INFLUENCES OF LONG-TERM CIGARETTE SMOKE EXPOSURE ON PHARMACOKINETICS OF THEOPHYLLINE, AND ON LIVER MICROSOMAL ENZYMES IN RATS

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SUMMARY

The influences of long-term cigarette smoke exposure on pharmacokinetics of oral theophylline (20 mg/kg), and on liver microsomal enzymes which metabolize drugs were studied in rats. Animals were exposed to cigarette smoke for 20 min each in the morning and evening every day for 26 days in the pharmacokinetic study, and 27 days for the enzyme assays. Theophylline was administered 13 h after the last exposure to smoke, and plasma concentrations were measured using HPLC. Plasma concentrations of theophylline during the absorption phase and 6 h after oral administration were lower in the long-term cigarette smoke-exposed group than in the control group. In the smoke-exposed group, the AUC and K_a were lower, and the K_e was slightly higher than in the control group. Liver weight and the ratio of liver weight to body weight were lower in the smoke-exposed group, and cytochrome b_5 content and NADPH-cytochrome P-450 reductase activity were higher, but cytochrome P-450 content did not differ from the control group. These results indicate that long-term exposure to cigarette smoke suppresses theophylline absorption from the gastrointestinal tract, accelerates its elimination, and affects liver microsomal enzymes which metabolize drugs.

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KEY WORDS

theophylline, long-term, cigarette smoke exposure, pharmacokinetics, microsomal enzymes, rats

INTRODUCTION

Theophylline is widely used as a bronchodilator in clinical medicine. It is well known that theophylline is mainly eliminated by biotransformation in the liver and by urinary excretion of the metabolites. The therapeutic and toxic effects of theophylline are closely related to its plasma concentration. The therapeutic dose range of theophylline is very narrow, and monitoring of plasma concentration is required in clinical treatment /1-3/. It has also been reported that long-term smoking shorten the half-life of theophylline, increases its clearance, and reduces its plasma concentration in young adults /4, 5/. Thus, cigarette smoking may increase the theophylline requirement. This higher rate of theophylline elimination is reported to be caused by induction of the liver microsomal enzyme system /6-8/. We have already observed in animal studies that in the absorption phase, plasma theophylline concentrations can be lowered by acute exposure to cigarette smoke /9/. However, most people who smoke do so for the long term. Systemic studies of the effect of cigarette smoke on the absorption of oral theophylline are insufficient. Therefore, in the present experiment, we studied the influence of long-term exposure to cigarette smoke on the pharmacokinetics of oral theophylline. We also assayed liver microsomal enzymes which metabolize theophylline in rats after long-term exposure to cigarette smoke.

MATERIALS AND METHODS

Animals

Twelve male Wistar rats weighing 216-233 g were used to study the pharmacokinetics of oral theophylline. They were divided into two groups: one was the long-term cigarette smoke-exposed group, and the other was the non-smoking restrained control group. To assay liver microsomal enzymes, 16 male Wistar rats weighing 159-177 g were used, and were divided into two groups as described above.

Animals were housed 3-4 per cage in 26 x 36 x 25 cm plastic-walled cages, and were maintained on a 12 h light-dark cycle (lights on from 8:00 to 20:00) at room temperature of 20-22°C and approximately 60% relative humidity. Food was given *ad libitum* to the smoke-exposed group, and the control rats were given the amount of food that was taken on the preceding day in the smoke-exposed group. Thus, the food intake was nearly the same in the two groups. Both groups were given free access to water.

Chemicals

Pure powdered theophylline (Katayama Chemicals, Japan) was suspended in 0.5% sodium carboxymethyl cellulose solution and given orally at 20 mg/kg in 2 ml/kg of body weight by gastric tube. 2-Hydroxyethyl theophylline (Tokyo Kasei, Japan) was used as an internal standard (IS). Reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Kohjin, Japan. Cytochrome *c* (Type III) was purchased from Sigma, USA. All other chemicals used were of reagent grade and were obtained from commercial sources.

Cigarettes

The cigarettes used in the present experiment were "Long-Peace®" filter cigarettes supplied by Japan Tobacco Inc., Japan. Each cigarette weighed 1.023 g. Contents of nicotine and tar in the smoke were 2.2 and 23 mg per cigarette, respectively, when two-thirds of the cigarette was consumed using a smoking machine (the inhalation conditions were as follows: inhalation volume - 35 ml, duration - 2 sec and interval - 1 min).

Apparatus for cigarette smoking

The Hamburg II Smoking Machine (Borgwaldt) was used for exposing the animals to cigarette smoke. The apparatus consists of a smoking head, to which up to 30 cigarettes can be attached, a smoke channel, a smoking chamber slide piece, an inhalation chamber and 10 animals holders. The cigarettes attached to the smoking head were lit individually and the smoking head was turned. The smoke of the lit cigarettes was pumped to the smoking chamber, mixed with air at

a ratio of 1:7 and sent to the inhalation chamber. In the inhalation chamber, each animal in the holders was exposed to the smoke. In the present experiment, 15 cigarettes were lit initially and the remaining 15 cigarettes were lit after the first 15 cigarettes had burned out. The inhalation duration was 2 sec and the frequency was 15/min. Six or 8 animals were exposed simultaneously.

Theophylline pharmacokinetics

1. Cigarette smoke exposure and theophylline administration

Animals in the cigarette smoke-exposed group were held in the animal holders of the smoking machine and exposed to cigarette smoke for 20 min each in the morning and evening every day for 26 days. The control rats were held in the animal holders for the same period, but were not exposed to the cigarette smoke. Body weights were 258-300 g in the cigarette smoke-exposed group and were 290-328 g in the control group. Theophylline at a dose of 20 mg/kg was administered orally 13 h after the last exposure to cigarette smoke and plasma concentrations of theophylline were measured by high performance liquid chromatography (HPLC).

2. Blood collection and pretreatment for HPLC

Blood samples to determine the plasma theophylline concentrations were collected in capillary tubes (60 μl, Miles Sankyo Co.) from the tail vein of each rat. The proximal part of the tail vein was carefully incised with a knife (approximately 1 mm) to cause bleeding. The blood was sampled 0.5, 1, 2, 4, 6, 8 and 12 h after the administration of theophylline. Plasma separation was performed by centrifugation at 5,396 *g* for 3 min, using a hematocrit centrifuge (Compur M 1100, Miles Sankyo Co.). Twenty-μl of the obtained plasma were used to determine the plasma drug concentrations.

To pretreat the samples of plasma which contained theophylline, 20 μl of plasma was added to 200 μl methanol solution containing the IS (4 μg/ml) and mixed for 20 sec. After centrifugation at 7,000 *g* for 5 min, 20 μl of the supernatant was injected onto a HPLC column.

3. Determination of plasma theophylline concentrations by HPLC

The plasma drug concentrations were determined by HPLC [Hitachi Type 655-11 – automatic sampler (Type 655A-40), processor (Type 655-61) and UV detector (Type 655A)]. A stainless steel column packed with C-18 10 μm μBondapak (Waters Assoc.) was used and the column was maintained at room temperature. The sample was injected by an automatic sampler. The mobile phase was acetonitrile and 0.01 M acetate buffer, pH 4.0 (1:12 in volume) and the flow rate was 1.5 ml/min. Theophylline and the IS were detected at 280 nm (0.01 AUFS).

4. Pharmacokinetics

The area under the plasma concentration-time curve (AUC) and the mean residence time (MRT) were obtained from the plasma drug concentration-time data of each animal with a personal computer program for model-independent moment analysis /10/. The absorption rate constant (K_a), the elimination rate constant (K_e) and the volume of distribution (V_d) were estimated by non-linear least squares fit (program MULTI) of data from each animal /11/.

Assay of liver microsomal enzymes

1. Cigarette smoke exposure

Animals in both groups were treated in the same way as those in the theophylline pharmacokinetics experiment, except that they were exposed to cigarette smoke for 27 days. The body weights of animals were 285-308 g in the cigarette smoke-exposed group and were 284-303 g in the control group. The liver microsomes of all rats in both groups were prepared 20 h after the last cigarette smoke exposure.

2. Preparation of liver microsomes

To prepare microsomes, the animals were sacrificed by drawing blood from an inferior artery under anesthesia with ether. The livers were removed rapidly and placed into ice-cold 1.15% KCl solution. They were homogenized in 4 volumes of 1.15% KCl solution using a

loose-fitting Potter homogenizer with a teflon pestle. The homogenate was centrifuged at 9,000 *g* in a refrigerated centrifuge (Hitachi 20PR-52) at 4°C for 10 min. The precipitate was discarded and the supernatant was centrifuged again for 10 min. The precipitate was discarded and the supernatant was centrifuged at 100,000 *g* in an ultracentrifuge (Hitachi 70P-72) at 4°C for 60 min. The supernatant fluid was discarded and the pellet was resuspended in 2 ml of 1.15% KCl solution and centrifuged again at 100,000 *g* for 60 min. The resultant microsomal pellet was resuspended in 2 ml of 0.1 M phosphate buffer (pH 7.4) to measure the contents of cytochrome P-450 (cyt. P-450) and cytochrome b_5 (cyt. b_5), and NADPH dependent cytochrome P-450 (NADPH-cyt. P-450) reductase activity. Microsomal protein was determined by the method of Lowry *et al.* /12/.

3. Measurements of cyt. P-450 and cyt. b_5 contents, and NADPH-cyt. P-450 reductase activity

Cyt. P-450 and cyt. b_5 contents were assayed spectrally according to the method of Omura and Sato /13, 14/, using an extinction coefficient of 91/mM/cm for cyt. P-450 and 185/mM/cm for cyt. b_5. NADPH-cyt. P-450 reductase activity was measured by the method of Phillips and Langdon /15/, using an extinction coefficient of 21/mM/cm for reduced cytochrome *c*.

Statistics

The data were statistically evaluated by the two-tailed Student's unpaired t-test.

RESULTS

Theophylline pharmacokinetics

The retention times on HPLC were 8.1 min for theophylline and 10.1 min for the IS. There was no confounding peak in the chromatogram of blank plasma, indicating a good separation between the theophylline and the IS. The time courses of plasma theophylline concentrations in both groups after oral administration of 20 mg/kg are shown in Fig. 1. Plasma theophylline concentrations in the

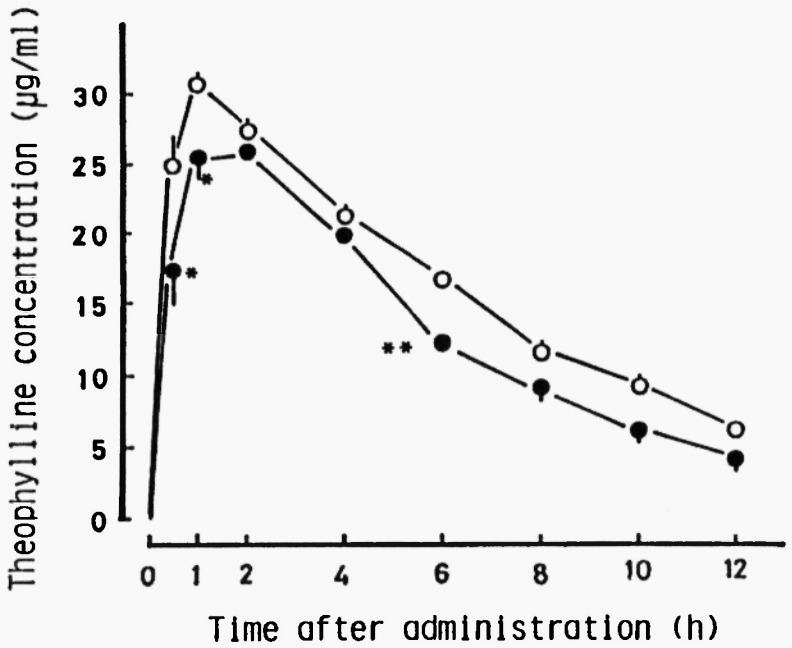

Fig. 1: Plasma theophylline concentration over time in rats after oral administration (20 mg/kg) in the long-term cigarette smoke-exposed group (●) and the control group (o). Each point indicates the mean with S.E.M. (vertical bar) of 6 animals. Significant differences in comparison to the control group at $*p<0.05$ and $**p<0.01$.

control group increased to approximately 32 μg/ml 1 h after the drug was given, and then gradually decreased to approximately 7 μg/ml at 12 h after administration. Plasma theophylline concentrations in the long-term cigarette smoke-exposed group were lower than in the control group at all times measured after administration. The concentrations in the smoke-exposed group reached approximately 27 μg/ml 2 h after administration, and then gradually decreased to approximately 4 μg/ml at 12 h after. There were significant differences between the two groups at 0.5 ($p<0.05$), 1 ($p<0.05$) and 6 h ($p<0.01$) after administration.

Table 1 shows the pharmacokinetic parameters of theophylline in the two groups. The AUC and K_a were significantly lower ($p<0.05$) in the smoke-exposed group than in the control group. The K_e in the smoke-exposed group was slightly higher than in the control group.

There were no significant differences in the MRT and V_d between the two groups.

Liver microsomal enzymes

Table 2 shows body weight, liver weight and their ratio in the two groups after smoke exposure for 27 days. There was no difference in body weight between the two groups. However, liver weight in the smoke-exposed group was significantly lower than in the control group ($p < 0.05$), and, as a result, the ratio in liver weight to body weight was also lower in the smoke-exposed group ($p < 0.01$).

The effects of long-term cigarette smoke exposure on cyt. P-450 and cyt. b_5 contents and NADPH-cyt. P-450 reductase activity in liver microsomes are shown in Table 3. Although there was no difference in cyt. P-450 content per mg protein, cyt. b_5 content per mg protein was higher in the smoke-exposed group than in the control group ($p < 0.001$). NADPH-cyt. P-450 reductase activity was greater in the smoke-exposed group ($p < 0.05$).

TABLE 1
Influences of long-term cigarette smoke exposure on pharmacokinetic parameters of theophylline in rats

	N	AUC (μ·h/ml)	MRT (h)	K_a (/h)	Ke (/h)	V_d (l)
Control	6	201.7+7.7	4.62±0.10	3.15±0.62	0.14±0.01	0.021±0.001
C. smoke	6	167.9±8.6*	4.34±0.17	1.61±0.28*	0.22±0.04+	0.020±0.002

Each value is the mean±S.E.M. Theophylline was administered orally 13 h after the last cigarette smoke exposure. AUC, the area under the plasma concentration-time curve; MRT, the mean residence time; K_a, the absorption rate constant; K_e, the elimination rate constant; V_d, the volumeof distribution; N, number of animals used; C. smoke, long-term cigarette smoke-exposed group.
+ $p < 0.1$; * $p < 0.05$ in comparison to the control group.

TABLE 2
Influences of long-term cigarette smoke exposure on body weight, liver weight and ratio of liver weight to body weight in rats

	N	Body Weight (g)	Liver Weight (g)	Ratio of Liver Weight to Body Weight
Control	8	293.6±3.9	7.80±0.14	0.027±0.001
C. smoke	8	290.8±3.6	7.15±0.21 *	0.025±0.002 **

Rats were exposed to smoke for 27 days. Weights were measured 20 h after the last cigarette smoke exposure. Each value is the mean±S.E.M. N, number of animals used; C. smoke, long-term cigarette smoke-exposed group.
*$p < 0.05$; **$p < 0.01$ in comparison to the control group.

TABLE 3
Influences of long-term cigarette smoke exposure on liver microsomal enzymes in rats

	N	Cyt. P-450 (n mol/mg protein)	Cyt. b_5 (n mol/mg protein)	NADPH-Cyt. P-450 Reductase (n mol/mg protein/min)
Control	8	0.765±0.033	0.333±0.010	50.89±1.08
C. smoke	8	0.742±0.027	0.379±0.009 ***	55.99±1.95 *

Each value is the mean±S.E.M. N, number of animals used; C. smoke, long-term cigarette smoke-exposed group.
*$p < 0.05$; ***$p < 0.001$ incomparison to the control group.

DISCUSSION

It is well known that the therapeutic efficacy and toxic effects of theophylline are closely related to its plasma concentration, and that the therapeutic dose range is very narrow /1-3/. Generally, people who smoke do so for many years, and long-term smoking shortens the half-life of theophylline and increases its clearance in young adults. This is because cigarette smoke activates the liver microsomal enzymes which metabolize theophylline /6-8/. The present data indicate that long-term exposure to cigarette smoke influences both the

absorption and the elimination of oral theophylline. The peak of plasma theophylline concentration during the absorption phase was lower after long-term exposure to cigarette smoke, and K_a was also lower. These findings suggest that theophylline absorption from the gastrointestinal tract is impaired by long-term cigarette smoke exposure.

Acute cigarette smoking accelerates gastric passage of the liquid component of meals /16, 17/ and hampers the transport of the solid component from the stomach to the intestines /18/. Cigarette smoking also inhibits the basal motor activity responsible for gastroduodenal motility /19/. Boyd *et al.* /20/ reported that nocturnal acid secretion was greater in smokers treated with an H_2 antagonist if they were allowed to smoke than if smoking was prohibited. Cigarette smoking inhibits the secretion of pancreatic juice and bicarbonate in light smokers, and heavy smokers have depressed pancreatic secretory rates during non-smoking periods /21/. These reports suggest that upper intestinal pH is decreased by cigarette smoking. Furthermore, cigarette smoking has both chronic and acute effects on basal gastric secretion and bile salt reflux /22/, and nicotine accumulates over 6 to 8 h of regular smoking and remains overnight, even during sleep /23/. Thus, smoking does not result in intermittent exposure to nicotine but in exposure that lasts 24 hours a day. The lower plasma concentrations of theophylline and the later peak in smoke-exposed rats in the present experiment may be related to abnormal absorption from the gastrointestinal tract. Furthermore, Sugawara and Nagaoka /24/ showed that theophylline penetration through the stomach wall in an isolated guinea-pig stomach preparation was positively correlated with pH. Since cigarette smoke causes a decrease in gastrointestinal pH, the absorption of theophylline after oral administration may be impeded.

The K_e in the smoke-exposed group was slightly higher than in the control group, suggesting that theophylline elimination was facilitated. Therefore, we investigated the effects of long-term cigarette smoke exposure on the enzymes which metabolize theophylline in liver microsomes. In the present data, although there was no significant difference in body weight between the two groups, the liver weight, and the ratio of liver weight to body weight, were significantly lower in the smoke-exposed group than in the control group. The cyt. b_5 content and NADPH-cyt. P-450 reductase activity were increased by long-term cigarette smoke exposure, but cyt. P-450 content did not

change. Acute cigarette smoke exposure did not affect these enzymes in liver microsomes (data not shown).

Recently, many studies have been reported of cytochrome P-450 as a genetic superfamily /25/. Of these superfamilies, the cytochrome P-450 IA subfamily is induced by polycyclic aromatic hydrocarbons (methylcholanthrene-type inducers), as in cigarette smoke, and cytochrome P-450 IA2, that may be confined to liver, is responsible for the primary metabolism of theophylline /26, 27/. In the present experiment, we measured only the total cytochrome P-450 content; the question of whether cytochrome P-450 IA2 was induced or not still remains. Further studies concerning the relation between theophylline metabolism in smokers and cytochrome P-450 isoenzymes are needed.

We also investigated the lipid peroxidation level in the livers of the long-term cigarette smoke-exposed rats. Mean lipid peroxidation level in the smoke-exposed group was higher than in the control group, indicating a lowered liver function. From these results, we can consider that stimulation of NADPH-cyt. P-450 reductase activity or the increased cyt. b_5 content per mg protein in liver microsomes, or both, may compensate for the relative impairment of drug metabolism caused by decreased liver weight and increased lipid peroxidation in the liver.

In the present experiment concerning long-term cigarette smoke exposure, the facilitation of theophylline elimination and the impediment of its absorption from the gastrointestinal tract were observed. Although the higher rate of theophylline elimination in smokers than in non-smokers has already been reported /4, 5/, the influence of cigarette smoke on its oral absorption has not been described previously. In the clinical application of oral theophylline in smokers, it is necessary to consider not only the change in the elimination rate, but also the reduced absorption from the gastrointestinal tract.

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