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Effects of chloroquine and primaquine on rat liver cytosolic *N*-acetyltransferase activity

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N-Acetyltransferase (NAT*) catalyzes the acetylation of a wide number of xenobiotics. The ability to acetylate xenobiotics has been shown to be a significant determinant in the predisposition to toxicity from several agents [1]. Alterations in acetylation, therefore, may have significant consequences.

While genetic factors appear to constitute the primary cause of variability in acetylation capacity, several drugs may also influence the ability to acetylate xenobiotics. It was reported recently that chloroquine may alter hepatic NAT activity in the rat [2]. In particular, single- and multiple-dose pretreatment with chloroquine was reported to reduce the acetylation of isoniazid (INH) and sulphadimidine (SDD) *in vivo*. Moreover, *in vitro* addition of chloroquine reduced the activity of NAT. Since the structure of chloroquine would not lead one to anticipate an interaction with NAT, we attempted to confirm the effect of this antimalarial on NAT activity.

* Abbreviations: INH, isoniazid; NAPA, *N*-acetylprocainamide; NASDD, *N*-acetylsulphadimidine; NAT, *N*-acetyltransferase; PA, procainamide; and SDD, sulphadimidine.

Methods

Chemicals. Procainamide (PA) was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Analytical standards of PA and *N*-acetylprocainamide (NAPA) were gifts from E. R. Squibb & Sons, Inc. (Princeton, NJ). *N*-Proprionylprocainamide, chloroquine, primaquine, acetyl-CoA, acetyl carnitine, and carnitine *O*-acyltransferase (EC 2.3.1.7) were purchased from the Sigma Chemical Co. (St. Louis, MO) and used as received.

Effects of antimalarials on NAT in vitro. The liver was removed from an untreated male Sprague–Dawley rat under ether anesthesia. Liver homogenate was prepared as described [3], and the 100,000 *g* supernatant (cytosol) was used as the source of NAT.

Cytosolic NAT activity was determined utilizing PA as the substrate in the presence of the required co-substrate acetyl CoA (0.42 mM). Incubation concentrations of PA were 0.15, 0.30 and 0.60 mM. Chloroquine or primaquine was added to a final concentration of 0.60 or 0.30 mM, respectively. Incubation mixtures (in quadruplicate) were brought to a volume of 900 μL with Sorensen phosphate buffer and maintained at 34° in a temperature-controlled water bath. Reactions were initiated by addition of 100 μL cytosol to each incubation mixture. Aliquots (100 μL) were withdrawn from the mixtures at 10 min and added to centrifuge tubes containing 1 M NaOH to terminate the reaction. NAPA concentration in the aliquots was determined by an HPLC method described previously [4]. NAT activity is expressed as nanomoles NAPA formed per minute per milligram of cytosolic protein.

Effect of chloroquine pretreatment on NAT activity ex vivo. Male Sprague–Dawley rats weighing 210–225 g received saline or chloroquine (10 mg/kg/day) intraperitoneally for 4 days prior to being killed. NAT activity was determined using a modification of the method described above which incorporated an acetyl CoA recycling system [5]. The recycling system consisted of acetyl carnitine (5 mM) and carnitine *O*-acyltransferase (1 unit/mL), with the incubation concentration of acetyl CoA reduced to 0.1 mM. Apparent K_m and V_{max} values were determined by fitting the data to the Michaelis–Menten equation using a nonlinear least-squares regression program (PC-NONLIN) with a weight of $1/y^2$.

Results and Discussion

NAT activity toward PA was not altered by the *in vitro* addition of chloroquine at equimolar concentrations (Fig. 1). Chloroquine at two to four times the PA concentration reduced NAPA formation by less than 10%. In contrast, the antimalarial primaquine (for which N-acetylation is a minor elimination pathway [6]) resulted in a 42–60% decrease in NAPA formation when incubated in cytosol at one-half to twice the concentration of PA (Fig. 1).

The potential for an indirect effect of chloroquine on NAT was examined by determining the apparent Michaelis–Menten parameters for NAT activity towards PA in liver cytosol from animals pretreated with chloroquine (10 mg/kg/day) for 4 days. The apparent K_m and V_{max} values in control and chloroquine-pretreated animals were essentially identical (Table 1). This experiment was repeated in a second group of animals (N = 5 in each group) with the same results (data not shown).

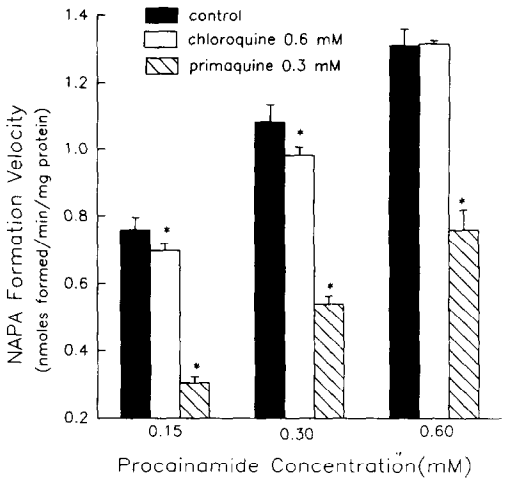

Fig. 1. Effect of *in vitro* addition of chloroquine or primaquine on the formation of *N*-acetylprocainamide (NAPA) by rat hepatic cytosol. Values are means of four determinations; bars represent 1 SD. Key: (*) $P < 0.05$ compared to control, using ANOVA with Tukey's test for repeated measures.

In the present investigation, the magnitude of reduction (<10%) in NAT activity following *in vitro* addition of chloroquine was much less than that reported by Nwankwo *et al.* [2] (66%). One difference between the two investigations is the model substrates chosen to probe NAT activity. For *in vitro* studies we utilized PA, whereas Nwankwo *et al.* [2] used INH. The observation that INH is a competitive inhibitor of PA acetylation in this model system [3] would suggest, however, that metabolism by different isozymes of NAT cannot explain the noted discrepancy.

Nwankwo *et al.* [2] also reported reductions in NAT activity *in vivo*, as measured by the urinary excretion of the acetylated metabolites of INH and SDD. In contrast, we found no effect of chloroquine pretreatment on NAT activity. In addition, studies in our laboratory [7], and

Table 1. Effect of chloroquine pretreatment on body weight, liver weight and apparent Michaelis–Menten parameters for *N*-acetyltransferase activity toward procainamide in rat liver cytosol*

Parameter	Control† (N = 5)	Chloroquine (N = 6)
Body weight (g)—Day 1	219 ± 4‡	218 ± 5
Body weight (g)—Day 5	247 ± 5	238 ± 13
Liver weight (g)	12.1 ± 1.2	10.5 ± 0.9§
$K_m \times 10^{-4}$ M	1.20 ± 0.40	1.00 ± 0.60
V_{max} (nmol NAPA formed/min/mg protein)	0.54 ± 0.04	0.52 ± 0.08

* Chloroquine (10 mg/kg/day) or saline was administered intraperitoneally for 4 days and animals were killed 24 hr after the last dose.

† Apparent Michaelis–Menten parameters were determined at an initial incubation acetyl CoA concentration of 0.1 mM in the presence of 5 mM acetylcarnitine and 1 unit/mL carniture *O*-acyltransferase.

‡ Data are means ± SD.

§ $P < 0.05$ compared to control, unpaired *t*-test.

reported by others [8], indicate that the total recovery of SDD and its acetylated metabolite (NASDD) in the rat is too low for this compound to be used as a model substrate for acetylation *in vivo*. Furthermore, although Nwankwo *et al.* reported that chloroquine reduces the deacetylation of NASDD *in vivo*, we were unable to detect any deacetylated compound after the administration of NASDD [7].

In summary, chloroquine caused only slight reductions in NAT activity when added *in vitro*, and had no detectable influence when animals were pretreated with it for 4 days. This would suggest that the previously reported reduced excretion of acetylated metabolites of INH and SDD following chloroquine pretreatment is not the result of inhibition of NAT. In contrast, we found that primaquine significantly ($P < 0.05$) reduced NAT activity when added *in vitro*, suggesting the need for further study with this agent.

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Comparison of the chromatographic characteristics of metabolites of tacrine hydrochloride in human serum and urine with those of *in vitro* metabolic products from hepatic microsomes

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Tacrine hydrochloride (THA) is a centrally acting cholinesterase inhibitor under investigation as a treatment for Alzheimer's disease [1–3]. It is unclear whether the adverse effects reported [4–6] are attributable to the parent compound or to a metabolite. Further development of this line of agents may depend upon an understanding of their metabolism and the structure and pharmacology of their metabolites. Kaul [7] found four metabolites of THA in rat urine using paper chromatography. The ultraviolet spectra of two resembled THA, one of which in the infrared spectrum revealed a cyclic carbonyl group. He suggested the formation of this C=O function could be through oxidative deamination which in biological systems would involve pyridoxal as the co-factor.

Metabolic products of THA have been observed in human and animal studies [5, 8, 9] using HPLC with ultraviolet or fluorescence detection. Their serum concentrations in patients receiving THA have been documented by this technique [10, 11]. Hsu *et al.* [12] have measured three metabolites in rat plasma which they postulate to be hydroxylated products.

Summers *et al.* [5] described a rapid conversion *in vivo* of THA to an apparent 1-hydroxy metabolite in rat, monkey and man. Hendrickson *et al.* [9] incubated rat hepatic microsomes with THA and added NADPH. One of the metabolites produced was identified tentatively as 9-hydroxylamine 1,2,3,4-tetrahydroacridine by *in situ* electrochemical characterization.

We describe here the metabolites found in human serum and urine and our attempt to produce corresponding metabolites *in vitro* from rat hepatic microsomes.

Materials and Methods

A Shimadzu liquid chromatography pump and RF535 fluorescence HPLC monitor (Dyson Instruments, Hetton, U.K.) and a Pye-Unicam CDP4 computing integrator (Philips Scientific, Cambridge, U.K.) were used with a 250 × 4.6 mm i.d. Shandon Hypersil 5ODS column (HPLC Technology, Macclesfield, U.K.) fitted with a Rheodyne 7125 valve (Dyson Instruments) and 100 μL loop. The excitation and emission wavelengths were 330 and 365 nm. The mobile phase was methanol–distilled water