Short Communication

COMBINED EFFECTS OF METHYL *n*-BUTYL KETONE AND METHYL ETHYL KETONE ON ACTIVITIES OF MICROSOMAL DRUG METABOLIZING ENZYMES IN RATSラット肝の薬物代謝酵素に及ぼす
MBK と MEK の混合影響

Key words: methyl *n*-butyl ketone; methyl ethyl ketone; drug metabolizing enzyme; cytochrome P-450; mixed solvent

Methyl *n*-butyl ketone (MBK) was found to induce polyneuropathy (MBK neuropathy), which affects a large number of workers at a plant producing plastic-coated and color-printed fabrics.^{1,2)} Since then, MBK has not been used in industries, but the toxicological interests have been discussed. Many investigators reported that the main causal agent of MBK neuropathy is not MBK itself, but its metabolite, 2,5-hexanedione (2,5-HD).³⁾ Therefore, microsomal drug metabolizing enzymes (cytochrome P-450) have been thought to participate in MBK neuropathy induced by the metabolite of MBK.

Methyl ethyl ketone (MEK) has no neurotoxicity itself, but Misumi *et al.*⁴⁾ reported that MEK enhances MBK neuropathy in experimental animals concomitantly exposed to MBK and MEK. We examined the combined effects of the administration of MBK and MEK on the activities of microsomal drug metabolizing enzymes.

Materials and methods. Animals: Male Donryu rats were obtained from Shizuoka Animal Co. and were housed in plastic cages with wood chips for bedding in a controlled room kept at $22 \pm 1^\circ\text{C}$ with a relative humidity of $55 \pm 5\%$ and a 12 h light/12 h dark cycle. All rats were allowed to acclimatize for at least 2 wk prior to the treatment. They were fed rodent feed (CE-2, CLEA Japan) and water *ad lib*. Organic solvent treatments: The rats (about ten weeks old) were injected subcutaneously with MBK, MEK, or a mixture of both for 3 days. The groups and dosages were as follows: (1) MBK 0.8 mmole/kg (MBK 0.8), (2) MBK 4.0 mmole/kg (MBK 4.0), (3) MEK 0.8 mmole/kg (MEK 0.8), (4) MEK 4.0 mmole/kg

(MEK 4.0), and (5) mixture of MBK/MEK 2.0/2.0 mmole/kg (MBK/MEK). Each group consisted of 5 rats. As the control, 9 rats were injected with saline solution (0.5 ml/kg). All rats were fasted 20 h after the last drug injection in individual wire bottom stainless steel cages, and then sacrificed by decapitation. Preparation of subcellular fractions: The removed liver was placed immediately into an ice-cold buffer consisting of 0.154 M-KCl in 0.1 M-potassium phosphate, pH 7.4. Microsomes in liver were prepared by the method of Alvares.⁵⁾ Drug metabolizing enzyme assay: Aminopyrine N-demethylase (AM) activity in the microsomes was determined by measuring formaldehyde production,⁶⁾ and aniline hydroxylase (AN) activity by measuring the formation of *p*-aminophenol.⁷⁾ The content of the microsomal cytochrome P-450 was determined by the method of Omura and Sato.⁸⁾ The protein concentration in the microsomal preparation was measured by the method of Lowry *et al.*,⁹⁾ using bovine serum albumin as a standard. Statistical analysis: Duncan's multiple range test was used to analyze the data.

Results. As shown in Table 1, the concentrations of microsomal protein in rat liver were higher in each of the tested groups than in the control. The microsomal cytochrome P-450 content increased in the MBK 4.0 and MBK/MEK groups. Activities of AM and AN per milligram protein increased in the MBK 4.0 and MBK/MEK groups. Although the dose of MBK was only 2.0 mmole/kg in the MBK/MEK group, AM activity in this group was significantly higher than that in the MBK 4.0 group.

AM activity per unit of cytochrome P-450 concentration in the MBK/MEK group was higher than that in the control group.

Liver weight in all groups was similar to that in the control.

Discussion. Couri *et al.*¹⁰⁾ reported that AN and AM activities were significantly enhanced two to threefold over the control values in the MBK/MEK group. In our present study, when 2.0 mmole/kg of MBK was injected to the rats of the MBK/MEK group in combination with equal mol of MBK, AM activity increased 1.3 times higher than that in the MBK 4.0 group (4.0 mmole/kg of MBK was injected), but the exposure to MEK alone did not show any effect on the cytochrome P-450 content or on AM and AN activities in the

Table 1. Effects of MBK and MEK on activity of drug metabolizing enzymes and on content of hepatic microsomal cytochrome P-450 in rats.

Group	n	Aminopyrine N-demethylase		Aniline hydroxylase		Cytochrome P-450		Microsomal Protein	Liver Weight
		nmol /mg-pro /20 min	nmol /nmol cyto.P450 /20 min	nmol /mg-pro /20 min	nmol /nmol cyto.P450 /20 min	nmol/mg-pro	nmol/mg-pro		
Control	9	28.46±4	36.36±6	6.75±0.98	8.77±1.30	0.779±0.097	23.7±1.1	3.61±0.47	
MBK 0.8	5	27.32±3.4	36.72±3.4	7.44±0.99	9.85±0.71	0.753±0.042	26.1±2.7**	3.87±0.27	
MBK 4.0	5	26.5±1.3**	41.6±8.0	9.85±1.11**	10.5±1.35	0.95±0.087*	27.2±1.4**	3.81±0.10	
MEK 0.8	5	29.0±4.9	41.6±9.4	6.78±0.35	9.62±1.27	0.702±0.031	27.3±2.0**	3.69±0.16	
MEK 4.0	5	32.4±4.5	42.7±5.4	7.1±1.06	9.73±1.30	0.709±0.161	28.7±1.8**	3.71±0.08	
MBK/MEK	5	32.7±2.5**	34.4±5.1**	10.02±1.44**	10.34±0.53	0.965±0.096**	29.1±1.3**	3.78±0.21	

Values are means ± SD.
Multiple range test was performed by Duncan's method. *: $p < 0.05$, **: $p < 0.01$ vs the control.
***: $p < 0.01$ between MBK/MEK and MBK 4.0.

liver. MEK enhanced AM activity in the mixed exposure group compared to both the control and the MBK 4.0 groups.

Microsomal protein in the MEK groups was higher than that in the control group, but the cytochrome P-450 content and enzyme activities in the MEK groups at 20 h after the last injection were similar to those of the control group in this study. However, when assayed at 8 h after the last injection, the cytochrome P-450 content increased in rats administered MEK (4.0 mmole/kg) (unpublished data). We could not describe the difference of induction mechanism of cytochrome P-450 in rats administered MBK or MEK in the present study.

In the future, to clarify the combined effect of MBK and MEK on the activities of microsomal drug metabolizing enzymes, we should determine the effect of MBK and MEK exposure on the pharmacokinetics of 2,5-HD, a metabolite of MBK, in the liver of rats administered MBK and MEK.

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References

- 1) Mendell JR, Saida K, Ganansia MF, et al. Toxic polyneuropathy produced by methyl *n*-butyl ketone. *Science* 1974; 185: 787-789.
- 2) Allen N, Mendell JR, Billmaier DJ, et al. Toxic polyneuropathy due to methyl *n*-butyl ketone. *Arch*

Neurol 1975; 32: 209-222.

- 3) Misumi J, Nagano M. Neurophysiological studies on the relation between the structural properties and neurotoxicity of aliphatic hydrocarbon compounds in rat. *Br J Ind Med* 1984; 41: 526-532.
- 4) Misumi J, Nagano M. Experimental study in the enhancement of the neurotoxicity of methyl *n*-butyl ketone by non-neurotoxic aliphatic monoketones. *Br J Ind Med* 1985; 42: 155-161.
- 5) Alvares AP, Mannering GJ. Two-substrate kinetics of drug-metabolizing enzyme systems of hepatic microsomes. *Mol Pharmacol* 1970; 6: 206-212.
- 6) Schenkman JB, Remmer H, Estabrook RW. Spectral studies of drug interaction with hepatic microsomal cytochrome. *Mol Pharmacol* 1967; 3: 113-123.
- 7) Imai Y, Ito A, Sato R. Evidence for biochemically different types of vesicles in the hepatic microsomal fraction. *J Biochem (Japan)* 1966; 60: 417-428.
- 8) Omura T, Sato R. The carbon mono-oxide-binding pigment of liver microsomes (II) Solubilization, purification and properties. *J Biol Chem* 1964; 239: 2379-2385.
- 9) Lowry OH, Rosebrough HJ, Plotnich HB, et al. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-273.
- 10) Couri D, Hetland LB, Abdel-Rahman MS, Weiss H. The influence of inhaled ketone solvent vapors on hepatic microsomal biotransformation activities. *Toxicol Appl Pharmacol* 1977; 41: 285-289.

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