# Studies on the effects of phenobarbital and endotoxin on the toxicity and metabolism of 6-mercaptopurine in mice

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*16 November 1976*

*Summary.* The administration of phenobarbital and endotoxin altered the toxicity and metabolism of 6-mercaptopurine in mice. These results indicate that the effects of 6-mercaptopurine may be modified by the activity of the drug-metabolizing enzyme.

6-mercaptopurine (6-MP) is a clinically useful antitumor drug, but little is known of its action mechanism or pathway of metabolism[2]. Coffey[2] and Walker[3] recently suggested that mechanisms other than xanthine oxidase inhibition could explain the allopurinol-induced potentiation of the antitumor activity of 6-MP. Among the hypotheses suggested for these mechanism is the possibility that pathways other than 6-MP oxidation may be responsible for the catabolism of 6-MP. Interestingly, Yoshimura[4] recently reported that 6-MP can be desulfurated into a biologically inactive substance in vitro by hepatic microsomal enzymes from rats. It is, therefore, of interest to observe the effects of both the metabolic stimulator phenobarbital[5] and the metabolic inhibitor endotoxin[6] on the toxicity and metabolism of 6-NP in mice.

*Materials and methods.* BALB/c male mice weighing 25 to 29 g were used. Endotoxin was purchased as a single lot of lipopolysaccharide from E. coli from Difco Laboratory (Detroit, Mich., USA). Endotoxin, phenobarbital and pentobarbital were dissolved in 0.9% NaCl, and 6-MP was dissolved in 0.1 N NaOH, so that 0.01 ml/g mouse weight would provide the desired dose when administered. Control mice were given an equal volume of 0.9% NaCl. All mice were injected i.p. Each group contained 3–4 mice and all experiments were carried out in duplicate. Livers from the mice treated with [8-$^{14}$C] 6-MP were extracted with 1.5 vol. of cold 0.5 M perchloric acid. Perchloric acid extracts were subjected for determination of thioinosinic acid (TIMP) by the same method previously described[7]. Sodium pentobarbital was administered at a dose of 35 mg/kg. Sleeping time was defined as the time between the loss and the recovery of the righting reflex. Pentobarbital hydroxylase activity was measured by the method of Kato[5] in 8,500 $\times$ g supernatant of 25% liver homogenates prepared in 1.5% KCl. Determination of pentobarbital was carried out according to the method of Brodie[8].

*Results.* BALB/c mice which were administered 100 mg of 6-MP/kg or 4 mg of endotoxin/kg survived the 14-day observation period; however 80% of the mice which received 6-MP 6 h after endotoxin at those doses died within 24 h. By contrast, other mice were treated with 80 mg of phenobarbital/kg each day for 3 days prior to challenge with 250 mg of 6-MP/kg, which was approximately the $LD_{50}$. These mice were resistant to the lethal effects of 6-MP.

6-MP must be converted to TIMP to be biologically active. Therefore, the effect of prior treatment on 6-MP anabolism in the liver was examined. Here the high levels of TIMP in mice treated with endotoxin contrasted sharply with those in control mice. But, in contrast, in mice treated with phenobarbital, TIMP formation was only half of that found in control mice. TIMP concentrations obtained in these experiments were consistent with the lethality of the combinations (table 1).

It is well known that phenobarbital[5] and endotoxin[6] alter the hepatic microsomal enzyme activity. It was found that by pretreatment with endotoxin, sleeping time following pentobarbital was prolonged significantly. On the other hand, administration of phenobarbital in mice reduced the pentobarbital sleeping time. Altered metabolism of pentobarbital by hepatic 8.500 $\times$ g microsomal supernatant fraction correlated well with the effects determined in vivo (table 2).

*Discussion.* From the data collected in these studies, it is

Table 1. The effects of phenobarbital and endotoxin on the lethality and metabolism of 6-MP in mice

| | 6-MP lethality (mg/kg) 0 | 100 | 250 | TIMP formation* (nmoles/g wet weight liver) |
|---|---|---|---|---|
| Control (saline) | | | 7/16 | 21.0 ± 2.0** |
| Phenobarbital (80 mg/kg 3 days) | 0/10 | | 2/16 | 11.4 ± 1.3 |
| p | | | <0.05 | <0.02 |
| Control (saline) | | 0/10 | | 24.3 ± 4.5 |
| Endotoxin (4 mg/kg) | 0/10 | 16/20 | | 52.0 ± 3.2 |
| p | | <0.01 | | <0.01 |

Mice were subjected to the experiments 24 h after the final injection of phenobarbital and 6 h after the injection of endotoxin. * Mice were killed 2 h after the injection of 6 mg of [8-$^{14}$C] 6-MP/kg. ** Data are the mean ± SE of 3 mice.

Table 2. The effects of phenobarbital and endotoxin on the pentobarbital sleeping time and pentobarbital metabolism in mice

| | Sleeping time (min) | Pentobarbital metabolism (nmoles/g wet weight liver/h) |
|---|---|---|
| Control (saline) | 36.3 ± 4.5(3)* | 338 ± 19(4) |
| Phenobarbital (80 mg/kg 3 days) | 0(3) | 473 ± 29(4) |
| p | <0.01 | <0.01 |
| Control (saline) | 43.0 ± 2.1(3) | 321 ± 17(4) |
| Endotoxin (4 mg/kg) | 88.0 ± 13.2(3) | 190 ± 40(3) |
| p | <0.05 | <0.05 |

Mice were subjected to the experiments 24 h after the final injection of phenobarbital and 6 h after the injection of endotoxin. * Data are the mean ± SE of 3 or 4 mice.

clear that the metabolism of 6-MP can be modified by the activity of the drug metabolizing enzyme in mouse liver. In many instances an increased rate of drug metabolism leads to decreased pharmacological action; however, in instances where the metabolite of a drug is more active than the parent compound, enzyme induction can lead to an increase in pharmacological activity of the drug. Since the increased levels in enzyme activity led to decreased toxicity, the present studies also confirmed the result reported by Yoshimura[4] that 6-MP can be catabolized in vitro by hepatic microsomal enzyme from rats.

The major pathway for the catabolism of 6-MP involves oxidation to thiouric acid by xanthine oxidase. An attempt to modify the catabolism of 6-MP by xanthine oxidase led to the development of allopurinol. Elion[9] reported that a reduction in doses of 6-MP was necessary when allopurinol was given concurrently, because allopurinol interferes with the enzymatic oxidation of 6-MP. However, there are some disagreements about the mechanism of the interaction between these drugs.

It has been shown that administration of allopurinol to healthy volunteers impairs the metabolism of antipyrine and coumarine, and further that this effect is caused by a reduction in hepatic microsomal cytochrome P-450 content[10]. In man, the proportion of inorganic sulfate generated is much higher following an oral administration of 6-MP, and lower following administration of allopurinol[9]. Yoshimura[4] also reported that the catabolism of 6-MP by hepatic microsomal enzyme was caused by desulfuration. These results also suggest that detoxification of 6-MP by hepatic microsomal enzyme may be more responsible for the catabolism of 6-MP.

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## ATPase activity in mercury intoxicated eels

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*Summary.* Eels intoxicated by lethal doses of $HgCl_2$ accumulate mercury in their gills. Mercury inhibits the ouabain-sensitive $Na^+K^+$ATPase activity of gills involving the rupture of the fish NaCl balance.

We have previously shown that the lethal effect of mercury ($HgCl_2$) added to seawater on the seawater-adapted eel Anguilla anguilla could be attributed to a disruption of the NaCl balance of the animal[2]. Indeed, when considering fishes, the gill appears to be the most likely site of attack by heavy metals and, in our case, no inhibition of the respiratory processes occurs[3]. Moreover the effects of mercury are similar whether isolated gills or whole animals are intoxicated[3]. It appears then that mercury acts in the gills on the osmoregulatory processes, either active or passive ionic movements. Since the work of Skou[4], there is agreement on the fact that the ouabain-sensitive $Na^+K^+$ATPase has a fundamental role in the active transport[5,6]. In this paper, we have tried to see whether the disruption of NaCl balance could be attributed to an inhibition of the gill ATPase activity.

*Material and methods.* Fresh water eels are adapted for at least 10 days to natural seawater. Each control fish is then placed in a polyethylene bag containing 10 l of continuously oxygenated seawater; the fishes to be intoxicated are trapped in an identical bag containing 10 l of oxygenated seawater with 1 ppm of $HgCl_2$. Both series of eels are kept under these conditions for 30 h. They are then killed and the blood is collected after section of the ventral aorta. The left gills are cut off for the ATPase activity measurement and the right ones for the determination of mercury.

Determination of mercury in the gills. The digestion of the sample is performed following the method described by Sandell[7] and modified by Mayer[8], in order to avoid the loss of mercury associated with the use of the concentrated nitric acid. The analysis of the solution is made by the dithizone technique[7].

Determination of Na, K and Cl in the plasma. The blood is centrifuged at 1085 × g for 10 min (Sorvall S centrifuge). The ions are determined in the supernatant. The analysis of sodium and potassium is performed using an Eppendorf flame photometer. Chloride is determined with the Marius Chlor-o-Counter.

1 The author wishes to express his sincere gratitude to Professor A. Disteche and Professor E. Schoffeniels for discussions during the investigation. This research was carried out in participation in the Belgian National Research and Development Program on the Environment-Water-Sea Project-Office of the Prime Minister-Interministerial Commission for Science Policy and Programmation.

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