Effects of Standard Cigarette Smoke and Nicotine-Reduced Cigarette Smoke on Plasma Concentrations of Indomethacin Administered Orally to Rats

Yutaka Gomita¹, Katsushi Furuno¹, Toshiko Yoshida², Ryozo Oishi², Kiyomi Saeki² and Yasunori Araki¹

Departments of ¹Hospital Pharmacy and ²Pharmacology, Okayama University Medical School, Okayama 700, Japan

ABSTRACT—The influence of acute exposures to standard (ST) and nicotine-reduced (NR) cigarette smokes on the plasma concentration of orally administered indomethacin (IM, 5 mg/kg) was investigated in rats. IM plasma concentrations in the ST- and NR-groups were lower than those in the non-smoking control group, while the lowered effect in the NR-group was slightly weaker than in the ST-group. These results suggest that the plasma concentrations of IM administered orally are lowered by the acute exposure of cigarette smoke, and this influence may be attributed largely to constituents other than nicotine in the cigarette smoke as well as slightly attributable to nicotine.

Keywords: Indomethacin, Plasma concentration, Cigarette smoke

It is recognized that the actions of many drugs are influenced by various factors such as environmental circumstances, emotional stress, and body conditions. Generally, a drug's effect may be altered by changes in the sensitivity of the site of its action and/or the pharmacokinetics of the drug itself. Cigarette or tobacco smoking may also exert an influence on drug action and pharmacokinetics. Indeed, there are numerous clinical studies concerning the influence of smoking on the pharmacokinetics of many drugs (1-4). The authors have already observed in animal studies that plasma concentrations of some drugs such as isosorbide dinitrate, nicorandil, theophylline and cimetidine were lowered by exposures to cigarette smoke (5-9).

On the other hand, indomethacin (IM) is widely used to treat inflammatory as well as body pains. Concerning the influence of cigarette smoking on IM pharmacokinetics, we have already observed that the acute exposure to cigarette smoke influences the plasma concentration of IM administered orally; i.e., being lower in the absorption phase in the cigarette smoke exposed group than in the non-smoking control group (10). However, it is unclear whether the lowered effects are attributed to nicotine in the cigarettes or tobacco smoke, constituents other than nicotine, or both. No sufficient basic study on the influence of cigarette smoke exposure on IM plasma concentration has been performed.

In the present experiment, we examined the influence of acute exposure to cigarette smoke in the absorption phase using the standard cigarette and nicotine-reduced cigarette to determine whether the influence of cigarette smoke is attributable to nicotine and/or constituents other than nicotine in the cigarette.

IM and O-desmethyl-indomethacin (O-DM-IM) pure powders were donated by Sumitomo Pharmaceuticals Co., Japan. IM was suspended in 0.5% sodium carboxymethylcellulose (Nacalai Tesque, Japan) and administered orally at a dose of 5 mg/kg in a quantity of 1 ml/kg body weight through a gastric tube using a 0.5 ml syringe. The plasma concentrations of IM and O-DM-IM were measured by high-performance liquid chromatography (HPLC), with butylparaben (Katayama Chemicals Co., Japan) as an internal standard. Other chemicals used were of analytical or reagent grade.

Cigarettes used in the present experiment were standard cigarettes (“Seven Star” without filter; mean weight, approximately 0.834 g/cigarette; nicotine content, 1.881%) and nicotine-reduced cigarettes (same as “Seven Star” except for the nicotine content (0.255%), i.e., the reduced nicotine content was 86%), which were supplied by the Smoking Research Founda-
Fifteen male Wistar rats (supplied by Charles River Japan) weighing 210–235 g were used in the present experiment. They were divided into three groups: standard cigarette exposed (ST) group, nicotine-reduced cigarette exposed (NR) group and the non-smoking control group. Four to five animals were housed together in 26 × 36 × 25 cm plastic-walled cages, and they were given food and water ad libitum, except for a fasting period of 24 hr before the experiment. Throughout the experiments, the animals were kept on a 12-hour light-dark cycle (light-on from 8:00 to 20:00 hr) at a temperature of 22 ± 1°C and approximately 60% relative humidity.

A smoking machine (Hamburg II, Borgwaldt, Germany) was used to expose the animals to cigarette smoke. In the present experiment, 15 cigarettes were initially lighted and the remaining 15 cigarettes were lighted after the first 15 cigarettes had burned out. Animals were exposed to the smoke (smoke : air, 1 : 7) for 8 min immediately after IM was administered (7–10). The duration of inhalation time from the smoking head and the frequency were 2 sec and 15/min, respectively. Five animals were exposed simultaneously to the smoke. Non-smoking control rats were held in the same holder for 8 min without exposure to the cigarette smoke.

Blood samples for measuring the plasma concentrations of IM and O-DM-IM were repeatedly collected from a tail vein using a capillary (60 μl, Miles-Sankyo Co., Japan) under the light local anesthesia of ethyl aminobenzoate ointment. The proximal part of the tail vein of the animals was carefully incised with a knife for sampling. The time points for blood sampling were 0.25, 0.5, 1, 2, 4 and 8 hr after IM administration. The measuring for 8 hr was decided in view of the peak-times of the plasma concentration of oral IM in the previous report (10). Plasma separation was performed by centrifugation at 10,000 rpm for 5 min in a hematocrit centrifuge (Compur M 1100, Miles-Sankyo Co., Japan). A 20-μl aliquot of the plasma obtained was transferred to an Eppendorf tube containing 50 ng of butylparaben as an internal standard. After the plasma protein was precipitated by adding 60 μl of acetonitrile, the mixture was centrifuged at 10,000 rpm for 20 min. A 20-μl aliquot of the supernatant was used.

The plasma concentrations of IM and O-DM-IM were simultaneously determined by an HPLC apparatus with UV spectrophotometric detection (10). The eluent was monitored at the wavelength of 254 nm. The calibration curves were constructed from the ratio of IM or O-DM-IM to the internal standard and IM solution diluted in plasma to concentrations of 0, 3, 6, 9, 15 and 30 μg/ml and O-DM-IM solution diluted in plasma to concentrations of 0, 5, 10 and 20 μg/ml. The concentrations of IM and O-DM-IM were obtained from the peak-area ratio of IM or O-DM-IM to the internal standard using those calibration curves.

Data obtained were evaluated statistically by means of the analysis of variance (ANOVA), followed by Duncan’s test.

The retention times of IM, O-DM-IM and the internal standard butylparaben on the HPLC chromatogram were 10.0, 5.9 and 6.7 min, respectively. These peaks were well-separated and no marked interfering peaks appeared in the chromatogram.

The curves of IM plasma concentrations in the ST-, NR- and non-smoking control groups after the oral administration of 5 mg/kg IM are shown in the upper graph (A) of Fig. 1. The IM plasma concentrations in the non-smoking control group increased rapidly and reached approximately 9.8 μg/ml at 0.25 hr after administration, and then they gradually increased to approximately 13.8 μg/ml at 8 hr after administration. On the other hand, IM plasma concentrations after oral administration in both cigarette smoke exposed groups were lower than in the non-smoking control group. There was a significant difference between the ST-group, NR-group and the non-smoking control group (F(2,72) = 16.1, P < 0.01, by ANOVA). Furthermore, there were significant differences in IM plasma concentrations at 0.5, 1 and 2 hr after administration between the ST-group and the non-smoking control group (P < 0.01, P < 0.01 and P < 0.05, respectively, by Duncan’s test) and at 0.5 and 1 hr after administration between the NR-group and the non-smoking control group (P < 0.01 and P < 0.05, respectively). However, there was no significant difference between both the cigarette smoke exposed groups, even though the concentrations were lower in the NR-group than in the ST-group.

The lower graph (B) of Fig. 1 shows the curves of O-DM-IM plasma concentrations in the ST-, NR- and the non-smoking control groups. In the non-smoking control group, the O-DM-IM plasma concentrations increased gradually and reached to approximately 1.8 μg/ml at 8 hr after IM oral administration. On the other hand, the O-DM-IM plasma concentrations in both the smoke exposed groups were lower than in the non-smoking control group. There was a significant difference between the ST-group, NR-group and the non-smoking control group (F(2,72) = 21.2, P < 0.01, by ANOVA). Furthermore, there were significant differences at 0.5, 2 and 4 hr after the administration between the non-smoking control group and the ST-group (P < 0.01, respectively) or NR-group (P < 0.05, respectively). Although concentrations were lower in the
ST-group than in the NR-group, there was no significant difference between both the cigarette smoke exposed groups.

The ratios of O-DM-IM to IM at each time after IM administration in the three groups were not significantly different, as shown in Table 1. The ratio values in the non-smoking control group increased with the increase in time after IM administration. The degrees of the increasing values in the ST-group and NR-group were smaller than in the non-smoking control group. Thus significant differences to the non-smoking control group were found at 2, 4 and 8 hr (P < 0.05, P < 0.01 and P < 0.01, respectively) after IM administration in the ST-group and at 4 and 8 hr (P < 0.01, respectively) after the administration in the NR-group.

The possibilities exist that patients to whom IM is administered for therapy as a non-steroidal anti-inflammatory drug have been smoking tobacco in the near or remote past, but there are few reports concerning the influence of tobacco smoke on the pharmacokinetics of IM. We have already found that in animals, acute exposure to Japanese commercial cigarette smoke influenced the IM pharmacokinetics; i.e., the plasma concentrations of IM and O-DM-IM, a major metabolite of IM, were lower in the cigarette smoke exposed group after oral administration of IM (10). However, it is not clear whether its influence is dependent upon nicotine and/or a component other than nicotine in the cigarette smoke.

In the present experiment, both the ST and NR cigarette smokes decreased the IM and O-DM-IM plasma concentrations in the absorption phase. The decreased effect in the NR-group was slightly weaker than in the ST-group even if there was no significant difference between the two cigarette smoke exposed groups. Thus, the lowering effects by cigarette smoke exposure on

---

**Table 1.** The ratios of O-desmethyl-indomethacin (O-DM-IM) to indomethacin (IM) at each time after IM administration in the standard and nicotine-reduced smoke exposed groups and the non-smoking control group

| Groups                        | Hours after IM administration |
|-------------------------------|------------------------------|
|                               | 1       | 2       | 4       | 8       |
| Non-smoking control           | 0.041 ± 0.021 | 0.070 ± 0.026 | 0.098 ± 0.015 | 0.129 ± 0.020 |
| Standard cigarette smoke exposed | 0.020 ± 0.011 | 0.041 ± 0.026* | 0.047 ± 0.008** | 0.084 ± 0.011** |
| Nicotine-reduced cigarette smoke exposed | 0.036 ± 0.024 | 0.047 ± 0.018 | 0.063 ± 0.023** | 0.089 ± 0.019** |

Each value represents the mean ratio (± S.E.M.), O-DM-IM/IM. *P < 0.05, **P < 0.01, in comparison to the non-smoking control group (Duncan's test). There were significant differences between the two kinds of cigarette smoke exposed groups and the non-smoking control group, but no significant difference was noted between the standard cigarette smoke exposed group and the nicotine-reduced cigarette smoke exposed group.
IM and O-DM-IM plasma concentrations may be due largely to components other than nicotine in the cigarette smoke as well as slightly attributable to nicotine.

We have already shown that the pharmacokinetics of isosorbide dinitrate and nicorandil, vasodiators; theophylline, a bronchodilator; and cimetidine, an antiulcer drug, were influenced by components other than the nicotine in cigarette smoke, nicotine or both (5–9). The mode of the acute exposure effect of cigarette smoke for IM was intermediate between that for nicorandil (i.e., being largely dependent on constituents other than nicotine) and that for theophylline (i.e., being dependent on not only nicotine but also constituents other than nicotine).

The influence of the ST cigarette smoke containing nicotine-tar was consistent with the report of Yoshida et al. (10), in which the plasma concentration of IM in the absorption phase was markedly influenced by the exposure to commercial cigarette smoke (containing nicotine and tar). In determining the reasons for the lowering effect on the plasma drug concentration by the exposure to cigarette smoke, one should consider the observation that acute cigarette smoking inhibits the basal activity of gastroduodenal motility (11). Accordingly, the lowered plasma concentrations of IM following the exposure to cigarette smoke may be largely attributable to a functional disturbance of absorption in the gastrointestinal tract. On the other hand, in the study of Yoshida et al. (10), the plasma concentration of IM was not influenced by the cigarette smoke when administered parenterally (intravenously and rectally).

It is said that cigarette smoke contains about 4000 different components, and one of pharmacologically active constituents is nicotine. In the previous study, we have already observed that subcutaneous nicotine caused a decrease in the plasma IM and O-DM-IM concentrations after oral IM, which was similar to the effects of cigarette smoke exposure, and that nicotine did not cause such an effect after intravenous IM (10). Accordingly, the decreasing effects caused by nicotine and cigarette smoke exposure may have involved retardation of IM absorption from the gastrointestinal tract, i.e., being mediated by a reduction of gastric mucosal blood flow (12) and/or an inhibition of gastrointestinal motility (11). On the other hand, as constituents other than nicotine in the gaseous phase of cigarette smoke, carbon monoxide, carbon dioxide, nitrogen oxides, ammonia, volatile sulfur- and nitrogen-containing compounds and so on were also determined (13). Although it is possible that a number of constituents in cigarette smoke may affect the pharmacokinetics of oral IM, here we will discuss the possible effects of carbon dioxide as one of the influential compounds in cigarette smoke. Carbon dioxide causes vasodilation in blood vessels as a direct action and additionally causes increases in cardiac contractility and vasoconstriction by activating a sympathetic nervous system, i.e., having complex total systemic action. It is said that the overall effects in healthy humans are an elevation of systolic and diastolic blood pressure and an increase in pulse pressure, being determined by a balance of the opposing effects on local tissues and the sympathetic nervous system effect. The activation of the sympathetic nervous system by cigarette smoke exposure causes an increase in vasoconstriction, which may induce a retardation of drug absorbance from the gastrointestinal tract. Accordingly, in the present study, the decreased effects of IM plasma concentration in the two cigarette smoke exposed groups may largely be attributed to substances such as carbon dioxide present in the gaseous phase.

On the other hand, IM administered orally is rapidly absorbed and metabolized by hepatic microsomal enzymes mainly to O-DM-IM (14, 15), i.e., a concept supported by the present data. Considering the ratios of O-DM-IM to IM concentrations in the plasma, there was no significant difference between the ST- and non-smoking control groups and between the NR-group and non-smoking control groups. Accordingly, the cigarette smoke may not have any influence on the metabolism of IM.

In a parallel series of experiments, we determined the nicotine plasma concentration in the ST- and NR-groups (not described here). Mean nicotine plasma concentrations immediately after an 8-min exposure to cigarette smoke were 66.7 ng/ml in the ST-group and 12.6 ng/ml in the NR-group, the ratio of the nicotine concentrations in the plasma between the ST- and NR-groups was almost the same as that of the nicotine content in cigarettes between both groups. The suppressive effects in the absorption phase by the two cigarette smoke exposures were not proportional to the plasma concentration or the nicotine content in the cigarette. Therefore, the plasma concentration of IM after the exposure to the two types of cigarette smoke may not be related to the nicotine in the cigarette smoke, i.e., being largely if not wholly related to the constituents other than nicotine. However, the possibility that nicotine has some effect cannot be totally excluded since the lowering effect in the NR-group was slightly weaker than in that in the ST-group.

In conclusion, the present study showed that the acute exposure to two kinds of cigarette smoke cause decreases in IM and O-DM-IM plasma concentrations, probably by the delayed absorption from the gastrointestinal tract. It is also suggested that this effect may be
more largely attributed to components other than nicotine in the cigarette smoke.

Acknowledgments
This study was supported by a grant from the Smoking Research Foundation, Japan. The authors are indebted to Dr. Y. Ito for his valuable advice.

REFERENCES

1 Boston Collaborative Drug Surveillance Program: Clinical depression of the central nervous system due to diazepam and chlordiazepoxide in relation to cigarette smoking and age. N. Engl. J. Med. 288, 277–280 (1973)
2 Jenne, J., Nagasawa, H., McHugh, R., MacDonald, F. and Wyse, E.: Decreased theophylline half-life in cigarette smokers. Life Sci. 17, 195–198 (1975)
3 Jusko, W.J., Schentag, J.J., Clark, J.H., Gardner, M. and Yurchak, A.M.: Enhanced biotransformation of theophylline in marihuana and tobacco smokers. Clin. Pharmacol. Ther. 24, 406–410 (1978)
4 Dawson, G.W. and Vestal, R.E.: Smoking and drug metabolism. Pharmacol. Ther. 15, 207–221 (1982)
5 Gomita, Y., Eto, K., Furuno, K. and Araki, Y.: Effects of standard cigarette and nicotine-less cigarette smoke inhalings on nicorandil plasma levels in rats. Pharmacology 40, 312–317 (1990)
6 Gomita, Y., Furuno, K., Eto, K., Okazaki, M., Suemaru, K. and Araki, Y.: Effect of cigarette smoking on theophylline pharmacokinetics in rats. J. Pharm. Pharmacol. 43, 621–624 (1991)
7 Gomita, Y., Furuno, K. and Araki, Y.: Influence of standard and nicotine-reduced cigarette smoke on plasma concentrations of isosorbide dinitrate and its metabolites in rats. J. Pharm. Pharmacol. 43, 811–812 (1991)
8 Eto, K., Gomita, Y., Furuno, K., Yao, K., Moriyama, M. and Araki, Y.: Influences of cigarette smoke inhalation on pharmacokinetics of cimetidine in rats. Drug Metabol. Drug Interact. 9, 103–114 (1991)
9 Gomita, Y., Eto, K., Furuno, K., Mimaki, M. and Araki, Y.: Influences of exposure to cigarette smoke on concentration of nicorandil in plasma of rats. J. Pharmaceutical Sci. 81, 228–231 (1992)
10 Yoshida, T., Gomita, Y. and Oishi, R.: Effect of cigarette smoke on pharmacokinetics of oral, intrarectal, or intravenous indomethacin in rats. Naunyn Schmiedebergs Arch. Pharmacol. 344, 500–504 (1991)
11 Ertel, G., Herman, B., Murthy, S.N.S. and Dinoso, V.P.: The effect of smoking (S) on gastroduodenal motility (GDM), PH changes in the proximal duodenum (PD), and gastrointestinal hormones. Gastroentelogy (Abstract) 88, 1375 (1985)
12 Sonnenberg, A. and Hüsner, N.: Effect of nicotine on gastric mucosal blood flow and acid secretion. Gut 23, 532–535 (1982)
13 Jeffe, J.H.: Drug addiction and drug abuse. In The Pharmacological Basis of Therapeutics, Edited by Gilman, A.G., Rall, T., Nies, A.S. and Taylor, P., p. 522–572, Pergamon Press, New York (1990)
14 Hacker, H.B., Zacchei, A.G., Cox, S.V., Brodie, D.A. and Cantwell, N.H.R.: Studies on the absorption, distribution and excretion of indomethacin in various species. Biochem. Pharmacol. 153, 237–249 (1966)
15 Yesair, D.W., Callahan, M., Remington, L. and Kensler, C.J.: Role of the entero-hepatic cycle of indomethacin on its metabolism, distribution in tissues and its excretion by rats, dogs and monkeys. Biochem. Pharmacol. 19, 1579–1590 (1970)