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Effect of Cigarette Smoking on Theophylline Pharmacokinetics in Rats

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Abstract—The effect of acute cigarette smoke inhalation on the plasma levels of theophylline administered orally and parenterally to rats has been studied. The animals were exposed to smoke containing low- or high-nicotine/tar concentration for 10 min immediately after oral, intraperitoneal (i.p.) or intravenous (i.v.) administration of theophylline. The plasma levels of theophylline when administered orally (20 mg kg^{-1}) were lower in the two cigarette smoke-inhaling groups than in the non-smoking restrained control group, with the lowest values in the high-nicotine/tar group. The plasma levels (8 and 12 h after administration) in the high-nicotine/tar group when theophylline was administered i.p. (10 mg kg^{-1}), were also slightly lower than in the non-smoking restrained control group but this was not significant. When theophylline was administered i.v. (5 mg kg^{-1}), there was no difference between the high-nicotine/tar group and the non-smoking restrained control group. These data indicate that cigarette smoke inhalation causes suppression or delay of theophylline absorption from the gastrointestinal tract.

Theophylline is frequently used for the therapy of bronchial asthma. It has been reported that its bronchodilatory action corresponds to the drug plasma level and that its therapeutic index is very narrow (Jenne et al 1972; Mitenko & Ogilivite 1973). Accordingly, therapeutic drug monitoring of theophylline plasma levels is advised.

On the other hand, evidence exists that the pharmacokinetics of theophylline are influenced by cigarette smoking (Jenne et al 1975; Hunt et al 1976), indicating that the therapeutic efficacy of theophylline may be affected in smokers. There are, however, no studies relating the acute influence of cigarette smoking to theophylline pharmacokinetics. In preliminary studies we observed lower plasma levels of theophylline induced by cigarette smoke inhalation when the drug was administered orally (p.o.), but it is unclear whether gastrointestinal absorption, distribution, or elimination is responsible. Intraperitoneal (i.p.) or intravenous (i.v.) administration does not show the effect of cigarette smoking on the drug absorption process from the gastrointestinal tract, and i.v. administration focuses only on the drug elimination process. In the present study, the acute influences of cigarette smoke inhalation on the plasma levels of orally and parenterally administered theophylline were investigated.

Materials and Methods

Drugs

Theophylline (Katayama Chemicals, Japan) was suspended in 0·5% sodium carboxymethylcellulose (CMC-Na) and was administered at a dose of 20 mg kg^{-1} p.o., 10 mg kg^{-1} i.p. or 5 mg kg^{-1} i.v. For determining theophylline plasma levels by HPLC, 7-(2-hydroxyethyl)-theophylline was used as internal standard.

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Cigarettes

'Peace' filter cigarettes (L-P) and 'Mild-Seven' filter cigarettes (M-S) supplied by Japan Tobacco Inc., Japan, were used. The weights of L-P and M-S were 1·023 and 0·938 g per cigarette, respectively. The contents of nicotine and tar when two-thirds of the cigarette was smoked by means of a smoking machine (inhalation volume, 35 mL; inhalation duration, 2 s) were 2·2 and 23 mg per cigarette for L-P, respectively, and 1 and 14 mg per cigarette for M-S, respectively.

Animals

Male Wistar rats, 280–335 g, were divided into 3 groups for p.o., i.p. and i.v. administration, and each group was further divided into 3 or 4 subgroups of L-P and/or M-S cigarette smoke-inhaling rats, non-smoking control rats restrained in animal holders, and non-smoking, non-restrained rats. Four or 5 animals were housed together in 26 × 36 × 25 cm plastic-walled cages, and free access was allowed to food and water, except for a 12 h fast before and during the experiment. The animals were maintained in a 12-h light-dark cycle (lights on from 0800 to 2000 h) at room temperature (22–24°C) and a relative humidity of approximately 60%.

Apparatus for cigarette smoking

A smoking apparatus (Borgwaldt, Type Hamburg II) was used for exposing the animals to cigarette smoke (Dontenwill 1970). The apparatus consisted of the smoking head to which up to 30 cigarettes can be attached, the smoking channel, the smoking chamber slide piece, the inhalation chamber and 10 animal holders for exposing the animals to smoke. The cigarettes attached to the smoking head were individually lit and the smoking head was turned. The smoke from the lighted cigarettes was mixed with air at a ratio of 1:7 and passed to the inhalation chamber. In the present experiment, 15 cigarettes were lit initially, and the remaining 15 cigarettes were lit immediately after the first 15 cigarettes had burned out. The animals were exposed to smoke for 10 min. The

inhalation duration of the smoking machine was 2 s and the inhalation frequency 15 min^{-1}. Between 5 and 7 animals were exposed to the smoke simultaneously.

Blood collection and extraction procedures

The proximal part of the tail vein was incised (approx. 1 mm) with a scalpel blade under superficial local anaesthesia with ethyl aminobenzoate ointment, and blood samples for the measurement of theophylline were collected in a capillary (60 μL, Miles-Sankyo Co.) from the same part of the tail vein. Plasma separation was performed by centrifugation at 11 500 rev min^{-1} for 3 min using a haematocrit centrifuge (Compur M 1100, Miles-Sankyo Co.); 20 μL of plasma was used for the determination of drug plasma concentration.

Twenty μL of plasma was added to 200 μL of a methanol solution containing the internal standard (4 $\mu g\ mL^{-1}$) and mixed for 20 s. After centrifugation at 10000 rev min^{-1} for 5 min, 20 μL of supernatant was injected onto the HPLC.

Determination of theophylline in plasma by HPLC

The theophylline plasma concentrations were determined by HPLC (Hitachi Type 665-11; automatic sampler Type 665-40, processor Type 655-61, UV detector Type 655A). A stainless-steel column (Waters Associates μBondapak C18; length, 300 mm; diameter, 3·9 mm; particle size, 10 μm) was used at room temperature. Acetonitrile:acetate buffer (0·01 M, pH 4·0), (1:12, v/v) served as the mobile phase. The flow rate was 1·5 mL min^{-1}. Theophylline and the internal standard were detected at 280 nm (0·01 aufs). The calibration curves were constructed using theophylline solution (1 mg/10 mL of methanol) diluted in plasma to concentrations of 5, 10, 20 and 30 $\mu g\ mL^{-1}$.

Pharmacokinetic parameters

The area under the plasma concentration-time curve (AUC) and the mean residence time (MRT) were obtained from the theophylline plasma concentration-time data of each animal using a personal computer program for model-independent analysis (Yamaoka et al 1978, 1982).

Statistics

Plasma concentration data obtained and pharmacokinetic parameters were statistically analysed by analysis of variance (ANOVA), followed by Duncan's test.

Results

The retention times of theophylline and the internal standard in the HPLC system were 8·1 and 10·1 min, respectively. No interfering peak appeared on the chromatogram of plasma from control rats.

Fig. 1 shows the curves of theophylline plasma levels in non-smoking restrained and non-smoking non-restrained groups when the drug was administered p.o. at a dose of 20 mg kg^{-1} (A), i.p. at a dose of 10 mg kg^{-1} (B) and i.v. at a dose of 5 mg kg^{-1} (C). There was no marked difference between the non-smoking restrained group and non-smoking non-restrained group after p.o., i.p. or i.v. administration.

Fig. 2 shows the effect of cigarette smoke exposure on the plasma levels of theophylline when administered p.o. (A), i.p. (B) or i.v. (C). As shown in Fig. 2A, in groups exposed to M-S or L-P cigarette smoke (M-S or L-P group), the theophylline plasma levels after p.o. administration were markedly lower than in the non-smoking restrained control group 0·5–1 h after administration. The peak concentration of theophylline (4 h after administration) in the L-P group was markedly higher than in the non-smoking restrained control and the M-S groups, i.e. the peak time of the theophylline plasma concentration was shifted to the later time by the exposure of L-P cigarette smoke. The plasma levels of theophylline during the elimination phase were lower in the L-P and the M-S groups than in both non-smoking groups with lowest values in the L-P group. There were significant differences between the M-S group and the non-smoking restrained group ($F(1:132) = 112{\cdot}2$, $P < 0{\cdot}01$), and between the L-P group and the non-smoking restrained control group ($F(1:132) = 158{\cdot}4$, $P < 0{\cdot}01$).

The theophylline plasma levels in the non-smoking restrained control and non-smoking non-restrained groups decreased gradually for 0·5 h after i.p. administration (Fig. 2B) reaching approximately 5 $\mu g\ mL^{-1}$ 12 h later. The theophylline plasma level in the L-P group decreased at almost the same rate as in both non-smoking groups, although drug level in the L-P group decreased slightly more rapidly. In addition, the plasma levels of theophylline in the

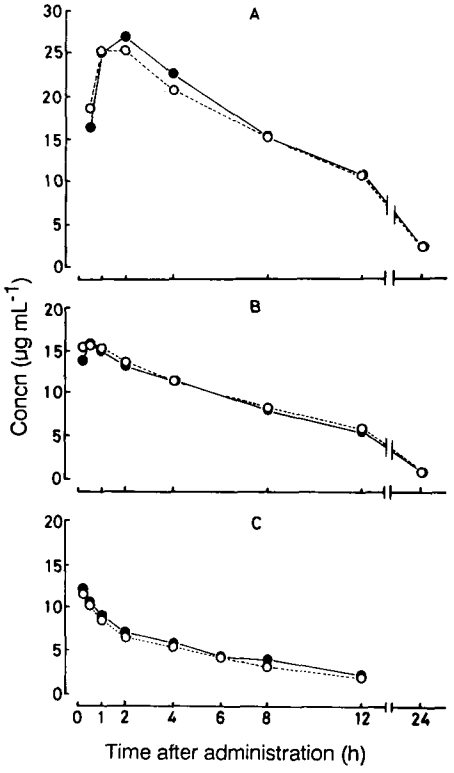

FIG. 1. Comparison of theophylline plasma levels in non-smoking restrained and non-restrained rats. Theophylline was administered orally at a dose of 20 mg kg^{-1} (A), intraperitoneally at a dose of 10 mg kg^{-1} (B) and intravenously at a dose of 5 mg kg^{-1} (C). The non-smoking restrained rats were held in the animal holder for 10 min immediately after drug administration. Each point indicates the mean value. ○, non-smoking non-restrained group (n = 12, 6 and 5 in groups A, B and C, respectively); ●, non-smoking restrained group (n = 12, 6 and 5 in groups A, B and C, respectively).

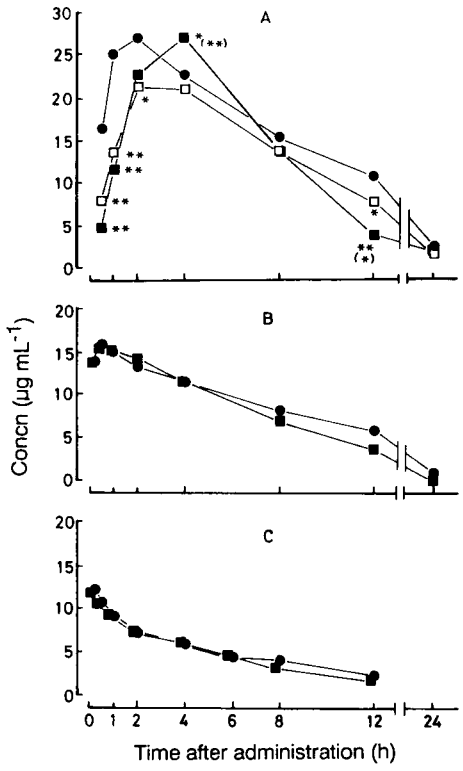

FIG. 2. Comparison of theophylline plasma levels in cigarette smoke-exposed and non-smoking restrained rats. Theophylline was administered orally at a dose of 20 mg kg^{-1} (A), intraperitoneally at a dose of 10 mg kg^{-1} (B) and intravenously at a dose of 5 mg kg^{-1} (C). The rats were exposed to cigarette smoke for 10 min immediately after drug administration. Each point indicates the mean value. ●——●, Non-smoking restrained group (control, n = 12, 6 and 5 in groups A, B, and C, respectively); □, "Mild-Seven" cigarette smoke exposed group (n = 7 in group A); ■, "Peace" cigarette smoke exposed group (n = 5, 6 and 5 in group A, B, and C, respectively). Significant differences in comparison with the non-smoking restrained group at $*P<0.05$ and $**P<0.01$, and with the "Mild-Seven" cigarette smoke-exposed group at $^{(*)}P<0.005$ and $^{(**)}P<0.01$ (by Duncan's test after ANOVA as two factors).

L-P group after i.v. administration (Fig. 2C) were not markedly different from those in both non-smoking groups.

Table 1 shows the pharmacokinetic parameters of theophylline when administered p.o., i.p. or i.v. Concerning the parameters when administered p.o., there were significant differences in the AUC values ($F(3:32)=8.0$, $P<0.01$) and in the MRT values ($F(3:32)=4.4$, $P<0.05$) between the groups of cigarette smoking and non-smoking treatment. The MRT value in the L-P group after i.p. administration was significantly lower than in the non-smoking restrained control group ($P<0.01$, by Duncan's test).

Discussion

It is known that therapeutic efficacy of theophylline is closely related to the plasma level.

Present data suggest that acute exposure of cigarette smoke for 10 min influences mainly the absorption phase of theophylline pharmacokinetics after p.o. administration of theophylline. Thus the rise of the theophylline plasma levels during the absorption phase was hampered by a single exposure of low- as well as high-nicotine/tar-containing cigarette smoke. The influence on the plasma levels by cigarette smoke exposure was more marked in the L-P group than in the M-S group, indicating a relationship to the nicotine/tar content of the smoke, possibly by inhibition of drug absorption from the gastrointestinal tract during the smoke exposure. The shifting of the peak time of the drug plasma level in the L-P group supports this hypothesis. On the other hand, after parenteral administration, although the MRT value in the L-P group was shortened following i.p. administration, the theophylline concentration-time curves and the pharmacokinetic parameters showed no marked differences between the groups.

In a parallel series of experiments, not described here, we determined the nicotine plasma concentration in smoke-exposed rats. Mean nicotine plasma levels immediately after 10 min exposure of cigarette smoke were 212 ± 54 (s.e.) ng mL^{-1} in the L-P group and 70 ± 11 ng mL^{-1} in the M-S group. The plasma levels of theophylline after the exposure of these types of cigarette smoke may be related to the nicotine content, but the possibility cannot be excluded that other components in the cigarette smoke may influence the theophylline pharmacokinetics.

The control animals in the present experiment were restrained in the animal holder of the smoking machine, and might not be expected to fully mimic the situation associated with being forced to breathe smoke which did not contain nicotine or tar. It was, however, considered that the experiment with the non-smoking restrained control group was sufficient to investigate the effects of smoking, as there were no differences in theophylline plasma levels and the pharmacokinetic parameters between the non-smoking restrained control group and the non-smoking non-restrained group.

It is reported that acute cigarette smoking accelerates gastric passage of the liquid component of meals (Grimes & Goddard 1978) and inhibits the basal activity of gastroduodenal motility (Ertel et al 1985). Furthermore there is the possibility that the animals not only inhaled the cigarette smoke but also swallowed it and that these factors have influenced the gastric pH and/or motility, resulting in an inhibition of drug absorption from the gastrointestinal tract; the lower plasma levels of theophylline following cigarette smoking after p.o. administration which have been observed in the present experiment may be related to a functional disturbance of absorption in the gastrointestinal tract.

Theophylline is absorbed rapidly after p.o. administration and metabolized to 1-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid, and 10% of the dose administered is excreted via the kidneys in unmetabolized form (Cornish & Christman 1957; Rall 1985; Hunt et al 1976). Sugawara & Nagaoka (1984) showed that theophylline penetration through the wall of stomach in the guinea-pig isolated stomach preparation was positively correlated with the pH. Since cigarette smoke causes a decrease of the gastrointestinal pH, the absorption of theophylline after p.o. administration may be impeded.

When theophylline was administered i.p. in the present experiment, facilitation of drug elimination was observed. As the period of cigarette smoking was 10 min, the lower plasma levels of theophylline after p.o. and parenteral administra-

Table 1. The area under the plasma concentration-time curve (AUC) and the mean residence time (MRT) of theophylline in cigarette smoke-exposed rats, non-smoking restrained control rats and non-smoking non-restrained rats.

Groups	n	AUC ($\mu g\ h\ mL^{-1}$)	MRT (h)
p.o. administration			
non-smoking non-restrained	12	286·6 ± 11·6	7·51 ± 0·14
non-smoking restrained	12	292·8 ± 8·6	7·51 ± 0·13
M-S smoke exposed	7	238·6 ± 10·1*	7·27 ± 0·13
L-P smoke exposed	7	219·4 ± 9·7**	6·57 ± 0·20**(*)
i.p. administration			
non-smoking non-restrained	6	158·9 ± 5·5	7·13 ± 0·10
non-smoking restrained	6	150·7 ± 8·8	6·93 ± 0·33
L-P smoke exposed	6	129·9 ± 6·8	5·57 ± 0·22**
i.v. administration			
non-smoking non-restrained	5	56·4 ± 2·5	4·35 ± 0·15
non-smoking restrained	5	63·6 ± 1·8	4·56 ± 0·04
L-P smoke exposed	5	58·6 ± 1·1	4·12 ± 0·11

Theophylline was administered orally (p.o.) at a dose of 20 mg kg^{-1}, intraperitoneally (i.p.) at a dose of 10 mg kg^{-1} and intravenously (i.v.) at a dose of 5 mg kg^{-1}. The AUC values were calculated for the first 24 h after p.o. and i.p. administration, and for the first 12 h after i.v. administration. Each value represents the mean ± s.e. Significant differences in comparison with the non-smoking restrained control group, $*P < 0.05$ and $**P < 0.01$, and the M-S cigarette smoke-exposed group, $^{(*)}P < 0.05$ (by Duncan's test after ANOVA as one factor); n, number of animals used; M-S, "Mild-Seven" cigarette; L-P, "Peace" cigarette.

tions probably cannot be attributed to the induction of drug-metabolizing enzymes, although nicotine and other components of cigarette smoke have been shown to cause the induction of microsomal drug-metabolizing enzymes (Hunt et al 1976).

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