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Toshiko Yoshida[*] Yoshinori Itoh[†]

Yutaka Gomita[‡] Ryozo Oishi[**]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,

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Toshiko Yoshida, Yoshinori Itoh, Yutaka Gomita, and Ryozo Oishi

Abstract

The release of indomethacin from fatty-base suppositories, which had been stored at a low (4 degrees C) and a high (25-30 degrees C) temperature for about one month, was examined in vitro and in vivo. In the in vivo experiments, the plasma indomethacin levels after administration of suppositories stored at different temperatures were measured in conscious and anesthetized rats. In the in vitro release test using a dialysis cell method, much lower amounts of indomethacin were released from the suppositories stored at a high temperature than from those stored at a low temperature. The melting point of suppositories stored at a high temperature was higher by approximately 2 degrees C than those stored at a low temperature. In conscious rats, the plasma indomethacin levels attained after the intrarectal administration of suppositories stored at a high temperature were slightly lower than those after the animals were given suppositories stored at a low temperature, but the difference was significant only 30 min after administration. In anesthetized rats, the plasma indomethacin levels were markedly lower than those in conscious rats, and the influence of the storage temperature on the plasma indomethacin levels was clearly observed. These results suggest that in conscious rats many factors such as a locomotor hyperactivity and enhancement of gastrointestinal motility caused by the rectal administration mask the real character of suppositories. The in vitro and in vivo results show that the release of indomethacin from fatty-base suppositories stored at a high temperature is less than the release from those stored at a low temperature.

KEYWORDS: indomethacin, suppository, quality contyol, bioavailability, in vitro release test

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Influence of Storage Temperature on Indomethacin Release from Fatty-Base Suppositories *In Vitro* and *In Vivo*

Toshiko Yoshida, Yoshinori Itoh, Yutaka Gomita[a] and Ryozo Oishi*

Department of Pharmacology and [a]Hospital Pharmacy, Okayama University Medical School, Okayama 700, Japan

The release of indomethacin from fatty-base suppositories, which had been stored at a low (4°C) and a high (25-30°C) temperature for about one month, was examined *in vitro* and *in vivo*. In the *in vivo* experiments, the plasma indomethacin levels after administration of suppositories stored at different temperatures were measured in conscious and anesthetized rats. In the *in vitro* release test using a dialysis cell method, much lower amounts of indomethacin were released from the suppositories stored at a high temperature than from those stored at a low temperature. The melting point of suppositories stored at a high temperature was higher by approximately 2°C than those stored at a low temperature. In conscious rats, the plasma indomethacin levels attained after the intrarectal administration of suppositories stored at a high temperature were slightly lower than those after the animals were given suppositories stored at a low temperature, but the difference was significant only 30 min after administration. In anesthetized rats, the plasma indomethacin levels were markedly lower than those in conscious rats, and the influence of the storage temperature on the plasma indomethacin levels was clearly obseved. These results suggest that in conscious rats many factors such as a locomotor hyperactivity and enhancement of gastrointestinal motility caused by the rectal administration mask the real character of suppositories. The *in vitro* and *in vivo* results show that the release of indomethacin from fatty-base suppositories stored at a high temperature is less than the release from those stored at a low temperature.

Key words : **indomethacin, suppository, quality control, bioavailability, *in vitro* release test**

Suppositories for antitumor, anticonvulsant, analgesic-antipyretic and anti-inflammatory agents are widely used because of certain therapeutic advantages inherent in this method of administration, such as avoidance of gastrointestinal irritation and first-pass elimination (1, 2). Indomethacin is one of the drugs most frequently used in suppository form. Quality control of suppositories is always required since drugs released from this form of preparation are influenced by many factors. The storage of suppositories at a high temperature causes a rise in the melting point and a decrease in the rate of release of the drugs. Although there are several *in vitro* tests to determine the quality of suppositories, such as drug-release rate (3-7), melting characteristics (8),

* To whom correspondence should be addressed.

liquefaction time (9) and reological properties (10, 11), the data obtained from these *in vitro* experiments do not always parallel those from *in vivo* bioavailability tests using conscious animals (12, 13). In the present experiments, therefore, we studied *in vitro* the quality of fatty-base suppositories, which had been stored at low and high temperatures, and compared the results of *in vitro* studies with the data on bioavailability of indomethacin from these suppositories in conscious and anesthetized rats.

Materials and Methods

Materials. Commercial fatty-base suppositories of indomethacin (Sawai Chemical Co., Osaka, Jakan) were used. The fatty-base consisted of Witepsol. They were stored at a low (4°C) or at (25–30°C) room temperature in summer for about one month. The indomethacine contents in the suppositories were not changed after these storage periods. Pure indomethacin was donated by Sumitomo Chemical Co. (Takarazuka, Japan). Sodium pentobarbital and phenobarbital were obtained from Dainippon Pharmaceutical Co. (Osaka) and Sankyo Pharmaceutical Co. (Tokyo, Japan), respectively. Other chemicals used were of analytical or reagent grade, obtained from Wako Pure Chemical Industries (Osaka) and Toyo Chemicals (Tokyo).

In vitro release experiments. The release of indomethacin from the suppositories was measured by the Paddle method (Japanese Pharmacopoeia-XI) modified by Fukuda *et al.* (14), in which a cellophane dialysis tubing was used instead of a basket and the dissolution menium was 900 ml of artificial plasma (pH 7.5 ± 0.1). At various times after the start of the experiment, 3 ml of medium of the compartment outside the cellulose tube was transferred to a glass cell for determination of indomethacin concentration by ultraviolet spectrophotometry at 254 nm.

In vivo experiments. Male Wistar rats weighing 190–210 g (Shizuoka Experimental Animals, Hamamatsu, Japan) were fasted for 24 h before the experiment. The suppositories cut in sizes corresponding to 40 mg/kg of indomethacin were inserted into the rectum, and the anuses were closed with alkyl-α-cyanoacrylate monomer (Aron Alpha, Sankyo Co., Tokyo). Some rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (20 mg/kg) combined with phenobarbital (100 mg/kg). They were treated with indomethacin 15 min after the loss of righting reflex and put on a plate maintained at a temperature of 37°C.

At various times after the rectal administration of an indomethacin suppository, a 60-μl blood sample was taken into a heparinized capillary tube from the tail vein of each rat. The blood was immediately centrifuged at 12,000 rpm for 3 min. To 20 μl of plasma an appropriate amount of butylparaven was added as an internal standard. The mixture was applied to a Bond Elute C_{18} column (1 ml column volume, Analytichem International, USA). After the column was washed twice with 1 ml of distilled water, indomethacin and butylparaven were eluted with 250-μl of methanol. A 20-μl aliquot of the eluate was injected into a high performance liquid chromatograph. Plasma indomethacin concentration was determined using a calibration curve made from the ratios of the indomethacin peak area to that of the internal standard. The high performance liquid chromatography system (Waters, Nihon Millipore, Tokyo) was composed of a pump (Model M 45 J), a spectrophotometer (Model 440 UV), an injector (Medel 710 B WISP), a recorder (Data module type 730) and a reverse phase column (μ-Bondapak C_{18}; 3.9 × 300 mm). The mobil phase was a mixture of 0.1 % H_3PO_4-CH_3CN (45:55 v/v). The flow rate was 1.5 ml/min, and the effluent was monitored at 254 nm.

Determination of melting point. The apparatus used for determination of the melting point of suppositories is described in Japanese Pharmacopoeia-XI. The immersion fluid used was water. Each suppository (1 cm in length) was inserted into a capillary tube opened at both ends. After heating, the time required for each sample to melt in the capillary tube was determined.

Statistical analysis. The data were evaluated for statistical significance by analysis of variance (ANOVA) and Student's t-test.

Results

In vitro release of indomethacin from suppository. The time courses of increases in indomethacin concentration in the compartment outside the cellulose tube which contained a suppository stored at a low or a high temperature are shown in Fig. 1. The indomethacin concentrations increased almost linearly for the first 90-min period.

The rate of release from suppositories stored at a low temperature was approximately twice that from suppositories stored at a high temperature.

Plasma indomethacin concentration after rectal administration of suppository. Fig. 2 shows the plasma indomethacin concentrations in conscious rats after the rectal administation of indomethacin suppositories stored at a low or a high temperature. The indomethacin concentrations increased up to 4 h after administration and remained relatively high even after 12 h. The means values for the indomethacin concentration in rats treated with suppositories stored at a high temperature were lower at all times examined than the values in rats treated with suppositories stored at a low temperature. However, when the indomethacin concentrations in these groups were analyzed by ANOVA, the difference was not found to be significant ($F = 2.73$, $p > 0.05$). A significant difference according to Student's t-test was observed only 30 min after the administration of suppositories ($t = 3.91$, $p < 0.01$).

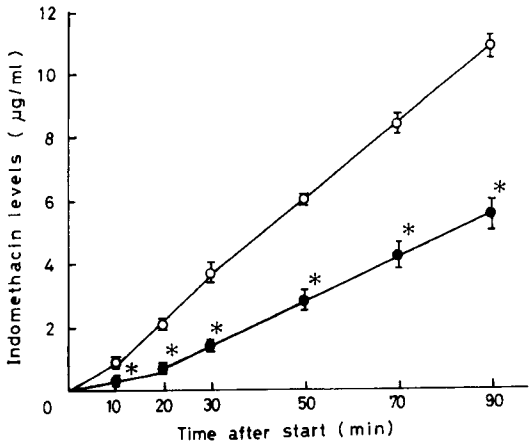

Fig. 1 *In vitro* release of indomethacin from suppositories stored at a low (○) and a high (●) temperature. The ordinate represents indomethahin concentrations in the compartment outside the cellulose tube. Each point represents the mean ± SEM of five suppositories. * $p < 0.01$, compared with the corresponding values for suppositories stored at a low temperature (Student's t-test).

Fig. 3 shows the effect of the storage temperature of suppositories on the plasma indomethacin concentrations after the rectal administration in anesthetized rats. The plasma indomethacin concentrations in anesthetized rats were 27–35 % of those in conscious rats, 0.5–2 h after administration (Figs. 2 and 3). In anesthetized rats, the administration of suppositories stored at a high temperature resulted in markedly lower plasma indomethacin levels than the corresponding values observed after the administration of suppositories stored at a low temperature ($F = 7.18$, $p < 0.01$).

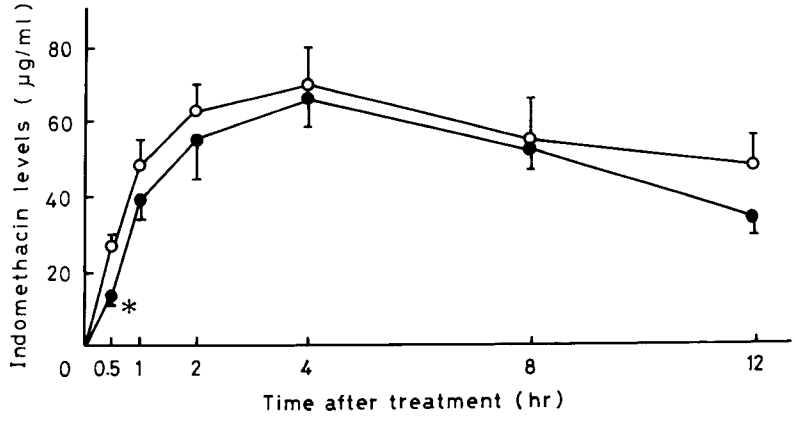

Fig. 2 Time course of changes in plasma indomethacin concentrations in conscious rats after the rectal administration of indomethacin suppositories stored at a low (○) or a high (●) temperature. Each point represents the mean ± SEM of five (○) or six (●) animals. * $p < 0.01$, compared with the corresponding value in the group given suppositories stored at a low temperature (Student's t-test).

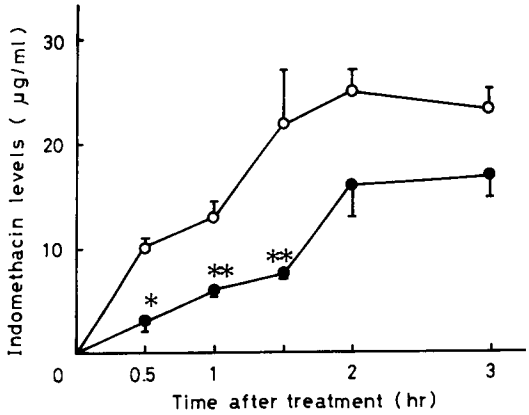

Fig. 3 Time course of changes in plasma indomethacin concentrations in anesthetized rats after the rectal administration of indomethacin suppositories stored at a low (○) or a high (●) temperature. Each point represents the mean ± SEM of five (○) or six (●) animals. * $p < 0.05$, * * $p < 0.01$, compared with the corresponding values in the group given suppositories stored at a low temperature (Student's *t*-test).

The differences were significant 0.5, 1 and 1.5 h after administration (Fig. 3).

Melting points of suppositories. The melting points of indomethacin suppositories stored at a low and a high temperature were 34.9 ± 0.1 °C (mean ± SEM, n = 10) and 37.3 ± 0.1 °C (n = 10), respectively, the difference being significant according to Student's *t*-test ($t = 8.49$, $p < 0.01$).

Discussion

Drug release from fatty-base suppositories is influenced by many factors such as size of drug particles, solubility of drugs and melting point of bases (6, 15–18). Therefore, quality control of suppositories is important. In this study, we have examined the properties of suppositories both *in vitro* and *in vivo* tests.

In the *in vitro* test using cellulose membrane, the release of indomethacin from suppositories stored at a low temperature was significantly faster than that from suppositories stored at a high temperature, which is consistent with the results reported by Yoshino *et al.* (19), who showed by the dialysis-cell method that the rate of release of brilliant blue from suppositories decreased with an elevation in storage temperature.

Ayres *et al.* (12) showed that the rate of *in vitro* release from suppositories containing various concentrations of ^{3}H-benzocaine roughly correlated with the *in vivo* bioavailability in rats. Vidras *et al.* (13) reported that the correlation between the rate of *in vitro* indomethacin release from various kinds of suppository bases and their *in vivo* bioavailability in rabbits was observed only at the initial period after administration into the rectum. In general agreement with these results, in the present experiments, when conscious rats were administered indomethacin suppositories stored at either a low or a high temperature, a significantly lower plasma indomethacin concentration was observed in animals given suppositories stored at a high temperature 30 min after treatment. However, the differences were generally slight as compared with those observed in the *in vitro* experement. To determine the reason for the difference between the data obtained in the *in vitro* and *in vivo* experiments, we examined the bioavailability of indomethacin in anesthetized rats. In such animals, plasma indomethacin concentrations were markedly lower than the corresponding values observed in conscious animals. This was probably due to decreased absorption of indomethacin resulting from reductions in gastrointestinal motility and regional blood flow caused by anesthesia (20). Notwithstanding the low plasma indomethacin levels atteined in anesthetized rats, a clear difference in the bioavalability of indomethacin as shown by the different plasma indomethacin levels was observed between animals given suppositories stored at a low and a high temperature.

Möes *et al.* (8) and Liversidge *et al.* (18) have demonstrated that the storage of fatty-base suppositories at a high temperature causes polymorphic changes and thereby results in an elevation of the melting point. Yoshino *et al.* (19) have also shown by X-ray diffraction analy-

sis, that the initial unstable crystal form in the suppository bases changed to a more stable crystal form after long term storage at a high temperature. In the present experiments, the melting points of suppositories at a low temperature were significantly lower than the values for suppositories stored at a high temperature, which is consistent with the results reported previously.

The results obtained in the present study suggest that although the rate of *in vivo* release of indomethacin from fatty-base suppositories is decreased by a high storage temperature, locomotor hyperactivity and enhanced gastrointestinal motility caused by the rectal administration of suppositories may increase the rate of melting of the suppositories and the absorption of indomethacin in conscious rats. Therefore, the difference between the rates of *in vivo* release of indomethacin from suppositories stored at a low and a high temperature may have been decreased in conscious rats. Because patients who take indomethacin suppositories are generally required to lie quietly, the difference in bioavailability of indomethacin between suppositories stored at different temperatures may be more marked than that expected from the results of experiments using conscious rats.

In conclusion, the present *in vitro* and *in vivo* experiments on fatty-base suppositories contaning indomethacin show that storage at a high temperature decreases the rate of release and bioavailability of the drug from suppositories.

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References

1. De Boer AG, Moolenaar F, De Leede LGJ and Breimer DD: Rectal drug administration: Clinical pharmacokinetic considerations. Clin Pharmacokin (1982) **7**, 285–311.
2. Kamiya A, Ogata H, and Fung H-L: Rectal absorption of nitroglycerin in the rats: Avoidance of first-pass metabolism as a function of rectal length exposure. J Pharm Sci (1982) **71**, 621–624.
3. Weiss AL and Sciarrone BJ: Release rates of salicylates from cocoa butter I. J Pharm Sci (1969) **58**, 980–982.
4. Thomas WH and McCormack R: The drug release characteristics of various rectal suppositories as determined by specific ion electrodes. J Pharm Pharmacol (1971) **23**, 490–494.
5. Pagay SN, Poust RI and Colaizzi JL: Influence of vehicle dielectric properties on acetaminophen bioavailability from polyethylene glycol suppositories. J Pharm Sci (1974) **63**, 44–47.
6. Kellaway IW and Marriott C: (1975) Correlations between physical and drug release: Characteristics of polyethylene glycol suppositories. J Pharm Sci (1975) **64**, 1162–1166.
7. Roseman TJ, Derr GR, Nelson KG, Lieberman BL and Butler SS: Continuous flow bead-bed dissolution apparatus for suppositories. J Pharm Sci (1981) **70**, 646–651.
8. Möes A and Jaminet F: Influence of aging of suppositories on rectal absorption of paracetamol. Pharm Acta Helv (1976) **51**, 119–125.
9. Setnikar I and Fantelli S: Liquefaction time of rectal suppositories. J Pharm Sci (1962) **51**, 566–571.
10. Baichwal MR and Lohit TV: Medicament release from fatty suppository bases. J Pharm Pharmacol (1970) **22**, 427–432.
11. Rutten-Kingma JJ, De Blaey CJ and Polderman J: Biopharmaceutical studies of fatty suspension suppository II. Influence of particle size and concentration on in vitro release of readily water-soluble compounds. Int J Pharm (Amst) (1979) **3**, 179–186.
12. Ayres JW, Lorskulsint D, Lock A, Kuhl L and Lskar PA: Absorption and distribution of radioactivity from suppositories containing ^{3}H-benzocaine in rats. J Pharm Sci (1976) **65**, 832–838.
13. Vidras NJ, Reid VE, Bohidar NR and Plakogiannis FM: Medicament release from suppository bases I: Physicochemical characteristics and bioavailability of indomethacin in rabbits. J Pharm Sci (1982) **71**, 945–949.
14. Fukuda T, Yoshida T, Eto K, Gomita Y and Araki Y: *In vitro* release of tegafur from a fatty-bases suppository and *in vivo* bioavailability of tegafur. Acta Med Okayama (1986) **40**, 195–200.
15. Krowczynski L: Changes in the complete deformation time of suppositories during storage. Acta Pol Pharm (1965) **22**, 157–162.
16. Parrott EL: Influence of particle size on rectal absorption of aspirin. J Pharm Sci (1975) **64**, 878–880.
17. Rutten-Kingma JJ, Polderman J and De Blaey CJ: Biopharmaceutical studies of fatty suspension suppositories I. Spreading *in situ*. Int J Pharm (Amst) (1979) **3**, 39–53.
18. Liversidge GG, Grant DJW and Padfield JM: (1981) Influence of physicochemical interactions on the properties of suppositories I. Interaction between the constituents of fatty suppository bases. Int J Pharm (Amst) (1981) **7**, 211–223.
19. Yoshino H, Kobayashi M and Samejima M: Changes of physicochemical properties of oleaginous suppository bases during storage and their effects on drug releare. Yakuzaigaku (1981) **41**, 102–112 (in Japanese).
20. Conway CM and Ellis DB: The haemodynamic effects of

short-acting barbiturates. Br J Anaesth (1960) **41**, 534–542. Received August 28, 1990; accepted September 21, 1990.