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Effect of cigarette smoke on lipid peroxidation and liver function tests in rats.

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Abstract

The effect of cigarette smoke on organ weights, lipid peroxidation and plasma biochemical parameters was investigated in male Wistar rats. Daily exposure (for 20 min twice a day) to cigarette smoke for 27 days caused a significant decrease in liver weight and a significant increase in lung weight. The smoke-exposure group showed increased lipid peroxidation in the liver, but not in the lung. In the smoke-exposure group, the GOT, gamma-GTP, total bilirubin and LDH values were significantly higher than those in the control group, while the plasma glucose value was significantly lower. These results suggest that cigarette smoking might induce liver injury by enhancing lipid peroxidation.

KEYWORDS: cigarette smoking, lipid peroxidation, liver function, rats

Brief Note

Effect of Cigarette Smoke on Lipid Peroxidation and Liver Function Tests in Rats

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The effect of cigarette smoke on organ weights, lipid peroxidation and plasma biochemical parameters was investigated in male Wistar rats. Daily exposure (for 20 min twice a day) to cigarette smoke for 27 days caused a significant decrease in liver weight and a significant increase in lung weight. The smoke-exposure group showed increased lipid peroxidation in the liver, but not in the lung. In the smoke-exposure group, the GOT, γ -GTP, total bilirubin and LDH values were significantly higher than those in the control group, while the plasma glucose value was significantly lower. These results suggest that cigarette smoking might induce liver injury by enhancing lipid peroxidation.

Key words: cigarette smoking, lipid peroxidation, liver function, rats

Tobacco smoking has been proposed as a risk factor in the pathogenesis of various diseases, such as obstructive lung diseases (1, 2), ischemic heart diseases (3, 4) and neoplasm (5). However, there are few reports which address the possible correlation between smoking and liver diseases. On the other hand, a recent report suggests that oxygen free radicals and other active oxygen species are involved in the adverse effects of tobacco smoking (6). Thus, in the present study, to clarify the effect of smoking on liver function and lipid peroxidation, we examined the weights of some organs, lipid peroxidation in the liver and lung, and plasma biochemical parameters in male rats exposed to cigarette smoke for 27 days.

Sixteen male Wistar rats (Charles River Lab., Atsugi, Japan) were used in the experiment. These animals each weighing 180-190 g were kept in groups of four in a room maintained on a 12h light/12h dark cycle (lights on at 0700) at $22 \pm 1^\circ\text{C}$ and *ca.* 60% relative humidity. The cigarettes used in the present study were commercial cigarettes (Long-Peace[®]; Japan Tobacco Co.). Each Long-Peace[®] cigarette weighs approximately 1.02 g, and contains 2.2 mg of nicotine and 23 mg of tar.

To expose the animals to cigarette smoke, the Hamburg II Smoking Machine (Borgwaldt, Hamburg, Germany) was used. The apparatus consists of the smoking head (up to 30 cigarettes can be attached), the smoking chamber slide piece, the inhalation chamber and animal holders (10 rats can be exposed to the smoke simultaneously).

In the experiment, 15 cigarettes in the smoking head were lighted initially and the remaining 15 cigarettes were lighted after the first 15 had burned out. The smoke from the lighted cigarettes was pumped into the smoking chamber, mixed with air at a ratio of 1:7 and sent to the inhalation chamber. The inhalation conditions were: inhalation volume, 35 ml; duration, 2 sec; and a frequency of 15 puffs/min.

The animals were exposed to cigarette smoke for 20

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^bPresent address: Pharmaceutical Analysis Center, Okayama Pharmacist Association, Okayama 700, Japan. The abbreviations used are: GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ -GTP, γ -glutamyl transpeptidase; CHE, cholinesterase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; TC, total cholesterol; TG, triglycerides.

min twice a day (at 0800 and 1700) for 27 days ($n = 8$). Control rats ($n = 8$) were handled in the same way except for the exposure to cigarette smoke. Since tobacco smoking is known to affect body weight and growth (7, 8) and since the blood biochemical parameters are influenced by obesity (9, 10), the control animals were allowed an amount of food equivalent to that consumed by the smoke-exposure animals on the day before. Body weight, and food and water consumption were measured every day (at 0730). After fasting for 20h following the last exposure to cigarette smoke, blood samples were collected from the inferior vena cava of animals under ether anesthesia. Subsequently, the liver, lung, heart, kidney and spleen were removed and weighed. Lipid peroxidation in lung and liver homogenates was estimated based on malondialdehyde (MDA) formation by the method of Ohkawa *et al.* (11). Plasma glucose was measured using a Mitsubishi Kasei glucose analyzer GL 101 (Mitsubishi, Tokyo, Japan), and all other biochemical parameters were measured using a Hitachi autoanalyzer 726 (Hitachi, Tokyo, Japan).

The results were statistically evaluated by the two-tailed Student's *t*-test or the Aspin-Welch method.

There was no difference in body weight gain between the cigarette smoke-exposure group and the control group throughout the experiment (data not shown).

Table 1 summarizes the wet weights of each organ and the lipid peroxidation in liver and lung homogenates. The liver weight in the cigarette smoke-exposure group was significantly lower than that in the control group ($P < 0.05$), whereas the lung weight in the cigarette smoke-exposure group was significantly higher ($P < 0.01$). Lipid peroxidation in the liver tended to be higher in the smoke-exposure rats than in the control group, but there was no difference in the lung between the groups.

As shown in Table 2, GOT, γ -GTP ($P < 0.01$), total bilirubin and LDH ($P < 0.05$) values in the cigarette smoke-exposure group were significantly higher than those in the control group. However, the plasma glucose level was significantly lower in the smoke-exposure group than in the control group ($P < 0.01$). The CHE and TG values tended to be higher in the smoke-exposure group. There were no significant differences in the GPT, ALP and TC levels between the groups.

The present experiments showed that exposure to cigarette smoke causes biochemical changes in the liver. Chow (12) showed that exposure to cigarette smoke for 7 days does not affect tissue weights or biochemical param-

Table 1 Effects of cigarette smoking on organ weights and lipid peroxidation in rats

Parameter	Control group ($n = 8$)	Smoking group ($n = 8$)
Organ weight (g of wet tissue)		
Liver	8.04 \pm 0.25	7.26 \pm 0.23*
Lung	1.31 \pm 0.04	1.73 \pm 0.10**
Heart	0.92 \pm 0.02	0.92 \pm 0.02
Kidney	2.09 \pm 0.04	1.94 \pm 0.06
Spleen	0.78 \pm 0.05	0.63 \pm 0.04
Lipid peroxidation ^a		
Liver	244 \pm 23	671 \pm 221 [†]
Lung	48.3 \pm 4.0	47.0 \pm 4.9

Each value represents the mean \pm SEM. ^aLipid peroxidation was estimated based on malondialdehyde formation (nmol/g of wet tissue/h). [†] $P < 0.1$, * $P < 0.05$, ** $P < 0.01$ vs. the corresponding control values.

Table 2 Effects of cigarette smoking on the plasma biochemical parameters in rats

Parameter	Control group ($n = 8$)	Smoking group ($n = 8$)
GOT (IU/l)	58.9 \pm 1.6	83.0 \pm 3.5**
GPT (IU/l)	21.4 \pm 1.7	21.1 \pm 1.8
γ -GTP (IU/l)	0.38 \pm 0.18	1.38 \pm 0.18**
CHE (Δ pH)	0.039 \pm 0.004	0.053 \pm 0.006 [†]
Plasma glucose (mg/dl)	128.1 \pm 3.6	89.9 \pm 6.1**
Total bilirubin (mg/dl)	0.10 \pm 0.0001	0.16 \pm 0.026*
ALP (K.A.U.)	18.1 \pm 0.74	15.3 \pm 1.32
LDH (U/l)	84.1 \pm 11.6	433.1 \pm 100.1*
TC (mg/dl)	50.3 \pm 2.6	45.4 \pm 1.9
TG (mg/dl)	53.9 \pm 4.4	61.0 \pm 5.2 [†]

Each value represents the mean \pm SEM.

[†] $P < 0.1$, * $P < 0.05$, ** $P < 0.01$ vs. the corresponding control values.

ters in rats. These findings suggest that short-term exposure induces little biochemical changes in rats. Yamada *et al.* (13, 14) reported that γ -GTP, TC and TG values increase in heavy smokers who have no drinking habit and speculated that those biochemical changes result from fatty liver. Clinical studies in patients with fatty liver have shown that biochemical parameters increase such as transaminase, γ -GTP and CHE levels and that lipid metabolism is often impaired (15, 16). In the present

study, the GOT and γ -GTP levels increased significantly and CHE and TG tended to increase in the cigarette smoke-exposure group. Thus, it seems likely that cigarette smoke caused the observed fatty degeneration of the liver.

On the other hand, in the present study, MDA levels in the liver tended to increase. Tobacco smoke is known to contain reactive free radicals, such as nitric oxide or nitric dioxide (17), and to activate neutrophils and/or macrophages to produce reactive oxidants such as superoxide or hydrogen peroxide (18, 19). Furthermore, it has been suggested that those oxidants and lipid peroxidation cause tissue injury and promote carcinogenesis (20). However, there have been no reports that smoking causes an increase in lipid peroxidation in the liver. Concerning the possible correlation between lipid peroxidation and liver diseases, CCl_4 -induced hepatotoxicity has been extensively studied. CCl_4 has been reported to inhibit secretion of lipoprotein from liver cells and to cause TG accumulation (21). Two mechanisms of this fatty degeneration in the liver have been proposed (22): One is lipid peroxidation of the liver cell endoplasmic reticulum induced by trichloromethyl radical or trichloromethylperoxyl radical, and the other is covalent binding of the CCl_4 metabolites to cell constituents. Therefore, in the present study, it is possible that the lipid peroxidation induced by cigarette smoke is responsible for the observed fatty degeneration in the liver.

In the present study, the 27-day exposure to cigarette smoke did not alter the MDA levels in the lung. While Chow (12) reported that MDA levels in the lung of rats significantly decreased after 3 or 7 days of exposure to cigarette smoke, Takahashi *et al.* (23) showed that MDA levels in the lung of golden hamsters significantly increased after 12 weeks of exposure to cigarette smoke, but not after 2, 4 or 8 weeks. Thus, it is likely that the smoking induces oxidative damage in the liver rather than the lung at the early stage of exposure to cigarette smoke.

In summary, exposure to cigarette smoke for 27 days induced a decrease in the liver weight, increased MDA levels and significant differences in the levels of GOT, γ -GTP, plasma glucose, total bilirubin and LDH. These results suggest that cigarette smoking enhanced lipid peroxidation, resulting in biochemical changes in the liver under the present experimental conditions. However, further studies are needed to clarify the effects on histopathology and lipid peroxidation in the liver after long-term exposure to cigarette smoke.

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