Transdermal Absorption of Propofol in Rats

Yuri TAKAHASHI,* a Keisuke YAMATO, a Hidero AKIYAMA, b Kazuyuki TSUJI, b Hiraku ONISHI, a and Yoshiharu MACHIDA a

a Department of Drug Delivery Research, Hoshi University; 2–4–41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan; and b Medical Information Services, Inc.; 3–6–18–403 Koyama, Shinagawa-ku, Tokyo 142–0062, Japan.

Received September 2, 2004; accepted January 22, 2005

Propofol (PF), a highly lipophilic anesthetic, has several desirable properties, such as the rapid onset and cessation of its effects upon intravenous infusion. In this study, the transdermal absorption of PF was investigated with the aim of the development of an alternative route of administration. PF solutions containing isopropyl myristate (IPM), ethanol or propylene glycol (PG) at various concentrations were prepared and applied to the abdominal skin of rats. Petrolatum and fatty alcohol propylene glycol (FAPG) ointments containing PF were also prepared and applied to the dorsal skin. Eyelid opening was measured and the ratio of the measured value to the initial value was calculated to evaluate the level of the pharmacological effect of the preparation. The PG solution containing 80% PF achieved higher plasma PF concentrations than the 100% PF solution. The PF-FAPG ointment produced a higher plasma PF concentration than the PF-petrolatum ointment. Furthermore, a drowsy state was confirmed after transdermal administration of 42% PF-FAPG ointment. These results indicate that the combination of PF and PG was appropriate for the transdermal absorption of PF, and PF was absorbed through the rat skin to an extent sufficient to cause a continuous sedative effect.

Key words transdermal drug delivery; skin permeability; propofol; skin irritation; ointment

MATERIALS AND METHODS

Materials PF was purchased from Clariant Corp. (Muttentz, Switzerland). Stearic acid and IPM were obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade) was purchased from Wako Pure Chemical Industries, Ltd. All other chemicals were obtained commercially as the purest grade available.

Animals Male Sprague–Dawley rats weighing approximately 200—240 g were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Hoshi University.

Test Solutions Containing PF Liquid state PF (100% PF), IPM solutions containing 20 and 60% (v/v) PF (PF-IPM), an ethanol solution containing 60% (v/v) PF (PF-ethanol) and PG solutions containing 20, 40, 60 and 80% (v/v) PF (PF-PG) were used as the test solutions.

In Vivo Experiment Using PF Solutions Rats were intraperitoneally anesthetized with pentobarbital sodium (40 mg/kg) and fixed on their backs. The hair of the abdominal skin was removed carefully with electric clippers and an electric shaver. A glass cell (bore: 3.4 cm, height: 3.0 cm, application area: 9.1 cm²) was placed on the clipped region using surgical adhesive. After the adhesive had hardened completely, the test solutions (1.0 g) were put into the glass cell. In order to prevent volatilization of the test solution, the glass cell was covered with aluminum foil. Blood samples were prepared and applied to the dorsal skin. Eyelid opening was measured and the ratio of the measured value to the initial value was calculated to evaluate the level of the pharmacological effect of the preparation. The PG solution containing 80% PF achieved higher plasma PF concentrations than the 100% PF solution. The PF-FAPG ointment produced a higher plasma PF concentration than the PF-petrolatum ointment. Furthermore, a drowsy state was confirmed after transdermal administration of 42% PF-FAPG ointment. These results indicate that the combination of PF and PG was appropriate for the transdermal absorption of PF, and PF was absorbed through the rat skin to an extent sufficient to cause a continuous sedative effect.

Key words transdermal drug delivery; skin permeability; propofol; skin irritation; ointment

MATERIALS AND METHODS

Materials PF was purchased from Clariant Corp. (Muttentz, Switzerland). Stearic acid and IPM were obtained from Kanto Chemical Co., Ltd. (Tokyo, Japan). Acetonitrile (HPLC grade) was purchased from Wako Pure Chemical Industries, Ltd. All other chemicals were obtained commercially as the purest grade available.

Animals Male Sprague–Dawley rats weighing approximately 200—240 g were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan). The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Hoshi University.

Test Solutions Containing PF Liquid state PF (100% PF), IPM solutions containing 20 and 60% (v/v) PF (PF-IPM), an ethanol solution containing 60% (v/v) PF (PF-ethanol) and PG solutions containing 20, 40, 60 and 80% (v/v) PF (PF-PG) were used as the test solutions.

In Vivo Experiment Using PF Solutions Rats were intraperitoneally anesthetized with pentobarbital sodium (40 mg/kg) and fixed on their backs. The hair of the abdominal skin was removed carefully with electric clippers and an electric shaver. A glass cell (bore: 3.4 cm, height: 3.0 cm, application area: 9.1 cm²) was placed on the clipped region using surgical adhesive. After the adhesive had hardened completely, the test solutions (1.0 g) were put into the glass cell. In order to prevent volatilization of the test solution, the glass cell was covered with aluminum foil. Blood samples were
(200 μl) were periodically collected from the jugular vein after application of the test solution. After centrifugation, the plasma was separated and stored at −70 °C until analysis. The abdominal skin under the test solutions was investigated visually when the examination ended.

**Measurement of PF Level in Plasma** The plasma concentration of PF was measured by HPLC using a fluorescence detector (excitation: 276 nm, emission: 310 nm) under the following conditions for HPLC.1,13 Chromatography was performed in a system consisting of a model LC-6AD pump (Shimadzu, Kyoto, Japan), a model RF-10A XL fluorescence detector (Shimadzu), and a model C-R7A plus Chromatopac (Shimadzu). Ethanol (200 μl) was added to the plasma (100 μl) and the mixture was centrifuged at 8000 rpm for 5 min after adequate agitation followed by standing for 10 min. Twenty microliters of the supernatant was injected into the HPLC system. Chromatographic separation was carried out on a Neopack C18 column (4.6 mm × 250 mm; Nishio Industry Co., Ltd., Tokyo, Japan) at room temperature. The mobile phase was composed of acetonitrile–water–phosphoric acid (85%): 60–40–0.2 and the flow rate was set at 1.3 ml/min.

**Stability Study for Solutions** Sixty percent (v/v) PF-ethanol and 60% (v/v) PF-PG with or without 10% (v/v) distilled water were incubated at 37 °C for 24 h. The solutions were diluted with ethanol at an appropriate concentration after incubation, and aliquots (20 μl) of the samples were injected into the HPLC system. HPLC conditions were the same as for the determination of PF in plasma.

**In Vivo Experiment Using PF Ointments** To evaluate the pharmacological effects of PF, ointments were applied to the dorsal skin of unanesthetized rats. A least 5 h before the application, the rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and the left femoral aorta was catheterized with a polyethylene tube (PE-10). Through this route, approximately 200 μl of heparin (sodium salt) diluted three-fold with saline was infused. The dorsal hair was carefully removed using electric clippers and an electric shaver. After the rat awakened, the ointments (0.5 g) were administered to the dorsal skin via an adhesive patch (application area: 9 cm²), as shown in Fig. 1. Blood samples (200 μl) were periodically collected from the catheter and the plasma concentration of PF was measured by HPLC. HPLC conditions were the same as for the experiments with PF solutions. Simultaneously, eyelid opening was measured at a predetermined time and the ratio of the measured value to the initial value was calculated to evaluate the intensity of the pharmacological effect as the eyelid opening reduction effect. A vernier caliper was used to measure the eyelid opening. The measurement was always performed using the right eye. The vernier caliper was separated from the eye by about 1 cm during the measurement.

**Preparation of Ointments** The composition of ointments is shown in Table 1. For the in vivo study, a petrolatum ointment, a fatty alcohol–propylene glycol (FAPG) ointment containing 20% (w/w) PF and a FAPG ointment containing 42% (w/w) PF were used. The petrolatum ointment was prepared by mixing PF and petrolatum in a mortar. FAPG ointments were prepared using previously reported methods with slight modification.8,12) PG and PF were added to stearic acid and dissolved in a water bath at 80 °C. The mixture was agitated in an ice bath at 600 rpm, and then stearyl alcohol melted in advance at 80 °C was added. After that, the FAPG ointment was mixed in a mortar until it became smooth.

**RESULTS**

Transdermal Absorption of PF from Solutions The plasma concentration profiles of PF after the transdermal administration of 20% and 60% (v/v) PF-IPM and 100% PF are shown in Fig. 2. Although the plasma concentration of PF increased in a manner dependent on the concentration of PF in the PF-IPM, the plasma concentration was low compared with that for 100% PF. The area under the plasma concentration–time curve \((AUC_{0→90})\) of 100% PF, and 20 and 60% (v/v) PF-IPM was 21444 ± 1485, 2070 ± 537 and 5209 ± 1460 ng·min/ml, respectively. The plasma concentration profiles of PF after transdermal administration of PF-ethanol and PF-

---

Table 1. Formulae of Ointments

<table>
<thead>
<tr>
<th>Ointment</th>
<th>Composition % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petrolatum Stearyl acid Stearic acid PG PF</td>
</tr>
<tr>
<td>20% PF-Petrolatum</td>
<td>80.0 — — — 20.0</td>
</tr>
<tr>
<td>20% PF-FAPG</td>
<td>— 24.8 5.2 50.0 20.0</td>
</tr>
<tr>
<td>42% PF-FAPG</td>
<td>— 24.8 5.2 28.0 42.0</td>
</tr>
</tbody>
</table>

---

Fig. 1. Schematic Representation of Administration of Ointments

---

Fig. 2. Plasma Concentration Profiles of PF after Transdermal Administration of PF and PF-IPM Solutions

Key: 100% PF (●), 60% PF-IPM (○), 20% PF-IPM (□). Each point represents the mean ± S.E. (n = 3 for 60% and 20% PF-IPM, n = 5 for 100% PF).
PG are shown in Figs. 3 and 4. There was an obvious decrease at 60 min after the application of PF-ethanol. The $AUC_{0-90}$ of PF-ethanol was 6811±732 ng · min/ml. Although the application of 20 and 40% (v/v) PF-PG brought about a lower plasma concentration than did 100% PF, a higher plasma concentration level was achieved by the application of 80% (v/v) PF-PG. The application of 60% (v/v) PF-PG resulted in about the same plasma concentration level as 100% PF. The $AUC_{0-90}$ of 20, 40, 60 and 80% (v/v) PF-PG was 9953±2919, 13040±1873, 27021±5169 and 46060±4839 ng · min/ml, respectively. A value approximately twofold the $AUC_{0-90}$ of 100% PF was obtained using 80% (v/v) PF-PG.

**Skin Irritation and Stability of Test Solutions**

Photographs of abdominal skin treated with PF solutions during the absorption experiment are shown in Fig. 5. No irritation was observed visually after the application of 100% PF (Fig. 5A). However, the color of the skin changed and necrosis was observed after the application of 60% (v/v) PF-ethanol (Fig. 5B). Although the skin paled after the application of 60% (v/v) PF-PG (Fig. 5C), the irritation was moderate compared with that caused by 60% (v/v) PF-ethanol. From the results of the stability study, as obvious peaks generated by decomposition were not observed in the HPLC profiles of the test solutions, it was considered that PF was stable in the test solutions. A photograph of abdominal skin treated with 100% ethanol is shown in Fig. 5D. Skin irritation was not observed visually after applying ethanol and PF independently. Hence, it was considered likely that the structure of PF was modified in the skin. Fuchs et al. reported that dithranol (anthralin), a polycyclic aromatic hydrocarbon, is converted in skin into several oxidized products, including persistent free radicals. The formation of free radicals is essential for dithranol-induced inflammation in the skin. PF oxidation by peroxynitrite (ONOO−) produces a short-lived radical (a phenoxyl radical). Also, under biological conditions, peroxynitrite is formed very rapidly from the reaction of nitric oxide with superoxide anion. Furthermore, in an in vitro study, addition of peroxynitrite to PF in methanol generated a color reaction corresponding to the formation of NO-PF within seconds. Thus, it was hypothesized that the formation of the phenoxyl radical resulting from the topical application of PF in ethanol caused the skin irritation. It was reported that skin irritation was inhibited by topical treatment with the free radical scavenger dl-α-tocopherol. Therefore, to investigate whether the skin irritation could be controlled by tocopherol, 60% (v/v) PF-ethanol with 10% (w/v) tocopherol was applied for 90 min under the same conditions as in the in vivo experiment described above for solutions. Observation of the abdominal skin (Fig. 5E) confirmed that the skin irritation was prevented by tocopherol. From the above results, it was considered that free radicals generated in the skin caused necrosis of the skin tissue, and that the plasma PF concentration was decreased as a result of the necrotic changes in the tissue after the administration of PF-ethanol. There are differences, such as the use of methanol versus ethanol, between this experiment and the previously reported experiments. Furthermore, it is not clear whether the formation of the phenoxyl radical is suppressed by the application of PF in PG. Further investigations will be needed to characterize the skin irritation caused by topical treatment with PF.

**Effect of the Administration of Ointments on Eyelid Opening**

The reduction in eyelid opening caused the ad-
Administration of ointments is shown in Fig. 6. An obvious decrease was observed 75 min after administration of 20% (w/w) PF-FAPG ointment compared with 20% (w/w) PF-petrolatum ointment. Shingu et al. 19) evaluated the anesthetic effect using a four-grade system after intravenous injection of PF to volunteers. The first grade is a sleeping level: there is no reaction when shaken. The second grade is a drowsy level: there is a reaction when shaken. The third grade is a sedative level: although the eyes open when the subject is called, the subject again sleeps immediately. The fourth grade is an awake level: the eyes are opened when the subject is called, and the subject does not fall asleep. When 42% (w/w) PF-FAPG ointment was administered to rats, a sedative level was observed after approximately 30 min. Furthermore, as the rats could hardly open their eyes though they reacted when shaken after 50 min, it was considered that the consciousness level progressed to the second grade at approximately 50 min. Shingu et al. also reported that anesthesia was induced in 72±13 s (mean±S.D., n=5) and maintained for 423±131 s (mean±S.D., n=5) after intravenous injection of PF (2 mg/kg) to volunteers. In contrast, though the eyelid opening was gradually reduced and a rapid sedative effect was not shown, a continuous sedative effect was observed after transdermal application of 42% (w/w) PF-FAPG ointment. This indicates the possibility that the continuous sedative effect was obtained as a result of maintaining the plasma PF level.

Transdermal Absorption of PF from Ointments The plasma concentration profiles of PF after transdermal administration of the ointments are shown in Fig. 7. Higher plasma concentrations were observed after treatment with 20% (w/w) PF-FAPG ointment than 20% (w/w) PF-petrolatum ointment. Furthermore, after application of 42% (w/w) PF-FAPG ointment, a considerably higher concentration was observed at 30 min and maintained during the experiment. Since in the eyelid opening experiment, the drowsy level appeared after 50 min, it was considered that there was a time lag between the rise of the plasma PF concentration and the appearance of an obvious sedative effect. Plots of the effect on eyelid opening versus the plasma PF concentration at 60 and 90 min are shown in Fig. 8. The data of 30 min were omitted because a sufficient sedative effect appeared not to have been obtained then. The eyelid opening showed a tendency to decrease as the plasma PF concentration increased.

DISCUSSION

The plasma concentration of PF increased continuously after the transdermal application of 100% PF. It is considered that it would take a long time for the permeation of PF to reach a steady state. Therefore, for the purpose of increasing the blood concentration of PF rapidly, some solvents were added to PF. IPM is known to be a useful lipophilic solvent for transdermal absorption. There have been several reports concerned with the enhancement by IPM of the transdermal absorption of drugs. 20—23) Since lipids in the stratum corneum contain large amounts of free and esterified long chain fatty acids, IPM partitions into this domain and increases the fluidity of the lipid portions of the stratum corneum. 24,25) In the present study, although the plasma PF concentration increased in a manner dependent on PF content, a significant enhancing effect was not observed after treatment with a mixture of PF and IPM because the PF was diluted by IPM. Furthermore, the affinity between PF and the solvent was high because both PF and IPM are lipophilic. Therefore, the release of PF from the mixture was sup-
pressed. In general, despite good partitioning into the stratum corneum lipids, the permeability of lipophilic molecules is low. This is probably due to the accumulation of lipophilic drugs in the stratum corneum because of low aqueous solubility. Thus, though ethanol was added to PF as a hydrophilic solvent, the plasma PF level did not increase remarkably. It was considered that the drug permeability was suppressed due to changes of the skin tissue, because obvious skin irritation was observed. In contrast, after treatment with 80% (v/v) PF-PG, the plasma PF concentration rapidly increased as compared with that after treatment with 100% PF. It was reported that PG penetrated through the skin barrier into the dermis and increased the solubility of solutes. It was reported that PG penetrated through the skin barrier into the dermis and increased the solubility of solutes. Thus, the permeation of PG must be an important factor influencing solute transport. Although PF is practically insoluble in water, it is partially soluble in a mixture of PG and water. Hence, it was considered that PG permeated the skin mixed with body fluids, and increased the solubility of PF in the hydrophilic body fluids. As the AUC0–90 of 80% (v/v) PF-PG was higher than that of 60% (v/v) PF-PG, it is considered that a higher PF concentration in the donor solution is advantageous because PF is a comparatively easily permeating drug. Thus, it was shown that PG was the most appropriate solvent of the three solvents tested for rapidly increasing the plasma PF concentration when the optimum amount of PG was used.

For evaluation of the pharmacological effects of topical application of PF, a transdermal dosage form which can be applied to unanesthetized rats is needed. In terms of safety, it is desirable that the materials of the base are used in conventional pharmaceutical preparations. Since the studies using PF solutions showed that PG was an appropriate solvent, FAPG ointment (which contains PG) was considered likely to be useful. Kaiho et al. reported that indomethacin was readily absorbed through the depilated abdominal skin of rats from an FAPG base, and they suggested that FAPG base could be a useful vehicle for percutaneous drug administration. Funke et al. reported that the combination of PG and fatty acid modified the barrier structure of the stratum corneum and allowed highly lipophilic antiestrogens to permeate easily through the skin. The synergistic permeation enhancement by PG and fatty acid is due to the enhanced solubility of fatty acid in the stratum corneum by PG and the enhanced PG permeability by fatty acid insertion between the intercellular lipids of the stratum corneum. Since the stearic acid contained in FAPG ointment is polymorphic, it may be susceptible to a process-induced variation in its final enhancing effect. Lin et al. reported that drug permeability was enhanced by increasing the cooling rate during the preparation of FAPG ointment, because the solubility of stearic acid in PG is increased by rapid cooling. In this study, at first, it was found that the plasma concentration of PF was variable and reproducibility was not obtained when FAPG ointment prepared by cooling at room temperature was used. Therefore, FAPG ointment prepared by agitation in an ice bath was used for in vivo experiments. Neither a sufficient concentration of PF in plasma nor an eyelid opening reduction effect was obtained with petrolatum ointment because petrolatum has lipophilic behavior and high affinity for PF. However, FAPG ointment produced good results. It was considered that permeation of PG into the skin was enhanced by stearic acid and transdermal absorption of PF that was dissolved in PG was thus increased. When 42% (w/w) PF-FAPG ointment was administered, a high plasma PF concentration was obtained at 30 min, apparently as a result of the rapid permeation of PF. The drug information form prepared by Astra Zeneca states that the clinically effective blood concentration of PF for anesthesia is 3—8 µg/ml. In this study, in spite of the low plasma concentration of PF (about one-tenth of the clinically effective blood concentration), a sedative or drowsy state was observed. Hence, it is considered that PF should be developed as a sedative or a hypnotic, although further research is needed to investigate the clinically effective blood concentration of PF for a sedative or a hypnotic effect. The transdermal permeability of PF may be increased by increasing the PF concentration in the preparation. However, there are limitations to the increase of the PF content in FAPG ointment. It is considered that it will be possible to make preparations containing a large amount of PF by using polymers.

In conclusion, the combination of PF and PG was useful for the transdermal absorption of PF. PF was absorbed through rat skin from PF-FAPG ointment to an extent that induced a continuous sedative effect, and a drowsy state after a lag. Further research is in progress to search for more effective enhancers and preparations for the transdermal delivery of PF.

Acknowledgements This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

REFERENCES


