PREVENTIVE EFFECT OF ISOFLURANE ON DESTRUCTION OF CYTOCHROME P450 DURING REDUCTIVE DEHALOGENATION OF CARBON TETRACHLORIDE IN GUINEA-PIG LIVER MICROSOMES

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SUMMARY

Effects of isoflurane on cytochrome P450 loss during anaerobic dechlorination of carbon tetrachloride in guinea pig liver microsomes were examined. Under anaerobic conditions, chloroform was produced from carbon tetrachloride by the microsomes in the presence of NADPH, and production of chloroform was increased 1.80 times by the addition of isoflurane. The concentration of microsomal cytochrome P450 decreased to 71.6% after 7 minutes incubation with carbon tetrachloride and NADPH. With the addition of isoflurane in the same incubation system, the decrease in cytochrome P450 was to 84.9%. The essential components for the loss of cytochrome P450 during the dechlorination of carbon tetrachloride were microsomes, NADPH and carbon tetrachloride. Addition of carbon monoxide reduced the cytochrome P450 loss to negligible. These findings indicate that isoflurane interacts with cytochrome P450 to prevent the cytochrome P450 destruction during the anaerobic dechlorination of carbon tetrachloride in guinea-pig liver microsomes. These results also suggest that the destruction of cytochrome P450 during anaerobic dechlorination of carbon tetrachloride in microsomes was caused by direct attack by the trichloromethyl radical, rather than by carbon tetrachloride-induced lipid peroxidation.

KEY WORDS

isoflurane, dechlorination, anaerobic, carbon tetrachloride, destruction

INTRODUCTION

Carbon tetrachloride is metabolized by the liver microsomal oxidase system, including cytochrome P450, to cleave the CCl_3-Cl bond, resulting in a trichloromethyl radical and producing chloroform. This radical combines with the heme group of cytochrome P450 during dechlorination /1/. During this reaction, cytochrome P450 loss has been observed. Toranzo *et al.* /2/ suggested that cytochrome P450 destruction was mediated mainly by direct attack by carbon tetrachloride metabolites rather than by carbon tetrachloride-induced lipid peroxidation.

Isoflurane, 1-chloro-2,2,2-trifluoro ethyl difluoromethyl ether, is an inhalational anesthetic which is hardly metabolized /3/. We have reported that isoflurane enhances the formation of chloroform from carbon tetrachloride in guinea-pig liver microsomes under anaerobic conditions /4,5/. The anaerobic dehalogenation of halothane in guinea-pig liver microsomes was also enhanced by isoflurane. The rates of reduction of cytochrome P450 and the formation of the intermediate-cytochrome P450 complex were accelerated dose-dependently in the presence of isoflurane in this reaction /4/.

In this study, we examined how isoflurane affects the degradation of cytochrome P450 during anaerobic dehalogenation of carbon tetrachloride by the guinea-pig liver microsomal mixed function oxidase system. We showed that isoflurane prevented the destruction of cytochrome P450 during the dehalogenation of carbon tetrachloride while enhancing the production of chloroform by guinea-pig liver microsomes.

MATERIALS AND METHODS

This study was carried out according to the Guidance on Animal Experimentation in Research Facilities for Laboratory Animal Science, School of Medicine, Hiroshima University.

Reagents

Analytical grade potassium hydroxide, sodium hydroxide, calcium hydroxide, carbon tetrachloride and chloroform were obtained from Katayama Chemical (Japan). Isoflurane for clinical use was obtained

from Dynabot (Japan). NADPH was purchased from Boehringer Mannheim (Germany). All other reagents were analytical grade.

Animals

Male Hartley guinea-pigs weighing 225-275 g were used. The animals were killed after 24 hours starvation, followed by excision of the liver. After perfusion through the portal vein with ice-cold physiological saline, the liver was homogenized in 0.05 M potassium phosphate buffer. Following centrifugation of the liver homogenate at 9,000 *g*, the supernatant was centrifuged at 105,000 *g*. The resulting sediment was resuspended in 0.1 M potassium phosphate buffer and used as the liver microsomal suspension.

Reaction system and measurements

The microsomes were suspended in 0.1 M potassium phosphate buffer (pH 7.4). A sample (0.9 ml) of the microsomal suspension from 0.1 g wet weight liver was sealed in a 12.5 ml test tube with a butyl rubber cap. The test tube was then perfused with deoxygenated nitrogen for 10 min to create anaerobic conditions. These suspensions were then pre-incubated at 37°C while being shaken 100 times per minute. After 10 minutes preincubation, 0.1 ml of 30 mM NADPH and carbon tetrachloride (final concentration: 86 μM) were added to start the reaction. The reaction was stopped by the injection of carbon monoxide through the cap and then the reaction mixture was subjected to cytochrome P450 measurement. To some tubes, isoflurane (final concentration: 370 μM) was injected through the caps by a micro-syringe before the preincubation.

Cytochrome P450 assay

Cytochrome P450 was measured by Omura's and Sato's method slightly modified /6/. The absorbance at 450 nm was recorded by a Beckman DC 640 spectrophotometer (USA). The experiment was performed at 25°C.

Gas chromatograhy conditions

The concentration of carbon tetrachloride, chloroform and isoflurane was determined by a HP-5890 Series II (Hewlett Packard,

USA) gas chromatograph equipped with a 30 m capillary column with an internal diameter of 0.55 mm (DB-624, Hewlett Packard, USA) at 90°C and a flame ionization detector at 250°C. A helium carrier stream of 1 ml/min was used. A 0.2 ml sample of gas in the test tubes was applied for analysis of carbon tetrachloride, chloroform and isoflurane.

Protein assay

The protein content of the microsomes was measured by the method of Lowry *et al.* /7/. The absorbance at 760 nm was recorded by a Beckman DC 640 spectrophotometer (USA). The experiment was performed at 25°C.

Statistical analysis

ANOVA and Bonferoni's test were used for statistical analysis of the results, with a p-value of less than 0.05 being considered significant.

RESULTS

Effect of isoflurane on the dehalogenation of CCl_4

Carbon tetrachloride undergoes dehalogenation by liver microsomes to produce chloroform. Chloroform formation was 0.113±0.10 nmol/0.1 g liver/min when 86 μM carbon tetrachloride and microsomes were incubated with NADPH. In the presence of isoflurane in this incubation system, chloroform formation was 0.203±0.012 nmol/0.1 g liver/min. This value was 1.80 times the value from the incubation mixture without isoflurane.

Time course of the change of cytochrome P450 content in the mixture of carbon tetrachloride, guinea-pig liver microsomes and NADPH

During the reaction of microsomes with carbon tetrachloride and NADPH, changes of microsomal cytochrome P450 content were observed. Fig. 1 shows the time course of the change of cytochrome P450 content in the mixture of carbon tetrachloride, guinea-pig liver microsomes and NADPH under anaerobic conditions. The amount of

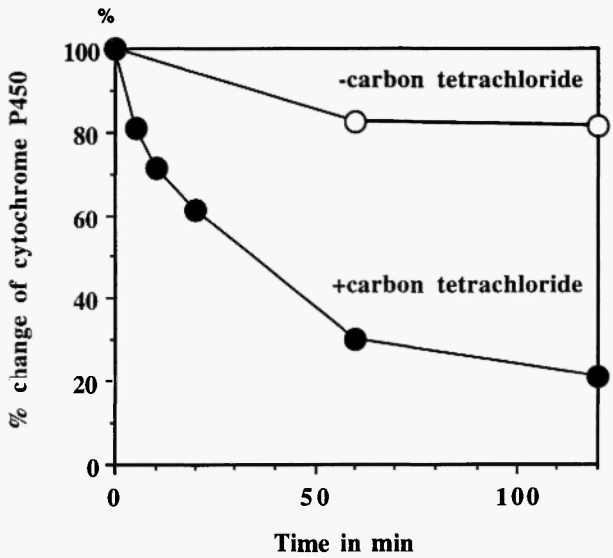

Fig. 1: Destruction of cytochrome P450 during dehalogenation of carbon tetrachloride. A sample (0.9 ml) of the microsomal suspension in 0.1 M potassium phosphate buffer (pH 7.4) from 0.1 g wet weight liver was sealed with deoxygenated nitrogen in a 12.5 ml test tube by a butyl rubber cap. Carbon tetrachloride (final concentration: 86 μM) and 0.1 ml of 30 mM NADPH were injected into the tubes through the cap. The reactions were performed at 37°C. The reaction was stopped by the addition of carbon monoxide. The concentration of cytochrome P450 in the reaction mixture decreased over time. Without carbon tetrachloride in the reaction mixture, the rate of cytochrome P450 decrease was lower.

cytochrome P450 in the reaction mixture decreased with time. The removal of carbon tetrachloride from the reaction mixture minimized the rate of cytochrome P450 decrease. The rate of decrease of cytochrome P450 is considered to be linear over 7 minutes incubation. Therefore, cytochrome P450 content was measured after 7 minutes incubation of carbon tetrachloride with guinea-pig liver microsomes in the presence of NADPH.

Essential component in the cytochrome P450 decrease

Cytochrome P450 was decreased to 71.6% of the control value by incubation of a reaction system consisting of guinea-pig liver micro-

somes, NADPH and 86 μM carbon tetrachloride under anaerobic conditions (Table 1). Addition of carbon monoxide resulted in a negligible decrease. The decrease in cytochrome P450 was prevented by the removal of NADPH or carbon tetrachloride from the reaction system. These findings show that the loss of the microsomal cytochrome P450 required carbon tetrachloride and NADPH and suggest that cytochrome P450 was destroyed during dehalogenation of carbon tetrachloride to produce chloroform in liver microsomes.

Effect of isoflurane on the decrease of cytochrome P450 during anaerobic dehalogenation of carbon tetrachloride

Addition of 370 μM isoflurane to the same incubation system resulted in a cytochrome P450 content of 84.9% of the control value (Table 1). This finding indicates that the loss of cytochrome P450 during dechlorination of carbon tetrachloride might be prevented by isoflurane.

DISCUSSION

As reported by Toranzo *et al.* /2/, loss of microsomal cytochrome P450 was observed in the mixture of guinea-pig liver microsomes, carbon tetrachloride and NADPH under anaerobic conditions (Fig. 1). In this system, the carbon tetrachloride underwent dehalogenation to produce chloroform. This microsomal cytochrome P450 loss was reduced in the absence of carbon tetrachloride or NADPH and was not observed in the presence of carbon monoxide (Table 1). These results strongly suggest that the microsomal cytochrome P450 loss was associated with the anaerobic dehalogenation of carbon tetrachloride by the liver microsomal oxidase system. This destruction of cytochrome P450 was reduced in the presence of isoflurane (Table 1). These results suggest that isoflurane prevented the destruction of cytochrome P450 during dechlorination of carbon tetrachloride by guinea-pig liver microsomes.

A possible mechanism for the dechlorination of carbon tetrachloride by liver microsomes is shown in Fig. 2. First, oxidized cytochrome P450 reacts with carbon tetrachloride to form a cytochrome P450-substrate complex, and this type I complex is then reduced by NADPH-cytochrome P450 reductase. Then carbon tetrachloride un-

TABLE 1

Cytochrome P450 loss during dehalogenation of carbon tetrachloride

	cytochrome P450 content (nmol/0.1 g liver)
control	2.18±0.05
complete system	1.56±0.06*
- carbon tetrachloride	1.92±0.03*
- NADPH	2.14±0.04
+ CO	2.18±0.04
+ isoflurane	1.85±0.04*
+ isoflurane, - carbon tetrachloride	1.91±0.05*

These values were obtained after 7 minutes incubation at 37°C (mean±SD; n=5). Control: microsomes incubated alone. Complete system: microsomes from 0.1 g liver, NADPH, and 86 μM carbon tetrachloride.
*: Significant, $p < 0.05$ compared to control.

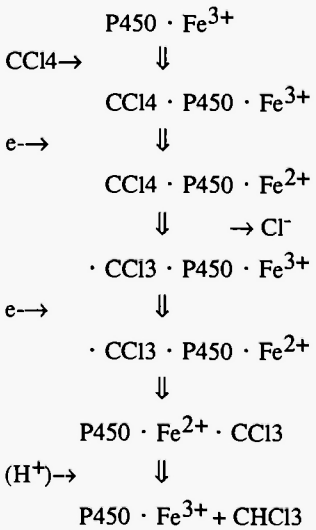

Fig. 2: A possible mechanism of dehalogenation of carbon tetrachloride.

dergoes cleavage of the CCl_3-Cl bond resulting in the formation of a trichloromethyl radical. After the reduction of cytochrome P450, the trichloromethyl radical binds to the heme molecule of cytochrome P450. Finally, this complex in turn decomposes with the formation of chloroform and oxidized cytochrome P450 /1/. Toranzo *et al.* /2/ have suggested that P450 destruction is mediated mainly by direct attack by carbon tetrachloride metabolites, trichloromethyl radicals, rather than by carbon tetrachloride-induced lipid peroxidation.

The stimulatory effect of isoflurane on the dechlorination of carbon tetrachloride in guinea-pig liver microsomes has been reported previously /4,5/. The Michaelis constant (K_m) for the dechlorination of carbon tetrachloride decreased, but the maximum velocity was unchanged in the presence of isoflurane /4,5/. The concentration of carbon tetrachloride used as a substrate of cytochrome P450 in this study was 86 μM, a lower value than the K_m value of 640 μM. Previous studies have also shown that isoflurane interacts with cytochrome P450, preventing the combination of carbon tetrachloride with cytochrome P450. Isoflurane also enhances the reduction of cytochrome P450 without interfering with NADPH-cytochrome P450 reductase, resulting in the enhancement of carbon tetrachloride dehalogenation by cytochrome P450 /4/. Isoflurane may accelerate the reduction of type I complexes and/or the reduction of the trichloromethyl radical complex, resulting in enhanced chloroform production (Fig. 2). In this study, chloroform formation increased to 1.80 times of the control value, while the destruction of cytochrome P450 during the dechlorination of carbon tetrachloride was reduced, in the presence of isoflurane (Table 1). These results might indicate that the speed of binding of the trichloromethyl radical to the heme group of cytochrome P450 after the reduction of cytochrome P450 was accelerated by isoflurane, resulting in reduction of cytochrome P450 destruction. These results also suggest that the destruction of cytochrome P450 during anaerobic dechlorination of carbon tetrachloride was mediated by the direct attack of the trichloromethyl radical rather than by carbon tetrachloride-induced lipid peroxidation.

In summary, these results show that isoflurane reduces the destruction of cytochrome P450 during the anaerobic dechlorination of carbon tetrachloride, and simultaneously enhances dechlorination. It is suggested that cytochrome P450 destruction is mediated by direct attack by the carbon tetrachloride metabolites, trichloromethyl radicals.

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