J Nutr Sci Vitaminol, 51, 374–376, 2005

Note

Influence of Cigarette Smoke on the L-Ascorbic Acid Metabolism and the Activities of Drug-Metabolizing Enzyme in Rats

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(Received December 3, 2004)

Summary This study clarified the influence of cigarette smoke on the L-ascorbic acid (AsA) metabolism and the activities of drug-metabolizing enzyme in rats. The test rats (group T) were exposed to weak sidestream smoke from cigarettes for 2 h, everyday for 57 days. AsA concentration in the tissues and excreted amount of AsA in urine of group T tended to be higher than those of control group (group C). The plasma AsA concentration and the activities of aniline hydroxylase and 7-ethoxycoumarin *O*-deethylase of group T were significantly higher than those of group C. There was no significant difference in the activity of UDP glucuronosyltransferase or in the liver cytochrome P-450 content between these two groups.

Key Words ascorbic acid, cigarette smoke, drug-metabolizing enzyme

It has been reported that cigarette smoke contains free radicals and other compounds which are capable of generating free radicals and carcinogens, such as tar and benzopyren (*1–5*). Inhalation of these substances would be capable of influencing the metabolism of L-Ascorbic acid (AsA), which is consumed as an antioxidant to scavenge radicals. It has also been reported that drug-metabolizing enzyme activities in AsA-deficient animals were lower than those in normal ones (*6*). This suggested that AsA might be required to maintain normal drug-metabolizing enzyme activity. Our previous paper showed that exposure of rats to relatively strong cigarette sidestream smoke increased the AsA concentration in tissues and the activities of drug-metabolizing enzymes (*7*). However, there is no clear evidence that weak cigarette sidestream smoke could increase the AsA concentration in tissues and the activities of drug-metabolizing enzymes. Therefore, in this study, to clarify the influence of weak cigarette sidestream smoke on the AsA metabolism and activities of drug-metabolizing enzymes, AsA concentrations in the plasma and tissues, the excreted amount of AsA in the urine, liver cytochrome P-450 content and activities of drug-metabolizing enzymes of the rats which were exposed to weak cigarette smoke for a long time were measured.

Materials and Methods

Experimental plan. Male Wistar rats 7 wk old (purchased from Nihon Clea Co., Tokyo, Japan) were divided into two groups, the control group (group C) composed of 7 and the test group (group T) composed of 8 animals. The breeding conditions for the rats were the same as those described in a previous paper (*7*). After a 14-d prefeeding, group T was exposed to sidestream smoke from cigarettes for 2 h every day for 57 d, and was given diet freely. The amount of diet given to rats in group C was controlled to be equivalent to the intake of group T on the previous day (pair-feeding).

The test animals were put in a stainless steel cage which was then placed in an acrylic cage with four small holes as outlets for ventilation. The top cover was closed. One cigarette (PEACE King Size, Japan Tobacco Inc., Tokyo, Japan) was lit for 3 min in the acrylic cage which filled the acrylic cage with smoke, after which the cigarette was put out, and this condition was maintained for 27 min. This passive smoking procedure which took a total of 30 min for one trial was repeated successively 4 times in a day for 57 d. This study was approved by the Committee on Animal Experiments of the Faculty of Human Life and Environmental Sciences, Ochanomizu University, and the animals were maintained in accordance with the faculty's guidelines for the care and use of laboratory animals.

In the pre-experimental and experimental period, the concentrations of AsA in the urine and plasma were measured. Blood was collected from the caudal vein. At the end of experimental period, the rats were killed under anesthesia with ether and blood was collected from the abdominal aorta, and the liver, adrenal glands, lungs and kidneys were removed.

Measurement of AsA and enzyme assay. The AsA concentrations in the plasma, tissues and urine were determined by the methods described in our previous paper (*7*). Activities of Aniline hydroxylase and 7-ethoxycoumarin *O*-deethylase and the liver cytochrme P-450 content were measured according to the methods described in the same paper (*7*). UDP glucuronosyltransferase activity was assayed using 4-nitrophenol as

the substrate (*8*). Protein was measured by the Lowry method (*9*).

Statistical analysis. Significant difference between groups was estimated by Student's *t*-test.

Results and Discussion

The growth response of group T was almost equal to that of group C (Fig. 1). Immediately after starting the cigarette smoke exposure test, the body weight gains of group T in the experimental period decreased as compared to those observed in the pre-experimental period, due to the reduction in diet intake caused by loss in appetite. Before the experiment, there was no difference in the excreted amount of AsA in the urine or AsA concentration in plasma between groups C and T (Fig. 2, Table 1). Throughout the experimental period, the excretion of AsA into the urine of group T seemed to be slightly higher than that of group C, and the plasma AsA concentration was apparently higher than that of group C (Table 1). At the end of the experiment, the AsA concentrations in the liver, adrenal glands, lungs and kidneys of group T tended to be higher than those of group C (Table 2). The results suggested that exposure of rats to cigarette smoke stimulates the biosynthesis of AsA in these animals. The activities of aniline hydroxylase and 7-ethoxycoumarin *O*-deethylase of group T were significantly higher than those of group C, and the activity of UDP glucuronosyltransferase was a little higher than that of group C, but not significantly different. There was no difference in the liver cytochrome P-450 content between these two groups (Table 3). It has been reported that supplementation of xenobiotics to animals stimulated activities of drug-metabolizing enzymes (*10*). Horio et al. have reported that the induction of UDP glucuronosyltransferase activity was closely related to the increase of AsA biosynthesis in rats supplemented with xenobiotics (*8*). The increased AsA biosynthesis which tended to cause the increase in the AsA concentrations of the tissues and plasma and in the excreted amount of AsA in the urine might be attributed to the induction of UDP glucuronosyltaransferase activity of the rats exposed to cigarette smoke. The chemical compounds in cigarette smoke which are considered to be mostly xenobiotics might stimulate the activities of drug-metabolizing enzymes and AsA biosynthesis.

In this study, the activities of drug-metabolizing enzymes and AsA biosynthesis of rats exposed to weaker cigarette smoke for a long time also tended to increase similarly to that of rats exposed to strong ciga-

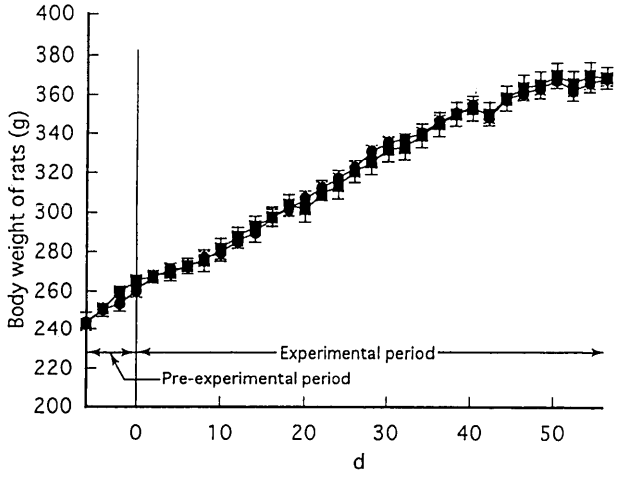

Fig. 1. Growth curves for rats exposed to cigarette sidestream smoke for 57 d. ●: Control group, ■: Test group. Symbols represent means and bars represent SE (n=7–8).

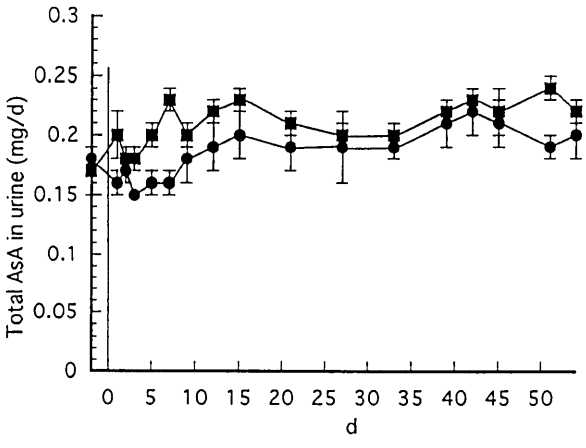

Fig. 2. Excretion of AsA in the urine of rats exposed to cigarette sidestream smoke for 57 d. ●: Control group, ■: Test group. Symbols represent means and bars represent SE (n=7–8).

Table 1. Concentration of AsA in plasma of rats exposed to cigarette sidestream smoke for 57 d.

Period	d	Control group (mg /100 mL of plasma)	Test group (mg /100 mL of plasma)
Pre-experimental	−2	0.312±0.006	0.324±0.022
Experimental	30	0.349±0.020	0.386±0.018
	51	0.333±0.019	0.412±0.023*
	57	0.537±0.022	0.622±0.026*

Each value is the mean±SE (n=5–8).
* Significantly different from the control group at $p<0.05$.

Table 2. Concentration of AsA in tissues of rats exposed to cigarette sidestream smoke for 57 d.

Tissues	Control group (mg /100 g of tissues)	Test group (mg /100 g of tissues)
Liver	4.45±0.66	7.96±1.88
Adrenal Glands	284±70	331±62
Lungs	23.2±1.9	27.1±1.5
Kidneys	4.91±0.29	5.63±0.40

Each value is the mean±SE (n=7–8).

Table 3. Content of liver cytochrome P-450 and activities of drug-metabolizing enzymes in rats exposed to cigarette sidestream smoke for 57 d.

	Control group	Test group
Cytochrome P-450 (nmol/mg of protein)	1.14±0.08	1.04±0.07
Aniline hydroxylase (nmol 4-aminophenol formed/min/mg of protein)	0.235±0.037	0.400±0.051*
7-Ethoxycoumarin *O*-deethylase (nmol 7-hydroxycoumarin liberated/min/mg of protein)	1.47±0.14	2.04±0.21*
UDP glucronosyltransferase (nmol 4-nitrophenol decreased/min/mg of protein)	10.9±1.7	15.4±2.2

Each value is the mean±SE (n=7–8).
* Significantly different from the control group at $p<0.05$.

rette smoke (*7*) for a relatively short time. Therefore, it could be assumed that the exposure to weak cigarette smoke for a long time could give a similar effect to the body as that of exposure to strong cigarette smoke for a relatively short time. It is then important to consider the influence of weak cigarette sidestream smoke on human health.

These results suggested that the amount of AsA required for the animals exposed to cigarette smoke seemed to be larger than that of animals which were not exposed. The increase in AsA requirement could be due to the increase in the amount of AsA consumed by scavenging radicals and for oxidants in the animals. Furthermore, the increased level of AsA concentration in tissues has been brought about by an increase in the activity of UDP glucuronosyltrransferase. Eventually, AsA could prevent any possible damages in animals which might be caused by chemical reactive compounds contained in cigarette smoke.

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