Effect of Chloroquine on Rat Liver Microsomes

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Summary Alterations in liver microsomal drug-metabolizing enzymes and phospholipids following oral administration of chloroquine to rats for 14 days were investigated. An increase in cytochrome P450 and cytochrome bs contents and an increase in the activities of NADPH cytochrome c reductase and NADH microsomal cytochrome c reductase were observed. A decrease in cholesterol and an increase in phospholipids were noted in the microsomal membranes, which altered the membrane fluidity, which may be of importance in controlling different membrane functions. The increase in different fractions of microsomal phospholipid is probably due to an increased incorporation of acetate into phospholipids.

Key Words: chloroquine, microsomes, liver, rat, phospholipids

Chloroquine (CQ), a cationic amphiphilic drug, is the most widely prescribed antimalarial in the world. Many biological effects of CQ, i.e., antimalarial action, retinal damage, and neurological side effects [1, 2], have been reported and are thought to be directly related to its lysosomotropism [3]. CQ is metabolized in the body by liver microsomal monoxygenases [4]. The major metabolites of CQ are desethylchloroquine and bisdesethylchloroquine [5]. Like the parent drug, both metabolites become highly concentrated in liver, heart, kidney, spleen, and lung [6]. Since CQ [7] and its metabolites [1] are associated with toxicity, an attempt was made to study the effect of CQ on drug-metabolizing enzymes and to correlate these findings with its toxicity.

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METHODS

Chemicals. Chloroquine diphosphate, 1,6-diphenyl-1,3,5-hexatriene (DPH), NADH, NADPH were purchased from Sigma Chemical Company, St. Louis, MO. [14C]Acetate was obtained from Bhabha Atomic Research Centre, Bombay, India. All other chemicals and solvents used were of analytical grade.

Animals. Male albino Wistar rats weighing 90-100 g were purchased from the Frederick Institute of Plant Protection and Toxicology, Padappai, Madras. They were housed in a well-aerated room and acclimatized to laboratory conditions for 1 week prior to use. Food and water were provided ad libitum.

Administration of chloroquine. The animals were divided into 2 groups. The experimental rats were orally administered CQ, dissolved in water, for 14 days. The dosage (100 mg/kg body weight/day) was selected according to Reasor and Hostetler [8]. For experiments on acetate incorporation, rats already treated with CQ as above and control rats were each administered [14C]acetate (5 μCi [41.9 mCi/mmol specific activity]/100 g body weight), suitably diluted in 50 mM unlabelled sodium acetate, intraperitoneally, according to Pugalendhi et al. [9].

Preparation of microsomes. The animals were fasted overnight and sacrificed by cervical dislocation 24 h after the last dose. For incorporation studies, the rats were killed 4 h after i.p. injection of [14C]acetate. The liver was homogenized in four volumes of ice-cold medium containing 1.15% KCl and 0.02 M Tris-HCl buffer, pH 7.4. The hepatic microsomes were prepared by differential centrifugation [10]. The homogenate was centrifuged for 15 min at 9,000 × g, and the resulting supernatant was centrifuged at 105,000 × g for 60 min to obtain the microsomes. The pellet was resuspended in 0.1 M Tris-HCl, pH 7.4. Activities of succinate dehydrogenase and acid phosphatase were assayed in the resuspended pellet to evaluate the respective mitochondrial and lysosomal contamination, if any.

Analytical procedures. Microsomal protein was estimated by the method of Lowry et al. [11]. Microsomal cytochrome P450 and cytochrome b5 contents were determined by the method of Omura and Sato [12]. NADPH cytochrome c reductase activity was assayed at 25°C by measuring the rate of reduction of cytochrome c at 550 nm [13]. NADH microsomal cytochrome reductase as assayed according to Strittmatter and Velick [14].

Analysis of lipids. Microsomal lipids were extracted by the method of Folch et al. [15]. Microsomal phospholipids [16, 17] and cholesterol [18] contents were then quantified. Phospholipids were separated according to Valimaki et al. [19] by TLC using chloroform : methanol : 6 M ammonia (65 : 35 : 5, v/v) as the solvent system. Iodine vapors were used to locate the respective fractions. The phosphate content of the various fractions was assayed according to Bartlette [17]. For experiments in which [14C]acetate was administered, individual phospholipid components were scraped off the TLC plates into scintillation vials and radioactivity estimated by use of a LKB-Pharmacia liquid scintillation counter (Sweden).
Fluidity measurement. Fluidity was measured by fluorescence polarization, with 1,6-diphenyl-1,3,5-hexatriene (DPH) used as the fluorescent probe [20, 21]. A 25-μl volume of membranes (microsomes) suspended in Tris-HCl buffer (0.1 M, pH 7.4) was taken in 3.0 ml of the Tris-HCl buffer, and 5 μl of 2 mM DPH solution in tetrahydrofuran was added to it. The mixture was mixed thoroughly for 20 min and kept at room temperature for another 30 min in the dark. Steady-state fluorescence polarization was measured at 37°C with an Amino Bowman spectrofluorimeter equipped with polarizers (excitation wavelength, 360 nm; emission wavelength, 428 nm). A fluorescence intensity value for the blank was subtracted as a correction for scattered light. Fluorescence polarization, \( P \), was calculated from the relation [22, 23] 

\[
P = \frac{I_{vv} - I_{vh}}{I_{vv} + I_{vh}},
\]

where \( I_{vh} \) and \( I_{vv} \) are the intensities of the horizontal and vertical polarized light, respectively.

Statistical evaluation. Comparisons between control and CQ-treated groups were made by Student’s \( t \) test. \( p \) Values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The data presented herein show that the administration of CQ at a level of 100 mg/kg body weight/day for 14 days resulted in an increase in liver microsomal phospholipids (Table 1). Phenobarbitone, a well-known inducer of microsomal enzymes, increases total microsomal phospholipid [24]. trans-Stilbene oxide, another inducer of microsomal enzymes, decreases the cholesterol content of the endoplasmic reticulum, thereby increasing membrane fluidity [25]. The significant increase in liver microsomal phospholipids and decrease in cholesterol (Table 1) led to an increase in membrane fluidity (Table 1), which affects the membrane functions, and therefore, the activities of the drug-metabolizing enzymes associated with it. We consider the increase in content in all the different phospholipid fractions (Table 2) to be due to an increased incorporation of acetate into microsomal phospholipids (Fig. 1).

Table 1. Effect of chloroquine on rat hepatic microsomal lipids, enzymes, and fluidity of the microsomal membrane at 37°C.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CQ-treated</th>
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<tbody>
<tr>
<td>Cholesterol (μg/mg protein)</td>
<td>29.7±0.85</td>
<td>25.9±2.82*</td>
</tr>
<tr>
<td>Phospholipid (μg/mg protein)</td>
<td>315±33</td>
<td>461±51***</td>
</tr>
<tr>
<td>Cytochrome P450 (nmol/mg protein)</td>
<td>0.58±0.05</td>
<td>0.72±0.10*</td>
</tr>
<tr>
<td>Cytochrome b₅₆ (nmol/mg protein)</td>
<td>0.171±0.016</td>
<td>0.204±0.020*</td>
</tr>
<tr>
<td>NADPH cytochrome c reductase (units/g protein)</td>
<td>93±17.1</td>
<td>135±28.3*</td>
</tr>
<tr>
<td>NADH cytochrome reductase (units/mg protein)</td>
<td>0.50±0.16</td>
<td>0.83±0.21*</td>
</tr>
<tr>
<td>Microsomal membrane fluidity (polarization×10⁻²)</td>
<td>2.9±0.18</td>
<td>5.2±0.58***</td>
</tr>
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</table>

Values are means±SD for 6 rats in each group. *\( p<0.05 \), **\( p<0.001 \) vs. control.
The cytochrome P450 monoxygenase system plays a central role in the oxidation of a wide variety of structurally unrelated compounds, including exogenous compounds such as pharmaceutical agents, chemical carcinogens, and other lipophilic xenobiotics as well as endogenous steroids and fatty acids [26, 27]. The observed increase in cytochrome P450 (Table 1) may be attributed to the above-mentioned increase in phospholipids, since lipid is essential for the enzymatic reduction of hemoprotein P450 [28]. Earlier reports have shown that the increased biotransformation of SRC-909 by phenobarbital or CQ pretreatment in rats indicates their P450 inducing capacity [29].

Studies in several laboratories have shown that cytochrome b₅ [30], which functions as an electron carrier between NADH cytochrome b₅ reductase and the terminal desaturase in the fatty acyl-CoA desaturase system [31, 32], may play a role in cytochrome P450-dependent reactions in microsomal membranes. Hence, the observed increase in cytochrome b₅ content (Table 1) may increase the activities of P450-dependent reactions.

The mixed-function oxidases are a membrane-bound enzyme system consisting of a source of NADPH, a flavoprotein reductase (cytochrome P450 reductase), and a hemoprotein (cytochrome P450), linked by a zwitterionic lipid, fluid

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Table 2. Effect of chloroquine on different fractions of rat hepatic microsomal phospholipids (μg/g tissue).

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>CQ-treated</th>
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<tbody>
<tr>
<td>Total phospholipids</td>
<td>5,962 ± 426</td>
<td>9,186 ± 812**</td>
</tr>
<tr>
<td>Lysophosphatidylcholine</td>
<td>565 ± 62</td>
<td>931 ± 105**</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>2,980 ± 214</td>
<td>4,910 ± 401**</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>103 ± 14</td>
<td>169 ± 21*</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>514 ± 49</td>
<td>727 ± 69*</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>1,130 ± 98</td>
<td>1,862 ± 201**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for 3 rats in each group. *p < 0.05, **p < 0.01 vs. control.
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coupler, phosphatidylcholine, which appears to be essential for the enzymic activity [33]. NADPH cytochrome P450 reductase participates in the metabolism of drugs, especially of those containing the quinone structure [34].

The increase in drug-metabolizing enzymes (Table 1) might be attributed to an increase in phospholipids, since CQ is a known phospholipidotic agent [35]. Jones and Wakil [36] have described a requirement for phospholipids by the microsomal NADH cytochrome reductase. Thus an increase in phospholipid content may lead to an increase in the activity of this enzyme. Electron transfer from NADPH to hemoprotein P450 was shown to be completely dependent upon the presence of microsomal lipid [28]. Therefore, the increase in microsomal lipid may have resulted in the increased activity of NADPH cytochrome c reductase. Hence, we assume that there is a relationship between CQ-induced phospholipidosis and drug-metabolizing enzymes.

Thus, CQ-induced increase in drug-metabolizing enzymes would lead to increased levels of metabolites of CQ, which are also associated with toxicity [1]. The therapeutic use of CQ has resulted in death from poisoning by the drug, which has been attributed to an effect on the cardiovascular system [37]. Essien and Ette [38] reported didesethylchloroquine to have greater potency than CQ in producing cardiodepression, and attributed this effect to its molecular structure. Hence, the toxic side effects of CQ and its metabolites need to be carefully considered in view of its wide therapeutic use.

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