STUDY ON THE METABOLISM OF VOLATILE HYDROCARBONS IN MICE —PROPANE, n-BUTANE, AND iso-BUTANE—

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Abstracts.....A study was conducted to establish whether volatile hydrocarbons, such as propane, n-butane and iso-butane, are metabolized in mice or not. In mice having inhaled these gases, isopropanol and acetone were yielded from propane, sec-butanol and methyl ethyl ketone from n-butane, and tert-butanol from iso-butane as the respective metabolites. In addition, liver microsomes were found to contain the enzymic system participating in these metabolisms. In vitro reactions with liver microsomes produced isopropanol from propane, sec-butanol from n-butane, and tert-butanol from iso-butane. It was assumed that hydrocarbons were first converted to (ω -1)-alcohols by microsomal enzyme system and then to corresponding ketones by alcohol dehydrogenase.

Key words: propane, butanes, isopropanol, sec-butanol, tert-butanol, mice, liver microsomes

INTRODUCTION

Tsukamoto et al. (1985) have reported that in male mice of ICR strain, isopropanol and acetone are detected in the blood and various organs as the metabolites of inhaled propane, and sec-butanol and methyl ethyl ketone from n-butane.

Bahima et al. (1984) found female rats of Wistar strain exposed to n-heptane for 6 hours a day for 12 weeks excreted 2-heptanol, 2-heptanone, 2, 6-heptanediol and 2, 6-heptanedione in the urine as main metabolites. Howd et al. (1982) studied a possible correlation between the chronic existence of hexane and 2, 5-hexanedione and the development of neurotoxic syndrome. They suggested that 2, 5-hexanedione was formed from hexane by the intervention of liver microsomes. Thus hydrocarbons are metabolized in vivo possibly in liver microsomes. It appears that the carbon atom at

(ω -1) position of a hydrocarbon molecule is mainly oxidized to its secondary alcohol, which is further oxidized to ketone.

The purpose of the present experiment was to detect the metabolites of hydrocarbons *in vivo* and to identify the metabolic enzyme system involved. Male mice of ICR strain were exposed to propane, *n*-butane and iso-butane and the resultant metabolites were identified. The *in vitro* metabolism with liver microsomes were also conducted.

MATERIALS AND METHODS

1) *In vivo* metabolism of propane, *n*-butane, and iso-butane in mice

Ten-week-old male ICR obtained from Charles River (Japan Inc. Tokyo) were used after one week of accommodation. The mice were placed in a glass desiccator, 18 cm in diameter and 15 cm in height. Propane (99.9% purity), *n*-butane (99.8%), and iso-butane (99.9%) were purchased from Nissan Shoji Co., LTD. Tokyo Japan. The volatile hydrocarbons were mixed individually with oxygen (Nihonsanso Co., LTD Tokyo, purity 99.5%) in a mixing box at a ratio of 200 ml/min to 100 ml/min and then introduced into the desiccator. After one hour exposure, the animals were killed by cervical dislocations and the blood and various organs were taken out immediately. The blood was deproteinized with 0.6 N perchloric acid (PCA) reagent. The organs were pulverized with liquid nitrogen and deproteinized with 0.6 N PCA reagent. The specimens were immediately centrifuged and the supernatants were analyzed for the metabolites by means of head space gas chromatography as reported by Tsukamoto et al. (1983, 1985) and Idota (1985). Acetone, isopropanol, methyl ethyl ketone, sec-butanol and tert-butanol (Wako Pure Chemical Indust.) were used as identification of the metabolites.

2) *In vitro* metabolism of propane, *n*-butane, and iso-butane with liver microsomes of mice.

Microsomes were separated from the mouse liver according to Kato's method (Kato 1966). The proteins in the microsomes were measured in the same manner as reported by Lowry et al. (1951). The method, by which Chen et al. (1984) had studied the metabolism of alcohol and ketone, were also adopted in the present study.

Glucose-6-phosphate dehydrogenase (G-6-PD), Glucose-6-phosphate (G-6-P) and nicotinamide adenine dinucleotide phosphate (NADP⁺) in microsomal assays were obtained from Boehringer Mannheim.

Saturated aqueous solutions of hydrocarbons were prepared to serve as the substrate for these enzymes. The saturating concentration was approximately 2.9 mM for propane, 6.7 mM for *n*-butane, and 5.8 mM for iso-butane. Three ml of each solution were used for individual incubation. NADPH-generating system consisted of 10 mM of MgCl₂, 0.3 mM of NADP⁺, 10 mM of G-6-P, 7 unit of G-6-PD, and 0.1 mM of EDTA. The amount of protein in microsomes was set at 5 mg. The incubation was carried out at 30°C for 1 hour and terminated by addition of 1.5 ml of 0.6 N PCA reagent. After centrifugation of the incubation mixture, 1 ml of the supernatants was subjected to head

space gas chromatography (Perkin-Elmer F 45) and analyzed for the metabolites.

RESULTS

1) In vivo metabolism of propane, n-butane, and iso-butane in mice.

After one hour inhalation of propane, unchanged gas was detected in the blood and various organs of the animals (Fig. 1). Acetone and isopropanol, the metabolites of propane, were also found in the blood and organs (Fig. 2). The blood and organs of the animals having inhaled n-butane were found to contain unchanged n-butane (Fig. 3) and sec-butanol and methyl ethyl ketone as the metabolites (Fig. 4) respectively. Iso-butane was demonstrated in the blood and organs of the animals having inhaled the gas (Fig. 5). Its metabolite demonstrated in the specimens was tert-butanol (Fig. 6) which was identified by means of JEOL GC/MS DX-300 (Fig. 7).

Fig. 1. Gas chromatogram of unchanged propane from the blood after one hour inhalation of propane gas in ICR mice in a chamber.

Gas chromatographic conditions for gas component : Perkin Elmer F 45 Head Space Analyzer.

Column : unibeads A 80-100 mesh 2 m × 3 mm ID

Temperature : column 90°C, inject, detector 140°C

Flow rate : N₂ 40 ml/min, H₂ 40 ml/min, Air 320 ml/min

Detector : FID, Range : 1

2) In vitro metabolism of propane, n-butane, and iso-butane with liver microsomes of mice

Saturated aqueous solution of propane was incubated with liver microsomes of mice in the presence of NADPH-generating system. The specimen was deproteinized with PCA reagent and its supernatants were analyzed by head space gas chromatography. As shown in Fig. 8, isopropanol was demonstrated but no peak of acetone was detected. The same enzymic reaction on n-butane yielded sec-butanol (Fig. 9). Similarly iso-butanol yielded tert-butanol (Fig. 10). Ketone was not detected from any of the substrates tested.

Fig. 2. Gas chromatogram of metabolites from the blood after inhalation of propane gas in ICR mice in a chamber.

Gas chromatographic conditions for metabolites: Perkin Elmer F 45 Head space Analyzer.

Colume: Chromosorb W PEG 600 (10%) 80-100 mesh 4 m × 3 mm ID

Temperature: column 75°C, inject, detector 140°C

Flow rate: N₂ 40 ml/min, H₂ 40 ml/min, Air 320 ml/min

Detector: FID, Range: 1

Fig. 3. Gas chromatogram of unchanged n-butane from the blood after one hour inhalation of n-butane gas in ICR mice in a chamber. Gas chromatographic conditions were same as described in Fig. 1 legend.

Fig. 4. Gas chromatogram of metabolites from the blood after inhalation of n-butane gas in ICR mice in a chamber. Gas chromatographic conditions were same as described in Fig. 2 legend.

Fig. 5. Gas chromatogram of unchanged iso-butane from the blood after one hour inhalation of iso-butane gas in ICR mice in a chamber. Gas chromatographic conditions were same as described in Fig. 1 legend.

Fig. 6. Gas chromatogram of metabolites from the blood after inhalation of iso-butane gas in ICR mice in a chamber. Gas chromatographic conditions were same as described in Fig. 2 legend.

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Fig. 7. Mass spectra of peak 3 (tert-butanol).

Conditions : GC : column : glass column 10% PEG-600 on chromosorb W(AW)
 80-100 mesh, 3 m×2.5 mm i. d. temperature : column 75°C, inject, detector 100°C
 flow : He 20 ml/min
 MS : apparatus : JEOL GC/MS DX-300, ion voltage 70 eV, ion current 300 μ A,
 temperature : separator 250°C, ion source 240°C

Fig. 8

Fig. 9

Fig. 10

Fig. 8. 9 and 10. Gas chromatograms from the specimens by the microsomal assays with propane (2.9 mM), n-butane (6.7 mM) and iso-butane (5.8 mM) respectively. The mixture was incubated for 1 hour at 30°C. The reaction was terminated by the addition of 1.5 ml of 0.6 N PCA reagent. After centrifugation of the incubation mixture, 1 ml of the supernatants was subjected to head space gas chromatography.

Gas chromatographic conditions were same as described in Fig. 2 legend.

DISCUSSION

We found alcohols and ketones as the metabolites of the inhaled volatile hydrocarbons *in vivo*. That is, isopropanol and acetone were yielded from propane, sec-butanol and methyl ethyl ketone from *n*-butane, and tert-butanol from iso-butane. Bahima et al. (1984) reported detection of 2-heptanol, 2, 6-heptanediol, and 2, 6-heptanedione produced as the metabolites of *n*-heptane. The production of these metabolites suggests the occurrence of (ω -1)-oxidation as the major metabolic pathway of hydrocarbon.

Therefore, experiments were further conducted to identify the enzyme system involved in these reactions. The results revealed the participation of microsomal enzyme in the presence of the NADPH-generating system. By enzymic reactions with microsomes, isopropanol was yielded from propane, sec-butanol from *n*-butane and tert-butanol from iso-butane. In the *in vivo* metabolism the blood and organs of mice having inhaled hydrocarbons were found to contain isopropanol and acetone from propane, sec-butanol and methyl ethyl ketone from *n*-butane, and tert-butanol from iso-butane. It might be presumed that propane and *n*-butane were first converted to secondary alcohols, i. e., iso-propanol and sec-butanol, with microsomes, and then to ketones, i. e., acetone and methyl ethyl ketone, by alcohol dehydrogenase respectively. In fact, it was confirmed that isopropanol and sec-butanol were metabolized by alcohol dehydrogenase as reported by Merritt et al. (1959) and Idota (1985). Tert-butanol, the only metabolite from the mice having inhaled iso-butane, is not thought to be metabolized by alcohol dehydrogenase. Cederbaum et al. (1980), however, reported that tert-butanol at a high concentration (35 mM) was metabolized by microsomes to acetone and formaldehyde. Since the concentration of iso-butane used in our experiment was 2-3 mM, the metabolism might proceed very slowly. This might be the reason for the detection of tert-butanol alone in mice having inhaled iso-butane.

Fig. 11 illustrates the metabolic pathway of volatile hydrocarbons in the liver. It was presumed that hydrocarbons were first oxidized by the microsomal enzyme to produce (ω -1)-alcohols which were, in turn, oxidized to (ω -1)-ketones by alcohol dehydrogenase.

Figs. 11. Metabolic pathway of volatile hydrocarbons

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