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Aminoquinoline antimalarials—Paradoxical regulation of hepatic tryptophan oxygenase and tyrosine aminotransferase by primaquine*

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THE 4-AMINOQUINOLINE antimalarials (of which chloroquine is a prototype) differ from the 8-aminoquinolines (of which primaquine is a prototype) in that the 4-aminoquinolines (4-AQ's) in general affect primarily the asexual erythrocytic forms of *Plasmodia*, whereas the 8-aminoquinolines (8-AQ's) generally affect the primary and secondary tissue (exoerythrocytic) stages of the parasite.[1] The mechanism of schizontocidal action of the 4-AQ's has been widely attributed to result from their binding to plasmodial DNA, and its consequent inhibition of DNA synthesis and function in replicative processes.[2–4] While the 8-AQ's have recently been demonstrated to interact with DNA *in vitro*,[5] it had been proposed earlier that antimalarial agents of this class are schizontocidal due to inhibition of electron transport systems in the parasites.[6] However, it was shown that both chloroquine and primaquine completely inhibited ^{32}P (orthophosphate) incorporation into DNA and RNA of *Plasmodia* at relatively high (10^{-4} M) concentrations *in vitro*.[7] It has also been found recently that both primaquine and chloroquine appreciably inhibited uridine-^{3}H incorporation *in vivo* into mouse liver RNA.[8] These findings would support a common mechanism of antimalarial action of both the 4-AQ's and the 8-AQ's, namely, inhibition of gene synthesis or function, or of both.

Chemical agents (physiologic substrates or drugs) which interact with DNA generally produce, if anything, either inhibition of DNA function and replication, e.g. actinomycin-D,[9] mitomycin C,[10] or opposite "anabolic" effects, e.g. steroids in certain tissues[11–14] or carcinogens such as benzpyrene.[15,16] Drugs of the actinomycin-D type inhibit induced enzyme synthesis by a mechanism generally believed to result from their interaction with DNA, and subsequent inhibition of genome-directed RNA synthesis.[9] Since chloroquine and primaquine both possess some actinomycin-D-like properties (see above), it was postulated that either or both of these drugs should inhibit genomic function *in vivo*. As a measure of genomic function *in vivo*, the hydrocortisone inducibility of two hepatic enzymes,

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TABLE 1. EFFECT OF CHLOROQUINE OR PRIMAQUINE ON INDUCTION OF HEPATIC TRYPTOPHAN OXYGENASE (TPO) AND TYROSINE AMINOTRANSFERASE (TAT) IN INTACT RATS

Treatments	Enzyme units/g liver (± S.E.)*	
	TPO	TAT
Control rats†	3·9 ± 0·4	68 ± 8
Chloroquine (CQ)‡	7·0 ± 0·6	97 ± 7
Primaquine (PQ)‡	9·7 ± 0·6	184 ± 11
Hydrocortisone§	15 ± 1·2	219 ± 23
Chloroquine‡. ‖ + Hydrocortisone§	18 ± 1·0	268 ± 6
Primaquine‡. ‖ + Hydrocortisone§	19 ± 0·8	382 ± 20

* Any two means joined by the same bracket do not differ significantly ($P > 0·05$) according to Student's *t*-test (two-tailed distribution). Each mean is representative of six animals.

† Propylene glycol, 2 ml/kg, subcutaneously for 6 hr.

‡ 100 μmoles/kg (CQ diphosphate = 52 mg/kg; PQ diphosphate = 46 mg/kg ip., 6·5 hr).

§ 83 μmoles/kg (30 mg/kg) subcutaneously for 6 hr (in propylene glycol).

‖ Administered 30 min prior to hydrocortisone.

TABLE 2. EFFECT OF CHLOROQUINE OR PRIMAQUINE ON INDUCTION OF HEPATIC TRYPTOPHAN OXYGENASE (TPO) AND TYROSINE AMINOTRANSFERASE (TAT) IN ADRENALECTOMIZED RATS

Treatments	Enzyme units/g liver (± S.E.)*	
	TPO	TAT
Control rats†	1·8 ± 0·1	18 ± 3
Chloroquine‡	1·2 ± 0·2	22 ± 6
Primaquine‡	2·0 ± 0	21 ± 1
Hydrocortisone§	17 ± 1·0	193 ± 15
Chloroquine‡ + Hydrocortisone‖	17 ± 1·8	287 ± 45
Primaquine‡ + Hydrocortisone‖	15 ± 1·5	376 ± 50

* Any two means joined by the same bracket do not differ significantly ($P > 0·05$) according to Student's *t*-test (two-tailed distribution). Each mean is representative of six animals.

† Propylene glycol, 2 ml/kg, subcutaneously for 6 hr.

‡ 100 μmoles/kg (see footnote ‡ in Table 1) at zero time and again at 12 hr; total time was 18 hr.

§ 83 μmoles/kg (30 mg/kg) subcutaneously for 6 hr (in propylene glycol).

‖ Administered 12 hr after the first aminoquinoline injection.

tryptophan oxygenase (TPO, EC 1.13.1.12) and tyrosine aminotransferase (TAT, EC 2.6.1.5), was studied in rats receiving the antimalarial compounds.

Male Sprague–Dawley rats weighing 150–250 g were obtained from Zivic-Miller Laboratories (Allison Park, Pa.) and were maintained on tap water (or 0·9% NaCl if adrenalectomized) and Purina Laboratory Chow. After drug treatments, animals were killed between 11:00 a.m. and 3:00 p.m. in order to minimize diurnal effects on enzyme activity.[17,18]

For enzyme assay, livers (perfused free of blood *in situ* with ice cold saline, and frozen on solid CO_2) were thawed and homogenized in 4 vol. of ice cold 0·15 M KCl containing 1 mM Na_2 EDTA and 5 mM Tris HCl (pH 7·4 at 2°). TPO was assayed in hepatic supernatant fractions which were prepared by centrifugation at 12,000–40,000 *g* for 10 min in hematin-activated (10^{-5} M) preparations by the Knox–Feigelson method.[19] TAT was assayed in supernatant fractions (spun likewise, but

for 20 min) by the method described by Diamondstone ,[20] except that assay tubes were incubated for 20 min at 30°. A unit of enzyme activity is defined as that amount required to catalyze the formation of 1 μmole of product during the period of incubation.

The administration of hydrocortisone for a 6-hr period to intact rats produced, as expected, a 3·9-fold increase in hepatic TPO activity and a 3·2-fold increase in TAT activity over control values (Table 1). However, the administration of either chloroquine or primaquine produced an unexpected increase in the activity of both enzymes (Table 1). Chloroquine pretreatment (30 min prior to hydrocortisone) did not inhibit either TPO or TAT induction by hydrocortisone. However, primaquine appeared to enhance significantly the inducing action of hydrocortisone on TAT. In the latter group, enzyme activity was approximately double that obtained with hydrocortisone alone (Table 1). It is noteworthy that, at the dose of aminoquinolines used (100 μmoles/kg), primaquine displayed greater activity than did chloroquine as a stimulant of basal enzyme levels.

The unexpected intrinsic stimulatory effect of the aminoquinolines on hepatic TPO and TAT was suggestive of an adrenocortical involvement, since these enzymes are of the "stress-inducible" type.[21] The experiment described in Table 1 was repeated with the following modifications: (1) adrenalectomized rats were used; and (2) the aminoquinolines were administered in two doses of 100 μmoles/kg each, 12 hr apart. Hydrocortisone was administered at the time of the second aminoquinoline injection. Under these conditions (i.e. adrenalectomy, etc.), the intrinsic TPO and TAT stimulating actions of both aminoquinolines were abolished (Table 2). Such a regimen also failed to produce inhibition of TPO induction by hydrocortisone, but did not abolish the superinduction of TAT in the primaquine plus hydrocortisone group (Table 2). This latter finding suggested that the superinduction of TAT in the primaquine plus hydrocortisone group was due to a true potentiation of hydrocortisone by primaquine.

The experiments described in this report were performed in an attempt to test the hypothesis that aminoquinoline antimalarials would inhibit enzyme induction as a measure of inhibited gene function. It is apparent from the data presented that, in spite of the widely described effects of aminoquinoline antimalarials (in particular, chloroquine) on nucleic acids, gene function (at least in rat liver) may not be significantly inhibited. This would tend to suggest that these drugs may have antimalarial actions which might be explained in terms other than those resulting from drug–DNA interaction. However, since the data presented in this report may have no bearing on the understanding of antimalarial drug action, such a proposition must be tempered with the possibility that biochemical events in rat liver may not be a suitable experimental model from which to extrapolate to a plasmodial system. Furthermore, cistrons other than those coding for TPO and TAT in fact may be inhibited, but our relatively narrow choice of inducible enzymes as models for the study of gene expression would not have detected such sites. However, the induction of these enzymes by hydrocortisone is adequately abolished by actinomycin D.[9] It is also apparent, with these same reservations considered, that host toxicity of these drugs may not necessarily be manifested as inhibited genomic function. Indeed, primaquine was found to promote rather than to inhibit TAT induction in rat liver.

While the mechanism of this induction-promoting phenomenon is unclear as yet, we have found additionally that primaquine does not affect the apparent rate of induced enzyme (TAT) decay (e.g. Refs. 22–25). That is, the exponential decay rates of TAT activity in hydrocortisone only or hydrocortisone plus primaquine treatment groups were both equivalent to a $T_{1/2}$ of 2 hr. Neither can this paradoxical effect be explained in terms of inhibition of hydrocortisone metabolism (e.g. Refs. 26, 27). Whereas primaquine added *in vitro* to a hepatic 10,000 *g* supernatant system* completely inhibited hydrocortisone α- and β-ring-A reductases at a concentration of $1{\cdot}5 \times 10^{-4}$ M, the drug retained its ability to potentiate hydrocortisone induction of TAT in rats receiving maximally effective doses (90 mg/kg) of the hormone. Indeed, if inhibition of hydrocortisone metabolism *in vivo* were occurring with primaquine to any appreciable extent, then the induction of TPO would also have been enhanced, but in fact was not. This follows because the dose of hydrocortisone used in this study is only approximately 70 per cent of that necessary to maximally induce TPO. Thus it would seem that the superinduction of TAT by hydrocortisone in primaquine-treated rats may be related to early events in the induction process and may be a phenomenon unique to TAT, e.g. by removal of an inhibiting regulatory process for TAT by primaquine similar to the paradoxical action of actinomycin D on TAT as reported by Garren *et al.*[30] and Tomkins *et al.*[31] However, this "paradoxical action" of actinomycin D on TPO and TAT has been challenged by others.[32–35] The relationship between

* Tris HCl (0·1 M, pH 7·6, at 37°), NADPH (0·1 mM), hydrocortisone sodium succinate (0·2 mM), rat liver 10,000 *g* supernatant equivalent to 25 mg liver, in a volume of 2·0 ml, assayed directly against a substrate-free blank at 37° by loss of absorbance at 248 mμ (indicative of reduction of steroid ring A[28,29]) in a Gilford 2400 spectrophotometer using a baseline absorbance offset technic and continuous recording.

this enigmatic action of primaquine and its ability to interact with DNA is not presently clear and is currently under investigation.

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