POTENTIATION OF TOLUENE TOXICITY BY HEPATIC ENZYME INHIBITION IN MICE

Kenichi KOGA

*Department of Pharmacology, Sapporo Medical College,
S. 1, W. 17, Sapporo 060, Japan*

Yoshio OHMIYA

*Department of Pharmacology, Akita University School of
Medicine, 1-1, Hondoh 1 chome, Akita 010, Japan*

Received December 15, 1977

Abstract.....The toxicity of toluene was evaluated in male mice by the narcotic effects and lethality. The i.p. LD₅₀ of toluene for male mice was calculated to be 1.15g/kg according to the method of Weil. Pretreatment with phenobarbital, produced a marked decrease in the number of sleep induced by a test dose (0.96 g/kg i.p.) of toluene. By contrast, pretreatment with a variety of hepatic enzyme inhibitors such as CCl₄, SKF 525 A, pyrazole and cyanamide was found to produce an increase in the number of sleep and death, accompanied with the prolongation of sleeping time. The present results suggest that the toxicity of toluene is enhanced by the inhibition of any step of metabolism.
Key words: Toluene toxicity; hepatic enzyme inhibition

Toluene is an organic solvent which has been used widely in industry and is contained in thinner as its major constituent. Toluene has an euphoric and central nervous system depressant action which is sought out habitually by some teenagers and responsible for the widespread practice of inhaling thinners. As many other aromatic hydrocarbons, toluene is now defined to be metabolized principally in the liver (Williams, 1959; Gillette, 1959). Accordingly, it is assumed that the neurotoxicity of toluene is modified by the varying hepatic function levels. In fact, it has been already reported that induction of hepatic microsomal enzymes with phenobarbital increased the tolerance of rats to the toxicity of toluene (Ikeda and Ohtsuji, 1971). In the present communication, it was intended to expand the studies to include the effects of a variety of metabolic inhibitors on the toxicity of toluene in mice.

MATERIALS AND METHODS

Treatment of animals

Male mice of ddN strain weighing 20 to 25 g were used throughout the course of experiment. Toluene and CCl₄ were dissolved in olive oil and phenobarbital, SKF 525A,

cyanamide and pyrazole in saline. CCl_4 (0.8 g/kg) and phenobarbital (75 mg/kg) were administered to animals 48 hr prior to the injection of a test dose (0.96 g/kg) of toluene. SKF 525 A (50 mg/kg), cyanamide (50 mg/kg) and pyrazole (100 mg/kg) were administered 30 min prior to toluene. Olive oil or saline alone was given as a corresponding vehicle control. Each treatment was performed by means of an intraperitoneal injection in a volume of 5 ml/kg.

Lethality

The number of death that occurred during a 24-hr period after the injection of geometrically graded dosages of toluene was recorded. The LD_{50} values were estimated by the moving-average method of Weil (1952).

Induction time and sleeping time

Induction time was measured as the time elapsing between the injection of toluene and the loss of righting reflex. Sleeping time was defined as the time interval between the loss and recovery of righting reflex. Results are presented as mean $\pm$ S.E.M. and the significance of differences between means was determined according to Student's *t* test.

RESULTS

Five groups of 6 mice each were observed for 24 hr following the injection of a graded series of doses of toluene and death was recorded. The cause of death appeared to be mainly respiratory failure. Lethality and LD_{50} of toluene for male mice are shown in Table 1. The LD_{50} for male mice was calculated to be 1.15 g/kg which is less than the LD_{50} of 1.64 g/kg for female rats reported by Ikeda and Ohtsuji (1971).

The induction time, sleeping time and mortality of mice pretreated with CCl_4 known as a potent hepatotoxin were compared with those of control animals (Table 2). It can be readily seen that CCl_4 pretreatment shortened significantly the onset of narcosis and prolonged its duration just 3 times. Lethal effects of toluene were remarkably enhanced by CCl_4 .

Table 3 summarizes the results of a series of other drugs tested in a similar fashion. Phenobarbital showed a marked protecting activity against the central nerve-depressing action of toluene and only 2 mice out of 19 pretreated with phenobarbital slept. The mean sleeping time of these 2 mice decreased significantly but total mortality was same as that of controls. By contrast, all mice pretreated with SKF 525 A fell into a sleep with the one exception. Sleeping time was prolonged over 2 times and mortality increased 4-fold by SKF 525 A, while induction time was not shortened. Cyanamide and pyrazole were found to produce the shortening of onset, prolongation of sleeping time and significant increase in mortality.

DISCUSSION

The main metabolic pathway of toluene is well documented (Williams, 1959 ; Gillette, 1959). Toluene is first oxidized to benzyl alcohol by NADH-dependent microsomal

Potentialiation of toluene toxicity

Table 1. Toluene-induced lethality in male mice.

Dose (g/kg)	No. of mice	No. of death
0.79	6	0
0.96	6	1
1.15	6	3
1.34	6	5
1.65	6	6
LD ₅₀ with 95% confidence limits (g/kg)	1.15 (1.04–1.31)	

Animals were injected intraperitoneally with a graded series of doses of toluene. The number of death that occurred during a 24-hr period after the injection of toluene was recorded.

Table 2. Effect of CCl₄ on toluene-induced narcosis and lethality in male mice.

Treatment	No. of mice	No. of sleep	Induction time (min)	Sleeping time (min)	Lethality (%)		
					1 hr	24 hr	48 hr
Control	7	5	20.6±1.6	35.0± 8.2	0	14.3	14.3
CCl ₄	15	15	12.0±1.0*	108.2±11.6*	46.7	66.7	73.3

Animals were injected intraperitoneally with 0.96 g/kg of toluene. CCl₄ (0.8 g/kg i.p.) was given 48 hr before the injection of toluene. Results of induction and sleeping time are presented as mean ± S.E.M. Significant differences from control are denoted by * P<0.01.

Table 3. Effect of various drugs on toluene-induced narcosis and lethality in male mice.

Treatment	No. of mice	No. of sleep	Induction time (min)	Sleeping time (min)	Lethality (%)			
					1 hr	24 hr	48 hr	72 hr
Control	20	13	18.1±0.7	40.0± 4.2	0	20	20	20
Phenobarbital	19	2	18.5±0.5	18.0± 2.0*	0	15.8	15.8	21.1
SKF 525A	13	12	19.5±1.2	94.9± 6.2*	0	23.1	69.3	84.6
Cyanamide	15	13	14.1±1.1*	56.0± 6.9**	26.7	46.7	60	66.7
Pyrazole	10	7	13.7±1.7*	80.2±16.0*	30	40	70	80

Animals were injected intraperitoneally with 0.96 g/kg of toluene. Phenobarbital (75 mg/kg i.p.) was given 48 hr and SKF 525A (50 mg/kg i.p.), cyanamide (50 mg/kg i.p.) and pyrazole (100 mg/kg i.p.) were given 30 min before the injection of toluene. Results of induction and sleeping time are presented as mean ± S.E.M. Significant differences from control are denoted by* P<0.01 and ** P<0.05.

enzyme system, which is by no means unique, but involved in many types of drug biotransformations. Benzyl alcohol is then oxidized to benzaldehyde by NAD-dependent alcohol dehydrogenase and benzaldehyde is further oxidized to benzoic acid by NAD-linked aldehyde dehydrogenase. Finally, benzoic acid is conjugated with glycine or glucuronic acid and excreted mainly into the urine.

CCl₄ and SKF 525A are established to inhibit the hepatic drug metabolizing enzymes and potentiate the effects of many drugs (cited by Conney, 1971). Enhancement

of toluene toxicity by these inhibitors is assumed to be attributed largely to the inhibition of the first step of oxidation. Since the microsomal enzyme inhibitors are shown to inhibit glucuronyl transferase which is involved glucuronide formation (cited by Mandel, 1971), the final step of metabolism may be also inhibited. Although glucuronide conjugation is far less important than glycine one in man and rabbits (Bray et al., 1951 ; Smith et al., 1959 ; Williams, 1959), both types of conjugations participate equally in the metabolism and excretion of toluene in mice (Koga, unpublished). On the other hand, based on the findings that the phenobarbital treatment did not alter the excretion of hippuric acid in rats after the administration of benzoic acid (Ikeda and Ohtsuji 1971), more doubt is still cast on the significance of the microsomal enzyme systems in the conversion of benzoic acid to hippuric acid.

Pyrazole is known to be a potent inhibitor of alcohol dehydrogenase (Goldberg and Rydberg, 1969). Just like disulfiram, cyanamide is alleged to produce a disturbance of alcohol metabolism through the inhibition of aldehyde dehydrogenase (Ferguson, 1956), which is a rather nonspecific enzyme and is reported to oxidize many aromatic and aliphatic aldehydes (Deitrich et al., 1962). Consequently, it is conceivable that pyrazole and cyanamide enhance the toluene toxicity by means of inhibition of the second and third step of metabolism respectively.

The present results concerning the effect of phenobarbital on the toluene-induced sleeping time and lethality were in agreement with those of Ikeda and Ohtsuji (1971). When toluene is administered through the intraperitoneal route as carried out in this study, an appreciable portion of the dose is expected to be metabolized in the liver by first pass effect. Such an interpretation can explain the shortening of induction time produced by the enzyme inhibitors used except SKF 525 A. In view of the fact that SKF 525 A stabilizes the biological membranes (Lee et al., 1968), it may retard the absorption of drugs.

SUMMARY

The toxicity of toluene was evaluated in male mice in terms of the narcotic effects and lethality. The i.p. LD_{50} of toluene for male mice was calculated to be 1.15 g/kg according to the method of Weil. Pretreatment with phenobarbital, a hepatic microsomal enzyme inducer, was shown to produce a marked decrease in the number of sleep induced by a test dose (0.96 g/kg i.p.) of toluene. By contrast, pretreatment with a variety of hepatic enzyme inhibitors such as CCl_4 , SKF 525 A, pyrazole and cyanamide was found to produce an increase in the number of sleep and death, accompanied with the prolongation of sleeping time. The present results suggest that the toxicity of toluene is enhanced by the inhibition of any step of metabolism.

ACKNOWLEDGEMENTS

The authors wish to thank Profs. Mamoru Tanaka and Kengo Nakai for their interest and support.

REFERENCES

- Bray, H. G., Thorpe, W. V. and White, K. (1951). Kinetic studies of the metabolism of foreign organic compounds 1. The formation of benzoic acid from benzamide, toluene, benzyl alcohol and benzaldehyde and its conjugation with glycine and glucuronic acid in the rabbit. *Biochem. J.*, **48**, 88-96.
- Conney, A. H. (1971). Environmental factors influencing drug metabolism. In "Fundamentals of Drug Metabolism and Drug Disposition" (LaDu, B. N., Mandel, H. G. and Way, E. L. ed.), pp. 253-278. Williams and Wilkins, Baltimore.
- Deitrich, R. A., Hellerman, L. and Wein, J. (1962). Diphosphopyridine nucleotide-linked aldehyde dehydrogenase I. Specificity and *sigma-rho* function.
- Ferguson, J. K. W. (1956). A new drug for alcoholism treatment. *Can. med. Ass. J.*, **74**, 793-795.
- Gillette, J. R. (1959). Side chain oxidation of alkyl substituted ring compounds. *J. biol. Chem.*, **234**, 139-143.
- Goldberg, L. and Rydberg, U. (1969). Inhibition of ethanol metabolism *in vivo* by administration of pyrazole. *Biochem. Pharmacol.*, **18**, 1749-1762.
- Ikeda, M. and Ohtsuji, H. (1971). Phenobarbital-induced protection against toxicity of toluene and benzene in the rat. *Toxicol. appl. Pharmacol.*, **20**, 30-43.
- Lee, I. P., Yamamura, H. I. and Dixon, R. L. (1968). The effect of β -diethylaminoethyl-diphenyl-propylacetate (SKF 525-A) on biological membranes-I. SKF 525-A-induced stabilization of human erythrocytes. *Biochem. Pharmacol.*, **17**, 1671-1681.
- Mandel, H. G. (1971). Pathways of drug biotransformation: Biochemical conjugations. In "Fundamentals of Drug Metabolism and Drug Disposition" (LaDu, B. N., Mandel, H. G. and Way, E. L. ed.), pp. 149-186. Williams and Wilkins, Baltimore.
- Smith, J. N., Smithies, R. H. and Williams, R. T. (1954). Studies in detoxication. 55. The metabolism of alkylbenzenes. (a) Glucuronic acid excretion following the administration of alkylbenzenes. (b) Elimination of toluene in the expired air of rabbits. *Biochem. J.*, **56**, 317-324.
- Weil, C. S. (1952). Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions in their use. *Biometrics*, **8**, 249-263.
- Williams, R. T. (1959). The metabolism of aromatic hydrocarbons. In "Detoxication Mechanisms", pp. 188-236. Chapman and Hall, London.