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A Comparative Study on Reductive Dehalogenation of Halothane in Liver, Kidney and Lung of the Rabbit

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ABSTRACT

The contents of cytochrome P-450 (P-450) and cytochrome b_5, and the activity of NADPH-cytochrome c reductase and the reductive metabolites of halothane, 2-chloro-1, 1-difluoroethylene (CDE) and 2-chloro-1, 1, 1-trifluoroethane (CTE) were measured in microsomes from the liver, kidney and lung of phenobarbital (PB) pretreated and untreated Japanese white strain rabbits.

Microsomal P-450 levels in the liver, kidney (renal cortex) and lung of the rabbits were 1.91 ± 0.35, 0.19 ± 0.04 and 0.42 ± 0.11 nmol/mg protein (mean ± SD), respectively. *In vivo* phenobarbital pretreatment (PB-pretreatment) increased the content of P-450 to 2.95 ± 0.40 nmol/mg protein (154%) in the liver and to 0.40 ± 0.11 nmol/mg protein (211%) in the kidney, but had little effect in the lung.

The activity of CDE formation was 0.72 ± 0.10, 0.08 ± 0.04 and 0.03 ± 0.01 nmol/mg protein/min in the liver, kidney and lung, respectively. PB-pretreatment enhanced the activity of CDE formation to 1.59 ± 0.49 nmol/mg protein (221%) in the liver, and to 0.29 ± 0.16 nmol/mg protein/min (363%) in the kidney, but showed little enhancement in the lung. The activity of CTE formation was 1.30 ± 0.19, 0.12 ± 0.04 and 0.09 ± 0.02 nmol/mg protein/min, in the liver, kidney and lung, respectively. PB-pretreatment enhanced the activity of CTE formation to 1.80 ± 0.44 nmol/mg protein/min (138%) in the liver, but caused only slight enhancement in the kidney and lung. PB-pretreatment markedly enhanced the activity of CDE formation in the kidney.

The authors conclude that cytotoxicity by reductive dehalogenation of halothane is possible not only in the liver but also in the kidney with PB-pretreatment.

Key words: *Halothane, Reductive dehalogenation, Microsome, Extrahepatic organs*

Halothane is among the commoest of the inhalational anesthetics. It had been previously thought that this drug was stable *in vivo*. However, Van Dyke et al[26] found that C^{14} halothane was metabolized in the mouse. The biotransformation of halothane is classified into oxidative and reductive metabolisms. Trifluoroacetic acid is excreted in the urine[22] as an oxidative metabolite. CDE and CTE are excreted in expiratory gas[17,24] as reductive metabolites. A radical intermediate produced in reductive halothane metabolism has been reported to cause hepatotoxicity[5,21,25]. Likewise, it has been reported that inorganic fluoride (F^-) from methoxyflurane causes nephrotoxicity[4]. It is well known that F^- is formed during reductive halothane metabolism[1,27]. However, halothane induced nephrotoxicity has not yet been reported.

The reductive dehalogenation of halothane occurs in the presence of P-450[1,8] and is facilitated by *in vivo* PB-pretreatment and under hypoxic conditions[16].

Studies on the biotransformation of anesthetics have mainly dealt with the liver. The metabolism of halothane in other organs has been reported by Blitt et al[2]. They observed the reductive biotransformation of halothane in microsomal preparations from kidney and lung. Their study, however, did not examine PB-pretreatment despite the fact that P-450 in rabbit kidney is enhanced by PB-pretreatment[12,18].

The present study, therefore, attempted to investigate the reductive dehalogenation of halothane in microsomes of the liver, kidney and lung in PB-pretreated rabbits. We examined the microsomal P-450 enzyme systems, P-450, cytochrome b_5 (b_5), NADPH-cytochrome c reductase (fp_2), and *in vitro* formation of reductive metabolites (CDE, CTE) of halothane.

METHODS

1) Animals and the preparation of microsomes

Japanese white strain rabbits (weighing 2.5-3kg, age 12 to 15 weeks old) were used. Five animals without PB-pretreatment were classified as the control group, and five other animals with PB-pretreatment were classified as the PB group.

Table 1. Effect of PB-pretreatment on the content of P-450, b_5 and fp_2 in the liver, kidney and lung microsomes

		P-450	b_5	fp_2	b_5/P-450
Liver	C	1.91 ± 0.35	1.12 ± 0.16	204 ± 38	0.59
	PB	2.95 ± 0.40 **	1.34 ± 0.41	199 ± 20	0.45
Kidney	C	0.19 ± 0.04	0.19 ± 0.06	29 ± 4	1.00
	PB	0.40 ± 0.11 **	0.30 ± 0.06 *	35 ± 7	0.75
Lung	C	0.42 ± 0.11	0.11 ± 0.03	137 ± 9	0.26
	PB	0.41 ± 0.08	0.10 ± 0.02	119 ± 20	0.24

All values except ratios are mean ± SD.
C = control group (n=5), PB = phenobarbital group (n=5)
P-450 = Cytochrome P-450 (nmol/mg protein), b_5 = Cytochrome b_5 (nmol/mg protein)
fp_2 = NADPH-cytochrome c reductase (nmol/mg protein/min)
Ratios were calculated from mean values of P-450 and b_5.
**$p<0.01$ versus control value. *$p<0.05$ versus control value.

Table 2. Effect of PB-pretreatment on the CDE and CTE formation by microsomal fractions from liver, kidney and lung

		CDE	CTE	CDE/CTE
Liver	C	0.72 ± 0.10	1.30 ± 0.19	0.55
	PB	1.59 ± 0.49 **	1.80 ± 0.44 *	0.88
Kidney	C	0.08 ± 0.04	0.12 ± 0.04	0.67
	PB	0.29 ± 0.16 **	0.14 ± 0.07	2.07
Lung	C	0.03 ± 0.01	0.09 ± 0.02	0.33
	PB	0.03 ± 0.01	0.09 ± 0.03	0.33

All values except ratios are mean ± SD.
C = control group (n=5), PB = phenobarbital group (n=5)
CDE = 2-chloro-1, 1-difluoroethylene (nmol/mg protein/min), CTE = 2-chloro-1, 1, 1,-trifluoroethane (nmol/mg protein/min)
Ratios were calculated from mean values of CDE and CTE.
**$p<0.01$ versus control value. *$p<0.05$ versus control value.

Animals in PB group were used for experiments after an intramuscular injection of 60mg/kg of PB for four consecutive days followed by a 24-hour fast. For the control group, a physiological saline solution was administered instead of PB. Animals were sacrificed by injecting air into the auricular vein in a cold room. The liver, kidney and lung were immediately removed and perfused with an ice-cold isotonic sucrose solution. The renal cortex and medulla of the kidney were macroscopically separated.

The renal cortex was regarded as the kidney in this experiment because the P-450 content of the medulla was 0.02 ± 0.01 nmol/mg protein, far less than that of the renal cortex (0.19 ± 0.04 nmol/mg protein).

The microsomal fractions were prepared by a standard method[6] and stored at −70°C before assay.

2) Assays for enzymes and the activity of reductive dehalogenation of halothane

The content of microsomal P-450 in the liver and kidney, and b_5 in the liver, kidney and lung were measured by Sato and Omura's method[20]. Microsomal P-450 of the lung was estimated using a dithionate difference spectrum[15]. The activity of fp_2 was determined by Masters' method[14].

The activity of CDE and CTE formation was measured as follows. Deoxygenated nitrogen was sealed in a 15 ml test tube with a silicon cap containing 1 ml microsomal suspension in a Tris-HCI solution (10 mM, pH 7.4). Each microsomal suspension was adjusted to a protein concentration of approximately 10mg protein/ml. Protein concentration was measured by Lowry's method[13]. The mixture was preincubated for 10 min at 37°C, and NADPH (5mM) was added. The reaction was initiated by the administration of halothane (9.4 μmol). After a 10 min incubation at 37°C, 0.5 ml of the gaseous mixture was injected into a gas chromatograph column for CDE and CTE analysis.

The results were analyzed by Student's unpaired t-test and were considered significant at a level of $p<0.05$. Data are shown as mean ± SD.

RESULTS

1) The effect of PB-pretreatment on the content of P-450, b_5 and fp_2 in the liver, kidney and lung microsomes is shown in Table 1.

The content of P-450 in the liver in control group was respectively more than 10.1 times and 4.5 times higher than that in the kidney and lung. PB-pretreatment significantly increased the content of P-450 in the liver (154%) and kidney (211%), but had little effect in the lung. The content of b_5 increased only in the kidney (158%) after PB-

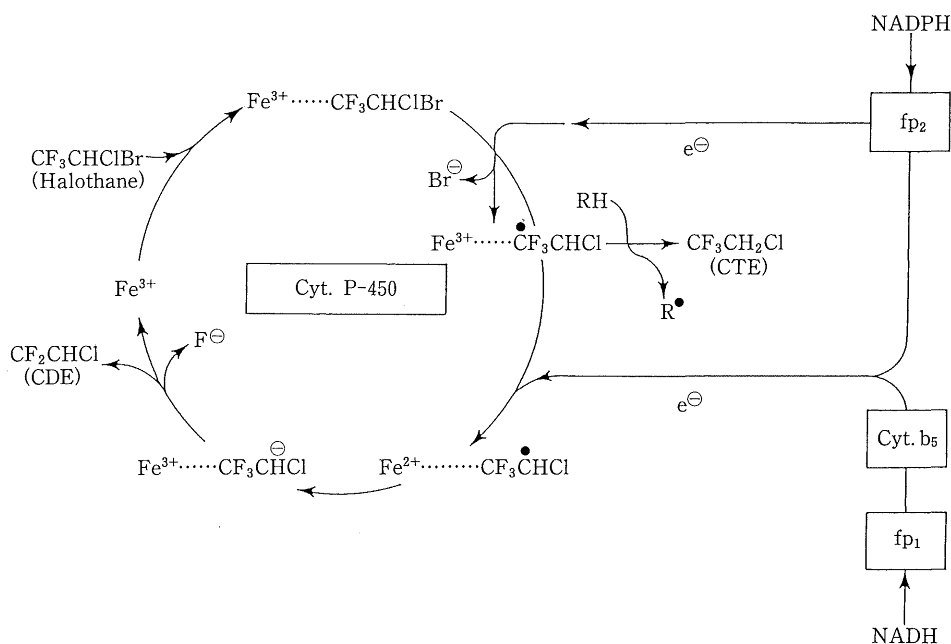

Fig. 1. The proposed pathway of the reductive dehalogenation of halothane

CTE is formed by one electron reduction and CDE is formed by two electron reduction. F^- is released when CDE is formed.

pretreatment. PB-pretreatment had little influence on the content of fp_2 in the liver, kidney and lung.

2) The effect of PB-pretreatment on the CDE and CTE formation by microsomal fractions from liver, kidney and lung is shown in Table 2.

The activity of CDE formation was enhanced by PB-pretreatment in liver microsomes (221%) and kidney microsomes (361%) but not in lung microsomes (100%). Regarding the activity of CTE formation, this was enhanced significantly in liver microsomes, slightly in kidney microsomes (117%) and not enhanced in lung microsomes (100%).

DISCUSSION

Blitt et al[2] reported that extrahepatic biotransformation may be an important factor in the disposition of volatile anesthetics showing that microsomes from rabbit kidney and lung metabolized halothane reductively.

Fiserova-Bergerova et al[7] showed that tissue concentration of CDE and CTE in rat kidney after halothane inhalation was not increased by PB-pretreatment. Their results suggested that P-450 may not be inducible for CDE and CTE formation in rat kidney by PB-pretreatment. In our study, the enhancement of CDE formation and the induction of P-450 were observed in rabbit kidney after PB-pretreatment. The discrepancy between their results and our results may be attributable to species differences. Indeed, it has been reported that P-450 is inducible in rabbit kidney[12,18] but not inducible in rat kidney[11] by PB-pretreatment.

We conclude that microsomes from the liver, kidney and lung of rabbits, metabolize halothane reductively and that PB-pretreatment enhances the reductive metabolism of halothane in rabbit kidney.

Fujii[10] reported that the concentration of CDE in expired gas from rabbits after halothane anesthesia increased markedly *in vivo* after PB-pretreatment, while CTE increased only slightly. Chikasue[3] reported that CTE formation showed a greater increase *in vitro* with PB-pretreated rat liver than CDE formation. It was explained that the discrepancy was most likely due to the difference of species as well as to the difference between *in vivo* and *vitro* methods. Our results in Table 2 show that CDE exhibited a greater increase than CTE in rabbit kidney undergoing PB-pretreatment. CTE is thought to be produced by one electron reduction and CDE produced by two electron reduction[1,19]. CDE formation depends on the content of b_5 because the second electron is supplied from b_5. A reconstituted system without b_5 demonstrated that CTE was the sole end product[9]. In the present study, the ratio of CDE/CTE was markedly high in kidney microsomes with *in vivo* PB-pretreatment (Table 2). The content of b_5 is enhanced in kidney microsomes. The b_5/P-450 ratio, however, is decreased by PB-pretreatment (Table 1). These data show that the formation of CDE did not, at least, solely depend on the content of b_5.

Figure 1 displays the metabolic pathway of reductive dehalogenation of halothane, from the report of Ahr et al[1]. It is well known that F^- as a metabolite of inhalational anesthetics causes renal injury[4] and that F^- is released when CDE is

formed[1,19]. Halothane has been believed not to cause renal injury because the serum level of F^- concentration either during or after halothane anesthesia is too low to cause renal injury[4,23]. However, the enhancement of CDE formation in rabbit kidney by PB-pretreatment may facilitate the accumulation of F^- in the kidney, suggesting a possible occurrence of renal injury during halothane anesthesia.

To summarize, in rabbits, the liver was the main organ responsible for the reductive dehalogenation of halothane. However, the kidney and lung in rabbits also metabolized halothane reductively. The reductive dehalogenation of halothane was enhanced in the kidney with *in vivo* PB-pretreatment.

Cytotoxicity due to the reductive dehalogenation of halothane may be possible not only in the liver but also in the kidney with PB-pretreatment.

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REFFERENCES

1. **Ahr, H.J., King, L.J., Nastainczyk, W. and Ullrich, V.** 1982. The mechanism of reductive dehalogenation of halothane by liver cytochrome P-450. Biochem. Pharmac. **31**: 383–390.
2. **Blitt, C.D., Gandolfi, A.J., Soltis, J.J. and Brown, Jr., B.R.** 1981. Extrahepatic Biotransformation of Halothane and Enflurane. Anesth. Analg. **60**: 129–132.
3. **Chikasue, F.** 1981. Study on the effect of multiple exposure of halothane on the rat liver microsomal enzyme system. Hiroshima J. Anesth. (in Japanese) **17**: 117–133.
4. **Cousins, M.J. and Mazze, R.I.** 1973. Methoxyflurane nephrotoxicity: A study of dose response in man. JAMA **225**: 1611–1616.
5. **De Groot, H. and Noll, T.** 1983. Halothane hepatotoxicity: relation between metabolic activation, hypoxia, covalent binding, lipid peroxidation and liver cell damage. Hepatology **3**: 601–606.
6. **Erster, L., Siekevitz, P. and Palade, G. E.** 1962. Enzyme-structure relationships in the endoplasmic reticulum of rat liver. J. Cell Biol. **15**: 541–562.
7. **Fiserova-Bergerova, V. and Kawiecki, R.W.** 1984. Effects of exposure concentrations on distribution of halothane metabolites in the body. Drug Metab. Dispos. **12**: 98–105.
8. **Fujii, K., Morio, M. and Kikuchi, H.** 1981. A possible role of cytochrome P-450 in anaerobic dehalogenation of halothane. Biochem. Biophys. Res. Commun. **101**: 1158–1163.
9. **Fujii, K., Miki, N., Sugiyama, T., Morio, M., Yamano, T. and Miyake, Y.** 1981. Anaerobic dehalo genation of halothane by reconstituted liver microsomal cytochrome P-450 enzyme system. Biochem. Biophys. Res. Commun. **102**: 507–512.
10. **Fujii, S.** 1978. Changes in volatile metabolites on shortly repeated halothane anesthesia and phenobarbital pretreated halothane anesthesia. Hiroshima J. Anesth. (in Japanese) **14**: 193–203.
11. **Jakobsson, S.W., Thor, H. and Orrenius, S.** 1970. Fatty acide inducible cytochrome P-450 of rat kidney cortex microsomes. Biochem. Biophys. Res. Commun. **39**: 1073–1080.
12. **Litterst, C.L., Mimnaugh, E.G. and Gram, T.E.** 1977. Comparative alterations in extrahepatic drug metabolism by factors known to affect hepatic activity. Biochem. Pharmacol. **26**: 749–755.
13. **Lowry, O.H., Rosebrough, N. J., Farr, A.L. and Randall, R.J.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265–275.
14. **Masters, B.S.S., Williams, Jr., C.H. and Kamin, H.** 1967. The preparation and properties of microsomal TPNH-cytochrome c reductase from pig liver. Methods Enzymol. **10**: 565–573.
15. **Matsubara, T., Prough, R.A., Burke, M.D. and Estabrook, R.W.** 1974. The preparation of microsomal fractions of rodent respiratory tract and their characterization. Cancer Res. **34**: 2196–2203.
16. **McLain, G.E., Sipes, I.G. and Brown, Jr., B.R.** 1979. An animal model of halothane hepatotoxicity. Anesthesiology **51**: 321–326.
17. **Mukai, S., Morio, M. and Fujii, K.** 1977. Volatile metabolites of halothane in the rabbit. Anesthesiololgy **47**: 248–251.
18. **Nariyama, M.** 1982. Studies on cytochrome P-450 from kidney cortex microsomes of rabbits treated with drugs. Medical Journal of Osaka City University Medical School (in Japanese) **31(4)**: 641–655.
19. **Nastainczyk, W., Ahr, H.J. and Ullrich, V.** 1982. The reductive metabolism of halogenated alkanes by liver microsomal cytochrome P-450. Biochemical Pharmacology **31**: 391–396.
20. **Omura, T. and Sato, R.** 1964. The carbon monoxide-binding pigment of liver microsomes. J. Biol. Chem. **239**: 2370–2378.
21. **Plummer, J.L., Beckwith, A.L.J., Bastin, F.N., Adams, J.F., Cousins, M.J. and Hall, P.** 1982. Free radical formation in vivo and hepatotoxicity due to anesthesia with halothane. Anesthesiology **57**: 160–166.
22. **Rehder, K., Forbes J., Alter, H., Hessler, O. and Stier, A.** 1967. Halothane biotransformation in man: A quantitative study. Anesthesiology **28**: 711–715.
23. **Sakai, T. and Takaori, M.** 1978. Biodegration of halothane, enflurane and methoxyflurane. Br. J. Anaesth. **50**: 785–791.
24. **Sharp, J.H., Trudell, J.R. and Cohen, E.N.** 1979. Volatile metabolites and decomposition products of halothane in man. Anesthesiology **50**: 2–8.
25. **Trudell, J.R., Bosterling, B. and Trevor, A.J.** 1982. Reductive metabolism of halothane by human and rabbit cytochrome P-450. Molecular Pharmacology **21**: 710–717.
26. **Van Dyke, R.A. and Chenoweth, M.B.** 1965. Metabolism of volatile anesthetics. Anesthesiology **26**: 348–357.
27. **Van Dyke, R.A. and Gandolfi, A.J.** 1976. Anaerobic release of fluoride from halothane. Drug. Metab. Disp. **4**: 40–44.