Effect of Side-Stream Cigarette Smoke on the Hepatic Cytochrome P450

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Abstract. The effect of inhalation of side-stream cigarette smoke on the hepatic microsomal cytochrome P450 was investigated. Rats were placed in a chamber of 0.1 m$^3$ in volume, in which cigarettes were burned at the rate of 1, 3, or 5 cigarettes per h, 8 h/day for 5 days. Cytochrome P450 and NADPH-cytochrome c reductase showed no significant changes; however, cytochrome $b_5$ increased significantly. On the other hand, the activity of aryl hydrocarbon hydroxylase (AHH) decreased in the rats treated with a high concentration of cigarette smoke. In order to study the changes of isoforms of cytochrome P450, western blot analyses were performed. The inductions of three kinds of isoforms, cytochromes P450IA1, IA2, and IIB1, were demonstrated immunochemically. However, there were disagreements between the results of the western blot analyses and the measurements of total cytochrome P450 content and AHH activity.

The exposure to cigarette smoke is one of the greatest risk factors in the induction of lung cancer in humans. Polycyclic aromatic hydrocarbons (PAHs) and nitrosamines are among the thousands of compounds identified in cigarette smoke and are known premutagens and/or precarcinogens. These premutagens and precarcinogens become genotoxic after the biotransformation catalyzed by the microsomal mixed function oxidase system (MFOS) (IARC 1986). Cytochrome P450 plays the most important role in the bioactivation of PAHs and nitrosamines by MFOS. It is well known that cytochrome P450 is induced by various xenobiotic treatments (Snyder and Remmer 1982). Microsomal enzyme activities enhanced by cigarette smoke are responsible for the biotransformation of PAHs and nitrosamines (Juchau et al. 1982). Enzyme induction in laboratory animals exposed to cigarette smoke has also been reported, and the data show that this response is highly dependent upon tissue, species, and type of exposure. Generally, aryl hydrocarbon hydroxylase (AHH) activity in the lung and kidney is induced by exposure to cigarette smoke (Abramson et al. 1977; Bilimoria and Ecobichon 1980). However, the effect of cigarette smoke on the hepatic microsomal enzymes is variable. Some researchers have shown that the hepatic AHH activity of rats was not inducible by cigarette smoke (Gielen et al. 1979; Bilimoria and Ecobichon 1980; Graziano and Dorough 1984), while others have observed some elevation in enzyme activity (Kushinsky and Louis 1976; Raunio et al. 1983).

Recently, it has been clarified that cytochrome P450 in liver microsome has multiple forms (Lu and West 1980). Considerable efforts have been directed toward identifying and characterizing various forms of microsomal cytochrome P450, and more than 18 isoforms have been purified in rats. The activity of biotransformation differs depending on the isoforms of cytochrome P450. In the case of rats, cytochrome P450IIIB1 catalyzes the hydroxylation of testosterone and the demethylation of benzphetamine most efficiently, but benz[a]pyrene (BaP) is catalyzed poorly. Cytochrome P450IAlA has very high catalytic activity for BaP and zoxazolamine hydroxylation and 7-ethoxycoumarine O-dealkylation (Ryan et al. 1979). Therefore, the catalytic activity is easily affected by the enhanced isoforms and xenobiotics which are the substrate of the enzyme; it is very important to investigate which isoforms of cytochrome P450 are induced by exposure to cigarette smoke.

In this paper, the authors investigated whether hepatic microsomal cytochrome P450 is induced or not, and which isoforms of cytochrome P450 are altered by the inhalation of side-stream cigarette smoke.

Materials and Methods

The cigarettes were purchased from Japan Tobacco Inc, Japan. The trade name of the cigarettes is "Mild Seven," which contains 13 mg tar and 1.1 mg nicotine per cigarette according to the manufacturer. Sodium phenobarbital (PB) was obtained from Wako Chemical Co. 3-Methylcholanthrene (MC) was purchased from Sigma Chemical Co. Male adult Wistar rats weighing 170–180 g were obtained from Clea Japan Inc, and were acclimated for 3 days before exposure. A standard diet (CE-2, Clea Japan Inc.) and water were supplied ad libitum. The animals were kept at 22°C on a 12-h light/dark cycle for the duration of the study.

The exposure chamber was built of stainless steel and was 0.1 m$^3$ in volume. The animals were transferred into the exposure chamber at 9:00 am. The filtered air flowed from top to bottom at the rate of 15 L/min (9 complete air changes/h). Cigarettes were burned in the cham-
ber at the rate of 1, 3, or 5 cigarettes/h until 5:00 pm (8 h). After exposure, the animals were taken out of the chamber and transferred to a steel net cage in another room. They inhaled fresh air until the exposure on the following day. The exposure continued for 5 days. Details of the cigarette smoke exposure system were reported by Tanaka et al. (1990). Sodium phenobarbital (80 mg/kg body wt) in saline and 3-methylcholanganthrene (20 mg/kg) in corn oil were injected into rats i.p. for 4 days. These rats were used as the positive controls.

The particles in the exhaust gas from the exposure chamber were collected on a glass fiber filter (Preliter AP, Millipore) every hour using a low volume air sampler. The PAHs in the particle matter were extracted from the glass fiber filter to 50 ml of ethanol and benzene (1:3) solution under sonication for 15 min. The extract was concentrated by using an evaporator after filtration. PAHs in the condensate were dissolved in ether and poured into a centrifuge tube. After the ether had evaporated, the PAHs that were dissolved in acetonitrile were applied to a high-performance liquid chromatograph (HPLC).

The conditions of HPLC were as follows; LiChrosorb RP-18 was packed into a stainless-steel column (150 × 4.0 mm I.D.). Acetonitrile-water (9:1) degassed by sonication for 10 min was used as the eluant at a flow rate of 1.0 ml/min. The measurements of BaP, benzo[k]fluoranthene (BkF), and benzo[ghi]perylene (BghiP) were carried out by the fluorescence method at an excitation wavelength of 368 nm and an emission wavelength of 406 nm. Details of the measurements were reported by Kodama et al. (1983). Animals exposed to cigarette smoke were sacrificed 16 h after the final exposure. All animals were sacrificed at 9:00–10:00 am to control for diurnal variabilities. Livers were perfused in situ with ice-cold 0.9% saline, blotted dry, weighed, mixed, and homogenized in 6 volumes of ice cold Tris-acetate buffer (pH 7.4), containing 0.1 M KCl and 1.0 mM EDTA. Liver microsomes were isolated by the method of Van der Hoeven and Coon (1974) with minor modifications. The details have been previously reported by Kawamoto et al. (1988, 1990).

Microsomal protein concentrations were assayed by the method of Lowry et al. (1951), using bovine serum albumin as the standard. The levels of cytochrome P450 and cytochrome b5 were determined by a Shimadzu double beam spectrophotometer (Model UV-210) using the method of Omura and Sato (1964). NADPH-cytochrome c reductase activity was measured as described by Strobel and Dignam (1978). AHH activities were assayed by the method of Nebert and Gelboin (1968) with minor modifications. 3-Hydroxybenzo[a]pyrene (3-OH-BaP) which was produced from BaP by AHH was measured fluorometrically, using HPLC. Details of the quantification of AHH activity were reported by Yashikawa et al. (1987).

A polyacrylamide electrophoresis of hepatic microsomes was performed in 8.5% polyacrylamide slab gels in the presence of sodium dodecyl sulphate as described by Laemmli (1970). A western blot analysis was done after transferring protein from the slab gel to nitrocellulose (NC) membrane (0.45 μm, Bio-Rad) by TransBlot SD (Bio-Rad). After blocking with 3% gelatin, the NC membrane was washed twice with 20 mM Tris, 500 mM NaCl (pH 7.5) containing 0.1% SDS and 0.2% Triton X-100® (washing buffer). The NC membrane was incubated with rabbit anti-serum against cytochrome P450IA1, IA2, or IIB1 for 1–2 h. The NC membrane was washed with a washing buffer solution twice, and then incubated with goat anti-rabbit IgG (H + L) conjugated with alkaline-phosphatase for 1 h. The final washing with Tris buffered saline following the washing buffer washing, the detection was carried out with an AP color development kit (Bio-Rad, Cat # 170-6432).

The antibodies against the three isoforms of cytochrome P450, that is, P-450(PB-1), P-450(MC-1) and P-450(MC-2), were kindly presented by Prof. Dr. Tsuneo Omura, Department of Molecular Biology, Graduate School of Medical Science, Kyushu University. The specificities of these three antibodies were reported by Kuwahara et al. (1984). Cytochrome P-450(MC-1), P-450(MC-2), and P-450(PB-1), named by Omura, correspond to cytochrome P450IA1, IA2, and IIB1, named by Nebert et al. (1987), respectively. In our report, the nomenclature of isoforms of cytochrome P450 conforms to that by Nebert et al.

### Results and Discussion

Figure 1 shows the average concentrations of PAHs in the exposure chamber during the exposure period from 9:00 am to 5:00 pm. The levels of BaP, BkF, and BghiP increased as the number of cigarettes consumed per hour increased from 1 to 5. The concentration of carbon monoxide in the chamber was 5–250 mg/m³ at 1 cigarette/h, 100–300 mg/m³ at 3/h, and 250–400 mg/m³ at 5/h.

A portion of the exposure should be through the gut, due to preening and to mucociliary clearance of inhaled particles. The latter process (mucociliary clearance) will result in a large fraction of the inhaled materials going to the gut. It is suspected that the change of hepatic cytochrome P450 is affected by the intake of the content of cigarette smoke through not only the lungs but also the gut. In daily life, humans who are exposed to side-stream cigarette smoke also swallow smoke particles by mucociliary clearance. Hence it is meaningless to separate the effect of inhalation from that of swallowing cigarette smoke particles.

Table 1 shows the changes of body weight, liver weight, relative liver weight (liver weight/body weight × 100), microsomal protein, cytochrome P450, cytochrome b5, NADPH-cytochrome c reductase, and aryl hydrocarbon hydroxylase of rats which were exposed to cigarette smoke at the rate of 1, 3, or 5 cigarettes/h. The body weights and liver weights decreased with the increase of the number of cigarettes. The reduction of dietary consumption was thought to be a main reason for the loss of body weight. However, the relative body weights were not changed by cigarette exposure. Microsomal protein concentrations also decreased with the increase of the number of cigarettes. Cytochrome P450 and NADPH-cytochrome c reductase showed no significant changes when exposed to cigarette smoke. Cytochrome b5 was increased significantly by the cigarette smoke exposure. The activity of cytochrome b5 in rats exposed to 5 cigarettes/h was about 1.6 times higher than that of control. The AHH activity was measured using BaP as a substrate. The AHH activity was not significantly changed by 1 or 3 cigarettes/h. In the case of 5 cigarettes/h, it decreased significantly (the Scheffe F-test was used to compare each group.)