efficacy of the washing procedure for removing metabolites from different organic solvents was checked with countercurrent dialysis, recoveries and chromatographic techniques.

The metabolic ratio 0·71 of unchanged chloroquine to total quinolines in the urine was preserved in the rats up to the 7th day after chloroquine administration (Table 3) although the 24 h excretion of the quinolines had decreased to about 0·5% of the amount eliminated in the first 24 h. Since the metabolic ratio in the urine appears to correlate closely with the ratio in the plasma, this finding suggests that chloroquine metabolism in vivo follows a first-order type of reaction, i.e. that a constant fraction of the available drug is metabolized with passing time.

Chloroquine metabolism in several mammals, including the rat, occurs mainly via a dealkylation process such that about 70% of excreted quinolines is unchanged chloroquine and the rest consists of the mono- and bi-dealkylated metabolites (McChesney et al 1956, 1966). The findings in the present study suggest that chloroquine metabolism is not readily changed by pyrogen in a mammalian species, and support earlier reports concerning the fraction of the drug that is recovered unchanged in urine.

When bacterial pyrogen suddenly floods the circulation, responses vary from hyperpyrexia to so-called endotoxic shock. Several investigators have studied the effects of pyrogens on the circulation and have shown with spirochetal pyrogen (Schofield et al 1968) and bacterial pyrogen (Bradley et al 1945) that the essential responses are peripheral vasoconstriction, an increase in heart rate, pulse pressure and an increase in total splanchnic blood flow.

Abolishing pyrexia with aspirin (Bradley et al 1945) does not block the increase in splanchnic blood flow. The antimalarial compounds (the aminoquinolines, the quinolinemethanols and the aminoacridines, represented by chloroquine, quinine and quinacrine respectively), have a high affinity for the melanocytic system of cells in the skin (Lindquist & Ullberg 1972) and for other tissues in general (Macomber et al 1966; Polet & Barr 1968; Wibo & Poole 1974). If standard doses of these drugs are administered in the febrile state, with the associated haemodynamic changes, toxic levels concentrations could be attained as has been found for quinine in man (Trenholme et al 1976) and as is suggested by the acute lethality of chloroquine in rats pretreated with pyrogen (Osifo & DiStefano 1978).

The pharmacokinetic parameters (Table 4) suggest that pretreatment with pyrogen significantly alters the initial pharmacokinetics of chloroquine. In the present study, the metabolism and renal elimination of chloroquine were not significantly altered by pyrogen treatment; these mechanisms may not be responsible for the increased K and the diminished T ½ in pyrogen-treated rats. It is possible that as the pyrogen-induced haemodynamic changes remit, and a restoration of normal flow to previously underperfused peripheral tissues supervenes, this apparently causes both the increased K and the diminished plasma T ½ compared with controls.

Table 4. Calculated half-life (T 1/2), apparent volume of distribution (AVD) and initial rate of decay constant (K) of chloroquine diphosphate (14 mg ml^{-1} kg^{-1} i.p.) in control and pyrogen-treated rats.

	T½ (h)		AVD** (Litres)		K	
	Control	Pyrogen-treated	Control	Pyrogen-treated	Control	Pyrogen-treated
Mean	7·63	6·88	16·5	10·0	0·09	0·101
s.d.	0·32	0·36	3·22	1·22	0·004	0·005
N	4	4	4	4	4	4
P	<0·02*		<0·01*		<0·02*	

s.d. = Standard deviation of means.
N = Number of regression lines.
* = Significant difference.
** = Normalized for body weight of 250 g.

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