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The Inductive Effect of Mepirizole on the Drug Metabolizing Enzymes of Rat Liver

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Many drugs induce the hepatic microsomal metabolism of drug and affect the pharmacological activity or toxicity of themselves or others. During investigation of the metabolic fate[2] of mepirizole [1-(4-methoxy-6-methyl-2-pyrimidinyl)-3-methyl-5-methoxypyrazole, DA-398], a new analgesic and anti-inflammatory agent,[3] this compound has been found to shorten the sleeping time of rats by hexobarbital and stimulate the metabolic activity of liver microsomes. This paper describes the results of these experiments.

Materials and Methods

Female Wistar rats weighing 70—80 g were used. Mepirizole was the gift from Daiichi Seiyaku Co. NADP, glucose-6-phosphate (G-6-P), and G-6-P dehydrogenase were purchased from Boehringer Co. Mepirizole dissolved in 0.9% saline was injected intraperitoneally (*i.p.*) in a dose of 80 mg/kg once a day.

Assay of Drug Metabolizing Activity——Rats were decapitated and the liver was homogenized with 4 volumes of 1.15% KCl containing 37.5 mM nicotinamide. The supernatant obtained by centrifuging at 9000 g for 20 min were used for enzyme assay.

The incubation mixture (3 ml) contained 50 mM Tris–HCl, pH 7.4, 10 mM $MgCl_2$, 2 mM G-6-P, 0.1 mM NADP, 0.5 mM aminopyrine or 0.1 mM aniline as substrate, and 9000 g supernatant corresponding to 200 mg of liver. Incubation was performed at 37° for 30 min in room atmosphere and terminated by the addition of 1.5 ml each of saturated $Ba(OH)_2$ and 20% $ZnSO_4$ for aminopyrine or of 1.5 ml of 20% TCA for aniline. The mixture was centrifuged at 10000 g for 15 min. Formaldehyde formed by aminopyrine demethylase was determined according to Nash.[4] *p*-Aminophenol formed by aniline hydroxylase was determined by the method of Imai, *et al.*[5]

For the kinetic analysis and the measurement of cytochrome P_{450}, microsomes were prepared from 0.25 M sucrose–0.001 M EDTA homogenates of liver by differential centrifugation. Microsome pellets washed with KCl–Tris were suspended in 1.15% KCl–0.025M Tris–HCl, pH 7.4. Incubation was performed at the presence of G-6-P dehydrogenase.[6] Protein was determined by Lowry, *et al.*[7] Cytochrome b_5 and P_{450} content and the spectral change of P_{450} with drug were determined by Remmer, *et al.*[8]

The sleeping time of rats was measured as time interval (min) from injection of 100 mg/kg of hexobarbital, *i.p.*, until restoration of the righting reflex.

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Result and Discussion

Inductive Effect of Mepirizole on the Drug Metabolizing Enzymes

The data of drug metabolism and microsomal cytochromes after the administration of mepirizole for 1,3 or 7 days are shown in Table I. Mepirizole enhanced significantly the activity of aminopyrine demethylase after 3 or 7 day-treatment and that of aniline hydroxylase after 1 or 3 day-treatment. The activity of aniline hydroxylase after 7 day-treatment was still higher than control but not significant. The content of P_{450} and the spectral change of P_{450} with hexobarbital or aniline were also increased by mepirizole treatment.

Table I. Inductive Effect of Mepirizole on Hepatic Microsomal Metabolism in Female Rats

	Control	Mepirizole, 80 mg/kg, *i.p.*		
		1 day	3 days	7 days
No. of rats	8	8	8	8
Body weight, g	103±2	109±3	97±5	102±4
Liver/body weight, g/100 g	5.24±0.18	5.05±0.14	4.71±0.14	4.94±0.09
9000 g supernatant				
Protein, mg/g liver	145±5	150±4	152±2	146±3
Metabolism, μmole/g/hr				
Aminopyrine	3.89±0.36	4.62±0.35	6.48±0.33[a]	7.03±0.49[a]
Aniline	0.89±0.06	1.20±0.07[a]	1.16±0.06[a]	1.06±0.10
Microsomes[b]				
Protein, mg/g liver	15.6	15.4	18.2	16.9
Cytochrome, $E\times10^{-3}$/mg/protein				
P_{450}	33.4	41.4	39.8	40.4
b_5	39.5	43.0	42.9	48.8
Binding with				
Hexobarbital	3.97	4.18	5.43	5.01
Aniline	5.00	9.47	9.47	9.83

The values represent mean ±standard error.
a) significantly different from control group
b) Prepared form pooled liver of each group.

There are two types in inducers of drug metabolizing enzymes, one is phenobarbital and another is polycyclic hydrocarbons such as 3-methylcholanthrene.[9] Phenobarbital induces the metabolism of more various kinds of drugs including aminopyrine and aniline. The inductive effect of polycyclic hydrocarbons is somewhat restricted and N-demethylation of aminopyrine is not induced by 3-methylcholanthrene. Phenobarbital does not change K_m for the metabolism of substrates such as aminopyrine but increases K_m for aniline hydroxylase.[10] 3-Methylcholanthrene decreases K_m for 3,4-benzpyrene hydroxylase which is not changed by phenobarbital.[11]

The effect of mepirizole on K_m and V_{max} values for enzymes are shown in Table II. V_{max} for both aminopyrine demethylase and aniline hydroxylase were increased by mepirizole. However, only the K_m for aniline hydroxylase was increased by mepirizole. From the point of kinetic view, mepirizole should be classified into phenobarbital type inducer.

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Table II. Effect of Mepirizole on the Kinetics of Drug Metabolizing Enzymes

	Control	Mepirizole
Aminopyrine demethylase		
K_m, mM	0.15	0.18
V_{max}, mμmole/mg/min	4.9	6.9
Aniline hydroxylase		
K_m, mM	0.031	0.054
V_{max}, mμmole/mg/min	0.69	1.17

These values represent typical experiments which have been repeated three times. Each value was obtained from pooling tissues from 5 rats. Mepirizole was administered for 4 days.

Effect of Mepirizole on the Sleeping Time by Hexobarbital

As shown in Table III, the sleeping time of rats by 100 mg/kg of hexobarbital, *i.p.*, was shortened significantly by the administration of mepirizole for 4 days. Tsurumi, *et al.*[12] reported that the hypnotic activity of mepirizole itself was reduced by the repeated treatment. These phenomenon are probably due to the induction of mepirizole metabolizing activity.

Table III. Effect of Mepirizole on Hexobarbital Sleeping Time

	Control	Mepirizole[a]
No. of rats	8	8
Body weight, g	92	92
Sleeping time, min[b]	72±7	39±4

a) Mepirizole was administered for 4 days.
b) Hexobarbital, 100 mg/kg, *i.p.* Values represent mean±standard error. Difference was significant at $p<0.05$.

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