

## Effect of a new 8-aminoquinoline antimalarial compound on hepatic microsomal mixed function oxidase system of mice

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A new 8-aminoquinoline derivative (compound 80/53) synthesized at the Central Drug Research Institute, Lucknow (India), has been found to be an active anti-relapse (tissue schizontocidal) compound. Compound 80/53 at 8.75 mg/kg  $\times$  4 days and primaquine at 7.00 mg/kg (base)  $\times$  4 days given orally to Swiss mice led to inhibition of the different components of the hepatic microsomal mixed function oxidase system to varying degrees. Compound 80/53 inhibited cytochrome P-450, aminopyrine-N-demethylase, aniline and benzo (a) pyrene hydroxylase, cytochrome b5 and heme content of the normal mice by 12, 14, 0, 57, 20 and 6 per cent respectively, whereas the inhibition caused by primaquine in these components was 25, 21, 17, 48, 26 and 6 per cent respectively. Thus, there was less inhibition of hepatic microsomal MFO system of mice by compound 80/53 as compared to that by primaquine.

A variety of compounds representing several classes of nitrogen heterocycles including quinolines are known to be established inhibitors of microsomal drug metabolising system. The hepatic microsomal mixed function oxidase system responsible for the metabolism and disposition of a variety of endogenous as well as exogenous compounds, consists of mainly cytochrome P-450, the terminal mono-oxygenase and a number of associated indices as aniline hydroxylase, aminopyrine-N-demethylase, arylhydrocarbon [benzo (a) pyrene] hydroxylase etc. Primaquine, the most important member of the 8-aminoquinoline group, is the only drug which is used in the treatment

of relapsing malaria, in spite of its known toxic effects on the host system such as induction of methaemoglobinaemia, haemolytic anaemia in G-6-PD deficient cases and toxicity in pregnant women. Primaquine and other 8-aminoquinolines, are also known to produce oxidative stress, and inhibit various components of hepatic microsomal mixed function oxidase (MFO) system both *in vivo* and *in vitro*<sup>1-5</sup>. A new compound 80/53 (Fig.) synthesized in the Central Drug Research Institute, Lucknow (India) is also an 8-aminoquinoline derivative which has primaquine like anti-relapse activity<sup>6</sup>. The effect of this compound on hepatic microsomal MFO system of mice has been compared with

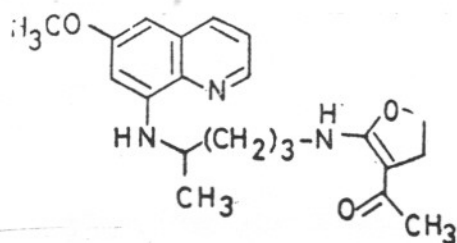


Fig. Compound 80/53.

that of primaquine, and the results are presented in this communication.

### Material & Methods

**Animals** : Colony bred albino mice (Swiss strain) weighing about 20-25 g, maintained in an air conditioned room (temperature  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ) free from infection, were used. Mice were used in three groups. One group of animals was given primaquine (7.00 mg base/kg/day), while the second group was given compound 80/53 (8.75 mg/kg/day) orally, for four consecutive days. These doses represent<sup>6</sup> the total course dose for anti-relapse efficacy against *Plasmodium cynomolgi* B. The control group was given the vehicle *i.e.*, normal saline, in the same manner as in the other two groups.

Overnight fasted mice of control and experimental groups were sacrificed by decapitation, and livers were excised and washed with chilled buffered KCl (150 mM KCl in 10 mM  $\text{NaH}_2\text{PO}_4$ - $\text{K}_2\text{HPO}_4$  buffer, pH 7.4) and homogenates (20%, w/v) were prepared using Potter Elvehjem homogeniser. Livers of three animals were pooled for each set of observation. The homogenates were centrifuged at  $11,000 \times g$  (20 min) to collect mitochondrial fraction, and the supernatant containing post-mitochondrial fraction were centrifuged at  $105,000 \times g$  (60 min) for obtaining microsomal pellet. Microsomes

were prepared according to Moule *et al*<sup>7</sup> and used immediately for various biochemical assays. All the procedure of homogenization and tissue fractionation were carried out at  $0-4^{\circ}\text{C}$ .

**Assay of enzymes** : Aniline hydroxylase<sup>8</sup>, aminopyrine-N-demethylase<sup>9</sup> and benzo (a) pyrene (arylhydrocarbon) hydroxylase<sup>10</sup> were assayed in post-mitochondrial fraction while cytochrome P-450, cytochrome b5 and heme were measured in the microsomal fraction according to the standard procedure<sup>11</sup>, using Shimadzu double beam recording spectrophotometer. Protein in the samples was measured according to Lowry method using bovine serum albumin as standard<sup>12</sup>. Statistical analysis was done by the Student's 't' test.

### Results & Discussion

The components of hepatic microsomal mixed function oxidase system of mice were inhibited to varying extent by the oral feeding of primaquine or compound 80/53. The post-treatment changes in various parameters investigated during the study were highly significant ( $P < 0.002$  to  $> 0.001$ ), as compared to that of untreated controls.

Primaquine-fed mice lost 12-15 per cent of their weight whereas the weight loss in mice treated with compound 80/53, was comparatively less ( $< 10\%$ ). The level of cytochrome P-450 decreased by 25 and 12 per cent in primaquine and 80/53 treated mice respectively (Table). The inhibition of aminopyrine-N-demethylase was more with primaquine while the benzo (a) pyrene hydroxylase activity was slightly more suppressed by compound 80/53. The inhibition of hepatic microsomal N and O dealkylation, which is mediated through cyt P-450 and

**Table.** Effect of primaquine and compound 80/53 on the hepatic microsomal mixed function oxidase system of mice

(Data are mean  $\pm$  SD of different observations (6 in number in each group each parameter))

| Parameter                                    | Normal            | Primaquine        | Compound 80/53     |
|----------------------------------------------|-------------------|-------------------|--------------------|
| 1. Cytochrome P-450                          | 1.29 $\pm$ 0.06   | 0.97 $\pm$ 0.08   | 1.13 $\pm$ 0.03*   |
| 2. Aminopyrine-N-demethylase                 | 1.28 $\pm$ 0.03   | 1.01 $\pm$ 0.11   | 1.10 $\pm$ 0.06†   |
| 3. Aniline hydroxylase                       | 0.06 $\pm$ 0.004  | 0.05 $\pm$ 0.004  | 0.07 $\pm$ 0.002*  |
| 4. Benzo (a) pyrene hydroxylase <sup>a</sup> | 0.213 $\pm$ 0.015 | 0.110 $\pm$ 0.033 | 0.087 $\pm$ 0.013† |
| 5. Cytochrome b5                             | 0.46 $\pm$ 0.04   | 0.34 $\pm$ 0.02   | 0.37 $\pm$ 0.02**  |
| 6. Heme                                      | 2.20 $\pm$ 0.05   | 1.95 $\pm$ 0.14   | 2.06 $\pm$ 0.07†   |

Activities are expressed as nmol product formed/min/mg protein; <sup>a</sup>change in relative fluorescence/min/mg protein.

Significance (primaquine vs 80/53) *P* values, \* < 0.001; \*\* < 0.02; † < 0.1

cyt P-448 mono-oxygenases, by primaquine is reported earlier<sup>1</sup>. It was surprising to note that in spite of being a potent inhibitor of benzo (a) pyrene (arylhydrocarbon) hydroxylase, compound 80/53 had not affected the aniline hydroxylase. The extent of inhibition, however, was significantly lower than that in benzo (a) pyrene hydroxylase. Both the compounds inhibited cytochrome b5 and levels of heme, the effect being more in primaquine treated mice as compared to the 80/53 fed animals. It is known that different primaquine derivatives (as N-acetyl and 5-hydroxy derivatives) and its metabolites (as carboxylic acid metabolites) effect the drug metabolising system of host to a varying extent<sup>4</sup>. Compound 80/53, which is an enamine analogue of primaquine, also inhibits different components of mixed function oxidase system to varying degree. The inhibition was less as compared to primaquine except in the case of benzo(a)pyrene hydroxylase where compound 80/53 was more inhibi-

tory. The present study clearly establishes that the new antimalarial compound is comparatively less hepatotoxic and offers promising potential as an antimalarial. It may be mentioned that the compound is also safe, as assessed by methaemoglobin toxicity in beagle dogs which was 3-4 times lower, in comparison to primaquine<sup>6</sup>.

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