Changes in Percutaneous Absorption of Fentanyl Patches in Rats Treated with a Sebum-Like Secretion

Tomonori Hayashi, a Hinako Kawaguchi, b Tsumugi Eifuku, b Hiroshi Matsuoka, b Atsufumi Kawabata, b and Noriaki Nagai a,b

a Kindai University Nara Hospital; 1248–1 Otodacho, Ikoma, Nara 630–0293, Japan; and b Faculty of Pharmacy, Kindai University; 3–4–1 Kowakae, Higashi-Osaka, Osaka 577–8502, Japan.

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The percutaneous absorption of a fentanyl (FEN)-patch is affected by various external factors including the volume of sebum secretion, which causes changes in the skin surface environment. In this study, we prepared a lard-based sebum-like secretion (SLS), and applied it to investigate the effect of different skin surface conditions on the drug penetration of a FEN-patch. In vitro work to test drug release using the Franz diffusion cell indicated that drug release was significantly suppressed by treatment with 5% SLS, which is equivalent to the amount of daily human sebum secretion. Conversely, in ex vivo experiments using rat skin, the amount of FEN that accumulated in the skin tissue of the 5% SLS-treated rats was higher in comparison with the non-SLS treated group. Furthermore, in vivo experiments indicated that the plasma FEN concentration in rats treated with the FEN-patch was significantly increased by treatment with 5% SLS. These results suggest that the sebum affected the release, accumulation, and absorption of FEN from the FEN-patch, and the FEN concentration in the blood was reflected by the balance of the suppression of drug release and the enhancement of drug accumulation in the skin with SLS.

Key words: fentanyl patch; percutaneous absorption; sebum-like secretion; skin; opioid

Introduction

Cancer is characterized by the development and unchecked growth of rapidly dividing, abnormal cells. Approximately 24–62% of cancer patients experienced cancer-related pain. In general, opioids such as fentanyl (FEN), are used to treat severe cancer pain. FEN has a low molecular weight, high lipid solubility, and is readily absorbed through the skin. In clinical settings, a commercially available FEN transdermal patch (FEN-patch) is most often used as the final drug in the palliative care of terminal cancer patients. However, previous reports showed that various factors such as temperature, body fat percentage, body mass index, nutritional status, cancer cachexia, and application to the site of skin damage affected the percutaneous absorption of FEN. In particular, the effect of temperature is addressed in the U.S. Food and Drug Administration (FDA) safety warning: a fever of 40°C is expected to increase blood concentration by 25%, and FEN blood levels could be approximately tripled by heating the skin for 4 h at the patch application site. Excessive FEN levels in the blood cause side effects including nausea, vomiting, somnolence, dizziness and respiratory depression. For FEN, the calculated odds ratios for the antinociception and respiratory depression were 3.03 and 2.54, respectively. Thus, the safety margin of FEN is low, and the safety index odds ratio (antinociception/respiratory depression) of FEN is 1.20. This report suggests that fentanyl shows little in vivo tissue selectivity and that a slight increase in fentanyl increases the risk of respiratory depression. Hence, the careful dose titration of highly effective opioids is important, as respiratory depression is a particularly dangerous side effect in patients treated with the FEN-patch. Teraoka et al. compared the residual amounts of FEN in FEN-patches after their administration to patients, and found that the residual amounts of FEN in the patches was significantly higher in patients with high levels of sebum. This work suggests that secreted sebum may also affect the penetration of FEN.

To study the relationship between sebum secretion levels and percutaneous absorption of FEN, it is important to select a substance to replace human sebum, since it is difficult to collect pure sebum from patients, and because the sebum can easily be denatured by oxidation. The human sebum is mainly composed of free fatty acids, triglycerides, wax ester, squalene, and the ratio of free fatty acids was enhanced by the oxidation of triglycerides. The free fatty acids are strongly related to the properties of sebum, and is characterized by a high content of oleic (35–42%), palmitic (21–25%), linoleic (9–17%), and stearic acids (15–18%). The fatty acid composition of lard is very similar to that of human sebum. These free fatty acids are secreted from the hair follicles to the skin surface. Nazzaro-Porro et al. reported that approximately 40–140 µg/cm² per day of sebum is secreted on the human thorax. Moreover, the amount of free fatty acids in human sebum is approximately 30%, and the daily secretion amount of free fatty acids is estimated to be approximately 30 µg/cm². In this study, we prepared a sebum-like secretion based on lard (SLS) and investigated the mechanism of drug release and percutaneous absorption of the FEN-patch in rat skin treated with SLS.

Experimental

Animals Seven-week-old male Wistar rats were purchased from Kiwa Laboratory Animals Co., Ltd. (Wakayama, Japan), and housed under normal conditions (7:00a.m.–7:00p.m. light, 25°C). Water and CE-2 formulation diet (Clea Japan Inc., Tokyo, Japan) were provided freely. The animal experiments were approved by the animal care and use committee.

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**Reagents**  The FEN patches (Fentos® tape, FEN-patch) were obtained from Hisamitsu Pharmaceutical Co., Inc. (Saga, Japan). Methanol, acetonitrile and acetic acid were purchased from Kanto Chemical Industry Co., Ltd. (Tokyo, Japan). Ammonium acetate, propyl p-hydroxybenzoate, sodium dihydrogen phosphate, sodium hydrogen phosphate, and isoflurane were purchased from Wako Pure Chemical Corporation (Osaka, Japan). Lard was obtained from Kishida Chemical Co., Ltd. (Osaka, Japan). The SLS was prepared to dissolve in saline, and the composition and ratio of free fatty acids were adjusted to the following: oleic (43.2%), palmitic (25.1%), linoleic (9.6%) and stearic acids (14.4%). A FEN-patch was incubated with SLS solution for 60 s, removed excess SLS solution. The SLS levels on FEN-patches treated with 0.1, 1 and 5% SLS were 0.34 ± 0.20, 2.99 ± 0.66 and 31.9 ± 1.10 µg/cm², respectively (n = 3), and the amount of SLS in the 5% SLS-supplemented FEN patch was similar to free fatty acids of sebum secreted daily on the human thorax. All other chemicals used were of the highest purity commercially available.

**HPLC Method**  FEN concentrations in the samples were determined by HPLC, using the LC-Net II/ADC system (JASCO Corporation, Tokyo, Japan). The conditions were as follows: mobile phase, solution consisting of 1% (w/v) ammonium acetate/methanol/acetonitrile/acetic acid (300/200/100/0.3); flow rate, 0.25 mL/min; column, Inertsil® ODS-3 column (3 µm, column size: 2.1 × 50 mm, GL Science Co., Inc., Tokyo, Japan); column temperature, 35°C; wavelength for detection, 258 nm; internal standard, 1 µg/mL propyl p-hydroxybenzoate.

**Extraction of FEN from FEN-Patch**  A FEN-patch was added to a solution of 0.23 M hydrochloric acid and 19 M methanol in a heat-resistant bottle, and stirred for 20 h at 50°C by a Bioshaker® (70rpm, BR-23FR MR, TAITEC, Koshigaya, Japan). After cooling to room temperature, the volume of the solution was brought to 50 mL using the methanol, and the extracted FEN in the solution was used as the sample for measurement.

**Drug Release from the FEN-Patch**  Drug release from the FEN-patch was measured using a Franz diffusion cell set with a 0.45 µm DURAPOR membrane filter (Merck KGaA, Darmstadt, Germany). The reservoir chamber was filled with phosphate buffer at pH 7.2 at 37°C. The SLS was administered to a FEN-patch for 60 s at room temperature, removed excess SLS solution, and then a FEN-patch with or without SLS was placed on the filter membrane of the Franz diffusion cell. Afterwards, 200 µL of sample was withdrawn from the reservoir chamber, and the same volume of buffer was replenished each time. The FEN-patch was incubated with SLS solution for 60 s, removed excess SLS solution, and the SLS-treated FEN-patch was placed on the skin. After treatment for 9h, the skins were collected, homogenized in methanol, and the homogenate was centrifuged at 20400 × g and 4°C for 15 min. Afterwards, 200 µL of sample was collected from the reservoir chamber from 0–9 h, and the same volume of buffer was replenished each time. The FEN concentrations in supernatants were measured by the HPLC method described above.

The data were analyzed by Eqs. (2)–(4):

\[
J_c = \frac{K_m C_{\text{FEN}}}{\delta} = K_p C_{\text{FEN}}
\]

\[
Q = J_c \cdot A \cdot (t - t_{\text{lag}})
\]

where \( t_{\text{lag}} \) is the lag time, \( \delta \) is thickness of the skin (0.071 cm, average for three rats), \( J_c \) is the FEN penetration rate, \( K_m \) is the skin/preparation partition coefficient, \( K_p \) is the diffusion constant within the skin, \( D \) is the diffusion constant within the skin, \( Q \) is the total amount of FEN (\( C_{\text{FEN}} \)) appearing in the reservoir solution at time \( t \), and \( A \) is the effective area of the skin (2 cm²). The \( \Delta \text{AUC}_{0-9h} \) was calculated according to the trapezoidal rule up to the last FEN measurement point (9h).

**FEN Concentration in the Blood of Rats Treated with the FEN-Patch**  The abdominal skin of rats was shaved with an electric clipper and razor, and a FEN-patch was applied to the affected area (2 cm²). After the application, blood (200 µL) was collected from the right jugular vein via cannulation and centrifuged at 15000 × g at 4°C for 20 min. The plasma FEN concentrations were measured by the HPLC method described above. The \( \Delta \text{AUC}_{0-24h} \) was analyzed according to the trapezoidal rule up to the last FEN measurement point (24h).

**Statistical Analysis**  The data are expressed as the mean standard error (S.E.) of the mean. Student’s t-test was used for statistical analysis, and a maximum p value of 0.05 (\( p < 0.05 \)) was chosen as the significance level.

**Results**

**Effect of SLS Treatment on Drug Release**  Figure 1 shows the drug release from SLS-treated FEN-patches in the in vitro model. Patches treated with 0 and 0.1% SLS released their entire drug load; FEN was not detected in the patch. However, the release of FEN was reduced by treatment with 1 and 5% SLS; the remaining FEN in the 5% group was higher than in the 1% group (Fig. 1A). The remaining amount of FEN in patches treated with 1 and 5% SLS were 0.5 and 5.3% of the original amount of FEN, respectively. Figures 1B and 1C show the release profile (B) and \( \Delta \text{AUC}_{0-9h} \) (C) of FEN from the patch in the Franz diffusion cell. The drug release from the 1% SLS-treated FEN-patches were similar to FEN-patches without SLS treatment. However, the treatment of 5% SLS prevents drug release: the \( \Delta \text{AUC}_{0-9h} \) of 5% SLS-treated FEN-patches was 79.9% of FEN-patches without SLS treatment. Moreover, the drug release rate constant (\( D \)) from the
The amount of FEN in the patch 9 h after the application of the FEN-patch. (B) Release of FEN from the FEN-patch through the SLS-treated membrane. (C) The $AUC_{0-9h}$ in the FEN-patch. $n = 3$, N.D., not detectable. * $p < 0.05$ vs. 0%. The 0–1% SLS did not affect the drug release; however, the release of FEN from the FEN-patch was prevented by the treatment with high SLS (5%).

Table 1. Pharmacokinetic Analysis of FEN-Patch Treated with SLS in in Vitro Drug Release

| SLS | 0%  | 0.1% | 1%  | 5%  |
|-----|-----|------|-----|-----|
|  $D$ ($\times 10^{-2}$h) | 2.83 ± 0.18 | 2.84 ± 0.11 | 2.83 ± 0.17 | 1.79 ± 0.08* |

$n = 3$, * $p < 0.05$ vs. 0% SLS.

5% SLS-treated FEN-patches was 63.3% of that from FEN-patches without SLS (Table 1).

**Effect of SLS Treatment on FEN Skin Penetration** Figure 2 shows FEN skin penetration from the SLS-treated FEN-patch using the *ex vivo* model. Figure 2A shows the amount of FEN in rat skin 9 h after FEN-patch administration. There was no difference between patches without SLS and the 0.1–1% SLS-treated patches. However, the accumulation of FEN was enhanced by the treatment of 5% SLS by 1.10-fold. Additionally, the penetration profile (Fig. 2B) and $AUC_{0-9h}$ (Fig. 2C) of FEN through the rat skin were strongly attenuated by the treatment of 5% SLS in experiments using the Franz diffusion cell (Fig. 2C). Table 2 summarizes the pharmacokinetic parameters calculated from the data in Fig. 2C. The penetration rate ($J$) of 5% SLS-treated FEN-patches was significantly lower (52%) than that in FEN patches without SLS treatment.

**Effect of SLS Treatment on FEN Blood Transfer** Figure 3 shows the *in vivo* changes in the plasma FEN concentration of rats administered FEN-patches treated with SLS. The amount of FEN remaining in the patch treated with 5% SLS was significantly lower (61%) than in the FEN patch without SLS (Fig. 3A). Figure 3B shows the behavior of plasma FEN concentrations after the application of the FEN-patch with or without 5% SLS treatment. A significant increase in FEN blood levels was observed in the SLS-treated rats compared to the non-SLS-treated group. However, the plasma FEN concentration in the non-SLS-treated rats was stable 6 h after application, while the 5% treated group continued to increase, and the levels were 2.19-fold higher at 24 h. Moreover, the $AUC_{0-24h}$ in the rats treated with 5% SLS was 1.92-fold higher than that in the non-SLS-treated group (Fig. 3C).

**Discussion**

The absorption of FEN through a transdermal delivery system is 1000-fold faster than using morphine, since FEN has a molecular weight of 336.5 g/mol and is also highly lipophilic (log $P_{ow} = 2.96$). Additionally, FEN is approximately 100-fold more potent than morphine. Due to the potency and fast delivery of FEN, changes in absorption *via* the transdermal route causes a potent analgesic effect and carries the potential for serious side effects. Ooi *et al.* reported that the
plasma FEN concentration in rats with dry skin treated with the FEN-patch was significantly lower compared to the plasma concentration of FEN in rats with wet skin.25) Furthermore, heating is also a factor that increases drug absorption from the FEN-patch.26,27) To prevent changes in FEN absorption by factors in the skin surface environment, the package insert of the Japanese FEN patch stipulates that the skin at the application site must be wiped clean through the non-use of use soap, alcohol and lotion et al. to modulate the absorption when the product is applied.14) However, changes in the skin surface environment after application cannot be regulated by the methods utilized in the package insert, and it is known that the volume of sebum secretion is the one such factor. In this study, we prepared SLS to replace free fatty acids in human sebum and investigated the effect of the amount of SLS on drug penetration from the FEN-patch.

First, we used a membrane in vitro drug release test, and then a rat skin ex vivo drug skin penetration test to investigate how SLS affects drug release in FEN transdermal formulations. In the in vitro drug release test, 0–1% SLS did not affect drug release. However, the drug release behavior was significantly suppressed by treatment with 5% SLS, and the release rate constant (D) was low (Figs. 1B and C, Table 1). After the experiment, the SLS was gelled on the donor side of the patch. The SLS gel may decrease the drug-releasing area of the patch, resulting in a decrease of the overall FEN release. We also measured the drug skin penetration in the ex vivo study. The penetration rate (Jc) of the FEN-patch was significantly lower in the 5% SLS-treated group. However, the amount of FEN that accumulated in skin of the 5% SLS-treated rats was higher in comparison to the group without SLS (Fig. 2). SLS is a hydrophobic substance and FEN is lipophilic; this interaction affects the drug solubility, and the high affinity between FEN and SLS enhances drug accumulation in the skin.23)
which could explain our observations. Moreover, oleic acid is the most abundant free fatty acid in SLS and is known to reduce the barrier function of the skin and increase the diffusibility of the entire stratum corneum by causing phase separation in the lipid domains of the stratum corneum.\textsuperscript{28,29} Touitou \textit{et al.} found that patches containing oleic acid formed pores on the surface of epidermal keratinocytes in rats,\textsuperscript{30} and Larucea \textit{et al.} also reports that oleic acid applied to mouse skin increases the transdermal absorption of tenoxicam.\textsuperscript{31} These results suggested that high SLS concentrations enhanced FEN uptake from the patch and the corresponding accumulation of FEN in the skin tissue. Additionally, the shift of FEN into the reservoir chamber may be prevented by the enhanced drug accumulation in the skin tissue. 

Next, we investigated SLS-mediated changes in plasma FEN concentration in rats treated with the FEN-patch. In contrast to the results in the drug release experiment (Fig. 1A), the residual amount of FEN in the patch was lower than that in non-SLS-treated rats 24h after the application of the FEN-patch (Fig. 3A). Furthermore, the plasma FEN concentration in the rats treated with the FEN-patch were significant increased by the application of 5% SLS (Fig. 3B). We investigated changes in the release and accumulation of FEN and found that high SLS caused the suppression of the drug release and the increase of drug accumulation in the skin. The skin is composed of the stratum corneum, epidermis, dermis, and subcutis (containing adipose) in that order from the surface, and the capillaries are located under the dermis. The \textit{ex vivo} test uses the skin of rats, the rat skin containing dermis were used. Therefore, it is not always necessary for the drug to pass all the way through the skin to enter the blood. Therefore, the inconsistency between Figs. 1–3 may be interpreted as being caused by differences in the experimental methods. Taken together, it was hypothesized that the uptake of FEN into the skin by high SLS was strongly worked than that in suppression of the drug release \textit{via} SLS, resulting in decrease the supplement of FEN in the \textit{in vivo}. Moreover, the enhancement of the drug uptake into the skin may cause the increase in the plasma FEN concentration since the capillaries are in the dermis\textsuperscript{32,33} (Fig. 4).

In previous reports, aging reduced sebum especially in men,\textsuperscript{3} and the bioavailability of the FEN-patch was different according to patient age: patients 75 year and older absorbed 50% of FEN in 72h, while patients younger than 65 year absorbed 66%.\textsuperscript{34} Additionally, the increase in plasma FEN concentrations occurred more slowly in elderly patients (mean age 73.7 year), as demonstrated by the mean half-time of 11.1h, compared with 4.2h in the younger group (mean age 32.7 year).\textsuperscript{31} In this way, our study supported these previous results, in that reduced levels of sebum in humans decreases the transdermal absorption of FEN. These results suggested that the sebum affected the release, accumulation, and absorp-

Fig. 3. Effect of SLS Content on the Plasma FEN Concentration in Rats Treated with the FEN-Patch

(A) The amount of FEN in the patch 24h after the application of the FEN-patch. (B) Changes in plasma FEN concentration in rats treated with the FEN-patch. (C) The \(\text{AUC}_{0-24h}\) in rats treated with the FEN-patch. \(n = 3–4 \), *\( p < 0.05 \) vs. 0%. The plasma FEN concentration in rats treated with the FEN-patch was significantly increased by the application of 5% SLS. Additionally, the total FEN release levels from tape treated with 5% SLS was also significantly higher in comparison with rats not treated with SLS.
In conclusion, we performed a basic experiment using SLS as a model of free fatty acids in human sebum, and showed that high SLS suppressed drug release of the gel formulation, as a model of free fatty acids in human sebum, and showed that high SLS suppressed drug release of the gel formulation, and enhanced the drug accumulation in skin due to the high hydrophobic affinity between FEN and SLS. Additionally, we found that the absorption of FEN via the FEN-patch with SLS was decided by the balance of the suppression of drug release and the enhancement of drug accumulation in skin with sebum.

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Conflict of Interest The authors declare no conflict of interest.

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