Communications to the Editor

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EFFECT OF SAFRAZINE, A MONOAMINE OXIDASE INHIBITOR BEARING A HYDRAZINE-TERMINAL, ON PHENYTOIN METABOLISM IN ISOLATED RAT HEPATOCYTES

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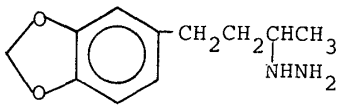
The inhibitory effect of safrazine, an antidepressant monoamine oxidase inhibitor containing a hydrazine terminal, on phenytoin behavior was investigated using isolated rat hepatocytes and rat liver microsomes. Biotransformation of phenytoin to form its oxidation metabolite, 5-phenyl-5-(p-hydroxyphenyl)hydantoin, was greatly inhibited in the liver cells. Examination of microsomes showed that the inhibition mode was a mixed type.

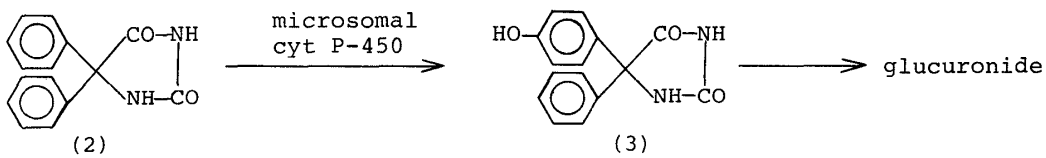
KEYWORDS——phenytoin monooxygenation; inhibition; safrazine; monoamine oxidase inhibitor; hydrazine compound; isolated rat hepatocyte; rat liver microsome

Safrazine (**1**) is the sole monoamine oxidase inhibitor (MAOI) being used only in Japan[1] as an antidepressant effective for some kinds of depression.[1,2] MAOI-antidepressants, such as iproniazid, isocarboxazide, nialamide, iproclozide and phenelzine, used to be employed in Japan and phenelzine is still being used widely in Europe and America. However, they are likely to induce marked hepatic injuries.[1] Yet, until now there have been almost no case reports on hepatitis or any other adverse reactions which have been induced by safrazine.



(1)

These results, of course, may be due partly to the fact that safrazine has not as yet been prescribed so frequently. On the other hand, we have studied the interactions of isonicotinic acid hydrazide (INH), an antituberculotic hydrazine derivative, with microsomal enzymes[3] and we have also studied its hepatotoxically active metabolites.[4] The observations obtained from our experiments suggest that the MAOIs bearing a free or alkyl-substituted hydrazine-terminals may give a rela-



(2) (3)

Chart 1

tively significant rise to reactive intermediates such as radicals, diazene derivatives, or their relatives. These probably contribute to the liver injuries[4] as well as to the inhibition of the drug metabolism catalyzed by the mixed function oxidase.[3] The recognition that safrazine is also a hydrazine derivative prompted us to investigate the behavior of this drug from the standpoint of metabolism, drug-drug interaction or cytotoxicity. First we elucidated the potency of the inhibitory effect of safrazine on phenytoin (**2**, PHT) behaviors in the isolated rat hepatocyte system and rat hepatic microsomes (Chart 1).

Isolated hepatocytes were prepared by the collagenase perfusion method according to previously reported protocols.[3,5] Microsomes were obtained from the livers of male Wistar rats pretreated with phenobarbital using an ordinary method.[3,6] The hepatocytes were incubated at 37°C in rotating round-bottomed flasks under a 95% O_2-5% CO_2 atmosphere at a cell concentration of 3 x 10^6 cells/ml in a Krebs-Henseleit buffer, pH 7.4, supplemented with 13 mM HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) and 10 mM glucose. The inhibition in rat liver microsomes was examined in the following mixture: 4.4 mg microsomal protein, 0.4 mM EDTA, 0.4 mM $MgCl_2$, 1.0 mM KCl, 0.05 M potassium phosphate buffer (pH 7.4), 1.0 mM NADPH, and varying amounts of the substrates. After the mixture (2.5 ml) was preincubated aerobically at 37°C for 90 s, enzyme reaction was initiated by adding NADPH and incubating for 10 min. Sample preparations and the assays of PHT and its oxidation metabolite, 5-phenyl-5-(p-hydroxyphenyl)hydantoin (**3**, 5-HPPH), were performed as described in the previous paper.[3]

When 80 μM safrazine was incubated with 80 μM PHT in the isolated rat hepatocyte system, the metabolic disappearance of PHT was strongly retarded as shown in Fig. 1. Consequently, 5-HPPH formation through oxidation was markedly inhibited so that only 4.6% of 5-HPPH for the control experiment was detected during the first 20 min period of incubation. That is, the formation amounts of 5-HPPH were 28.67±2.38 μM for the control groups and 1.33±0.46 μM for the safrazine-coadministered groups respectively (n = 3). Lineweaver-Burk plot, determined by the least squares method, of the inhibition of PHT p-hydroxylation in phenobarbital-pretreated rat liver microsomes showed that safrazine exhibited a mixed type inhibitory pattern (Fig. 2).

In view of the inhibition mechanism including the formation of active metabolites of hydrazine compounds, it is significant that the inhibition by safrazine is 95.4%. This is significantly higher than that of the corresponding moles of INH or acetyl hydrazine (AcHz), and the inhibition manner also differs from that of INH.

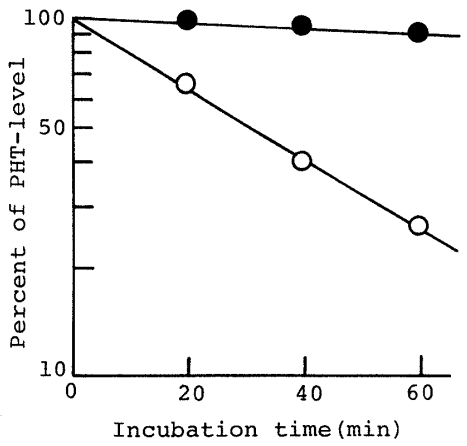


Fig. 1. Effects of Safrazine on PHT Oxidation in Isolated Rat Hepatocytes
○ : control, ● : 80 μM safrazine
The initial concentration of PHT was 80 μM/3x10^6 cells/ml. Each value represents the mean of three experiments.

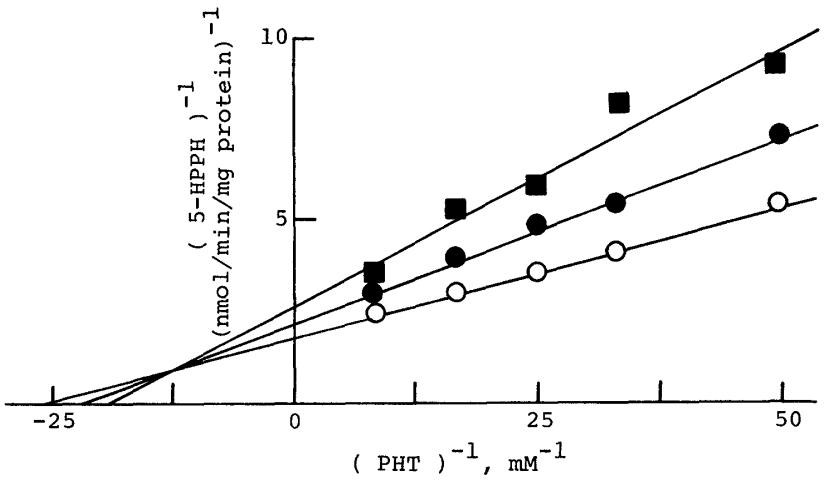


Fig. 2. Lineweaver-Burk Plot of the Inhibition of PHT p-Hydroxylation by Safrazine in Hepatic Microsomes from PB-pretreated Rats
○ : control, ● : 10 μM safrazine, ■ : 15 μM safrazine

This is shown as follows. Previously, we reported that INH and its metabolite inhibited PHT p-hydroxylation markedly in isolated rat hepatocytes, namely, the extent of inhibition compared to the control are 85.1 and 79.0%, respectively.[3] Kutt et al. showed that the mode of the inhibition by INH on PHT p-hydroxylation was non-competitive.[7] Further studies are in progress to elucidate the nature of the inhibition by safrazine and the related compounds.

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