Effect of Skin Surface Temperature on Transdermal Absorption of Flurbiprofen from a Cataplasm

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Transdermal absorption of flurbiprofen (FP) from a cataplasm (CFP) and its anti-inflammatory effect were investigated in the rat under various skin temperature conditions. As the skin temperature was raised, the plasma concentration of FP after application of the cataplasm increased significantly. It was demonstrated by a release and in vitro penetration experiment that skin penetration is the rate-determining step for absorption, and both release and penetration increased with a rise of temperature. Moreover, the in vivo transdermal absorption behavior was estimated by a deconvolution method from the plasma concentration data after intravenous administration and topical application of FP.

The Arrhenius plot of the in vitro penetration data at various temperature gave a nearly straight line and the activation energy calculated from the slope was 16.7 kcal/mol. The skin accumulation of FP decreased with a rise of temperature in the in vivo experiment while no significant change was seen in the in vitro experiment, suggesting participation of the increase of blood flow in the former experiment.

Though the anti-inflammatory effect was demonstrated at the normal skin temperature and under cooling, the effect was not found under warming. In addition, a considerable effect was observed with a control CFP which is free from FP when used under cooling.

From these results, it is suggested that the transdermal absorption of FP from CFP increased with a rise of skin surface temperature, and both factors, the concentration of FP absorbed and topical cooling, contribute to the anti-inflammatory effect.

Keywords—flurbiprofen; cataplasm; percutaneous absorption; plasma level; skin temperature; deconvolution; release rate; Arrhenius plot; skin accumulation; anti-inflammatory effect

Recently, local chemotherapy by percutaneous drug delivery has attracted renewed interest from the standpoint of optimization of drug delivery.1) Topical application should concentrate the drug in the tissues to which it is applied and diminish the drug concentration in other tissues and blood. It could also minimize the side effect of gastrointestinal damage and the first-pass effect observed in the case of oral administration.2)

Flurbiprofen (FP) is an orally active, nonsteroidal anti-inflammatory drug found to be effective in the treatment of rheumatoid arthritis, but its side effect of gastrointestinal damage has limited its utilization.3) Therefore, topical application of FP warrants investigation as an alternative to oral treatment. However, it is well-known that most drugs can not penetrate the skin readily, and many factors may influence the skin penetration.4) In this context, we have reported that a rise of skin surface temperature could increase the transdermal absorption of methyl salicylate from a cataplasm.5)

In the present study, transdermal absorption characteristics of FP from a cataplasm at various skin temperatures were investigated with both in vitro and in vivo techniques. In addition, the anti-inflammatory effects at various skin temperatures were evaluated.
Experimental

**Materials**—FP was obtained commercially (Nippon Zanbon Co., Ltd., Tokyo, Japan) and used without further purification. Styrene isoprene block copolymer was also obtained commercially (Kimura Industry Co., Ltd., Tokyo, Japan). All other chemicals were of reagent grade.

**Preparation of a Cataplasm of FP**—A cataplasm (CFP) containing FP was prepared by Sansho Pharmaceutical Co., Ltd. (Saitama, Japan). The formulation contains FP (2 mg), olive oil (115 mg), styrene isoprene block copolymer (78 mg) and other minor constituents (19 mg) in 14 cm². The CFP has a water-absorbent resin sheet on the drug reservoir phase to cool the skin surface. The structure of the cataplasm is shown in Fig. 1. CFP containing no FP was prepared to evaluate the net effect of cooling on inflammation.

**Control of Skin Surface Temperature**—In the *in vivo* absorption and the anti-inflammatory experiments, the skin surface temperature after CFP application was controlled by the following treatments. Condition A: A polyvinyl bag containing ice-water was put on the CFP. Condition B: Water was added to a water-absorbent resin sheet of the CFP. Condition C: No treatment was carried out. Condition D: A chemical warmer (Hakugen Co., Ltd., Tokyo, Japan) was put on the CFP. In the anti-inflammatory experiment, condition D' was used instead of condition D; in this case, the rat foot on which the CFP was applied was wrapped with a polyvinyl bag and placed in a water bath at 41°C.

By manual monitoring during each treatment, the skin temperature could be maintained within a narrow range, as shown in Table I. The skin surface temperature was measured with a thermometer having a platinum resistance sensor (model EH200-06, Chino Works. Ltd., Tokyo, Japan) during experiments.

**In Vivo Transdermal Absorption Experiment**—Male Wistar albino rats weighing 250—300 g, whose abdominal hair was removed with animal clippers and a shaver at 24 h before the experiment, were used under anesthesia with pentobarbital, given intraperitoneally. CFP (available area; 14 cm²) covered with a protective peel liner was applied on the abdominal skin, and treatment A, B, C or D was employed for controlling the skin temperature. After the temperature had reached a constant level, the liner was removed from the CFP to start the absorption experiment. Blood samples were collected from the jugular vein at fixed intervals. A mixture of 0.2 ml of plasma and 0.2 ml of MeOH was shaken and centrifuged, and FP in the supernatant was determined by HPLC.

**Intravenous Administration Experiment**—A polyethylene glycol solution of FP (2.5 mg/ml) was injected into the femoral vein of three rats weighing 250—300 g (dose; 500 µg/head), and the plasma concentration of FP was determined by high performance liquid chromatography (HPLC) at fixed intervals. The mean plasma levels of FP (Cₚ) versus time obeyed biexponential kinetics as represented by Eq. 1.

\[
C_p = 9.18e^{-2.15t} + 8.77e^{-0.16t}
\] (1)

**Release and in Vitro Penetration Experiments**—A diffusion cell similar to that reported by Loftsson and Bodor, was used for the release and *in vitro* penetration experiments. It has a 6.8 cm² donor area and a 49 ml receptor volume. Cellulose membranes (pore size 24 A, Visking Co., Ltd., U.S.A) were used as diffusion membranes for the release experiment. The full-thickness abdominal skin excised from a rat weighing 250—300 g, whose hair had been removed as in the *in vivo* experiment, was used for the *in vitro* penetration experiment. The diffusion cell and receptor medium, isotonic sodium phosphate-buffered saline (pH 7.4) containing kanamycin sulfate (100 ppm), were placed in a thermostated chamber at least 10 h before the experiment, in which the temperature was regulated at the mean of each *in vivo* experiment. Just before the experiment, the excised skin or cellulose membrane was mounted in the diffusion cell, the receptor phase was filled with the medium, and finally the CFP was applied on the skin or membrane surface of the donor side. The receptor phase was stirred with a magnetic stirrer covered with an adiabator

<table>
<thead>
<tr>
<th>Temperature condition</th>
<th>Abdomen</th>
<th>Foot</th>
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<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>A</td>
<td>24.7</td>
<td>23.4—25.8</td>
</tr>
<tr>
<td>B</td>
<td>34.2</td>
<td>33.6—34.7</td>
</tr>
<tr>
<td>C</td>
<td>36.5</td>
<td>35.0—37.2</td>
</tr>
<tr>
<td>D and D'</td>
<td>41.7</td>
<td>41.0—42.5</td>
</tr>
</tbody>
</table>

a) See the text.
which serves to prevent heat flow. At appropriate intervals, an aliquot (0.5 ml) of the receptor fluid was withdrawn and mixed with 0.5 ml of MeOH. After shaking followed by centrifuging, the supernatant was used for HPLC assay.

**Skin Accumulation**—At the end of the experiments, the skin removed from the cell in the *in vitro* experiment and that excised from the rat abdomen in the *in vivo* experiment were placed in a test tube containing a mixture of phosphate buffer solution (pH 7.4, 25 ml) and MeOH (25 ml). After homogenization followed by filtration, FP in the filtrate was determined by HPLC.

**Determination of Remaining FP in CFP**—At the end of the *in vitro* and *in vivo* experiments, the CFP was removed and placed in a test tube containing 20 ml of chloroform. The tube was shaken for 20 min, then 30 ml of MeOH was added and the tube was shaken again for 5 min. After centrifugation, the supernatant was subjected to HPLC assay.

**Anti-inflammatory Test**—The anti-inflammatory effect was evaluated in terms of the suppressive effect on the swelling edema according to the method of Riesterer and Jaques with minor modifications. The edema was induced by dropping a 100 g iron weight from 1 m height onto the right hind paw of the rats (150–200 g). A CFP or CFP containing no FP was immediately applied to the edema area and foot skin temperature was controlled as described above. The CFP was removed hourly and the volume of the right hind paw was measured by using a plethysmometer (model KN-357, Natsume Works Co., Ltd., Tokyo, Japan). After the measurement, the foot was again covered with the CFP. The swelling (percent) was calculated as $100 \times \frac{(b-a-1)}{a}$ (a, paw volume before producing the edema; b, paw volume measured hourly after producing the edema) in order to evaluate the effect.

**Analytical Method**—FP was determined by the use of an HPLC system according to the method of Albert et al. with minor modifications. The HPLC apparatus (LC-6A pump, Shimadzu Co., Ltd., Kyoto, Japan; model 7125 injector, Rheodyne Inc., California, U.S.A.) was equipped with a fluorescence spectromonitor (RF-530, Shimadzu Co., Ltd.). The column was a bonded octadecylsilane-silica gel (Fine SIL C18, Japan Spectroscopic Co., Ltd., Tokyo, Japan) of 10 µm particle size (250 × 4.6 mm i.d.) and was used at room temperature. A mixture of MeOH-0.01 M citrate buffer solution (75:25, v/v) was used as the mobile phase at a flow rate of 1.0 ml/min after being passed through a 0.45 µm pore size membrane filter (Toyo Roshi Co., Ltd., Tokyo, Japan). The peak was detected fluorometrically at 250 and 315 nm (excitation and emission, respectively). Standard solutions were chromatographed and calibration lines were constructed on the basis of peak-area measurements.

**Estimation of in Vivo Percutaneous Absorption Behavior of FP in CFP by Deconvolution**—For calculation, plasma FP concentrations after intravenous injection and percutaneous application of CFP were assigned as weight and output functions, respectively. Practically, the theoretical values obtained from Eq. 1 were used as the data for the weight function instead of experimental data. This served to prevent the divergence of solutions and to generate any desired datum corresponding to the other function. Mean plasma concentrations obtained in the *in vivo* transdermal absorption experiment (Fig. 2) were used as the data for the output function. In addition, the solutions were obtained after normalization of the data with respect to dose: 500 µg in intravenous injection and 2000 µg in percutaneous application.

**Analysis of Data with a Microcomputer**—The results of the intravenous administration experiment were analyzed on the basis of biexponential kinetics. The *in vivo* absorption behavior of FP estimated by deconvolution and the experimental data obtained in the release and *in vitro* penetration experiments were analyzed on the basis of single exponential kinetics. These analyses were performed by means of a microcomputer.

**Results**

**In Vivo Transdermal Absorption Experiment**

Figure 2 shows the plasma concentration of FP after application of the CFP on the rat abdomen under conditions A, B, C and D. As the skin temperature was raised, the plasma concentration of FP increased significantly. Under all conditions except for D, the plasma levels increased initially and thereafter a nearly constant level was maintained.

**Release and in Vitro Penetration Experiments**

The release of FP from CFP is illustrated in Fig. 3. The release was very fast under all conditions and the recoveries in 3 h were higher than 70% of the initial content. Figure 4 represents the penetration of FP through an excised rat skin. The penetration of FP was relatively slow and the recoveries were somewhat low in comparison with release from the CFP. The release and penetration were both greatly enhanced by a rise of skin temperature.

**Absorption Behavior Estimated by Deconvolution**

Since it is difficult to measure the transdermal absorption behavior of FP experimentally,
it was estimated by a deconvolution method as described in the experimental section. The time course of absorbed FP thus obtained (% of initial content) is shown in Fig. 5.

The absorption of FP was greatly enhanced by a rise of temperature and it was found that the flux under condition D was about 4 times higher than that under condition A.

**Skin Accumulation**

Figure 6 shows the skin accumulation of FP at 10 h in the *in vivo* and *in vitro* experiments.
A tendency for skin accumulation of FP to decrease with a rise of skin temperature was observed in the in vivo experiment, but not in the in vitro experiment.

**Anti-inflammatory Effect**

Anti-inflammatory effects of CFP at various skin temperatures are shown in Fig. 7. The effect was evaluated in terms of the suppressive effect on the swelling edema in the foot of rats produced by a strong blow. The edema was suppressed significantly under condition C and especially under cooling conditions A and B, but no effect was found under warming conditions.
Figure 8 shows the anti-inflammatory effect of temperature when CFP containing no FP was applied. The application under cooling conditions (A and B) suppressed the swelling significantly.

Discussion

In the present investigation, the in vivo and in vitro transdermal absorption and pharmacological effect of FP in the CFP were examined under various temperature conditions. A rise of skin temperature resulted in an increase of the FP plasma concentration (Fig. 2), as in the case of salicylic acid, carbinoxamine and methyl salicylate. Moreover, the release (Fig. 3) and skin penetration (Fig. 4) of FP were also enhanced with a rise of temperature.

Release data (Fig. 3) were analyzed by the use of Eq. 2:

\[ A = FA_0(1 - e^{-k_r t}) \]  

where \( A \) is the amount of FP appearing in the receptor phase, \( F \) is the available fraction of the applied dose (\( A_0 \)) and \( k_r \) is a release rate constant. On the other hand, penetration data were analyzed by the use of Eq. 3 in which a lag time was taken into consideration, based on the experimental data in Fig. 4:

\[ A = FA_0[1 - e^{-k_p(t - \tau)}] \]  

where \( k_p \) is a penetration rate constant and \( \tau \) is a lag time. Furthermore, the deconvolution data were analyzed by the use of Eq. 2 using \( k_a \) (absorption rate constant) instead of \( k_r \), where \( A \) is the absorbed amount of FP, since the lag times were practically not recognized in Fig. 5.

The values of parameters involved in the equations are presented in Table II. Our findings from Table II may be summarized as follows. (1) With a rise of temperature, all the rate

<table>
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<tr>
<th>Data of</th>
<th>Parameter</th>
<th>Temperature (°C)</th>
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<tr>
<td></td>
<td></td>
<td>A (24.7)</td>
</tr>
<tr>
<td>Release</td>
<td>( k_r ) (h(^{-1}))</td>
<td>0.94</td>
</tr>
<tr>
<td>Experiment(^a)</td>
<td>( F )</td>
<td>0.81</td>
</tr>
<tr>
<td>In vitro</td>
<td>( k_p ) (h(^{-1}))</td>
<td>0.04</td>
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<tr>
<td>penetration</td>
<td>( F )</td>
<td>0.57</td>
</tr>
<tr>
<td>Experiment(^b)</td>
<td>Lag time (h)</td>
<td>1.42</td>
</tr>
<tr>
<td>Deconvolution(^c)</td>
<td>( k_a ) (h(^{-1}))</td>
<td>0.03(^a)</td>
</tr>
<tr>
<td></td>
<td>( F )</td>
<td>0.36</td>
</tr>
</tbody>
</table>

\(^a\) Analyzed on the basis of single exponential kinetics with an available fraction (\( F \)). \(^b\) Analyzed on the basis of single exponential kinetics with an available fraction (\( F \)) and a lag time. \(^c\) This value was obtained by analysis at a fixed \( F \) value of 0.36, the value for conditions B and C; good convergence was usually not obtainable without such fixing.

| Table III. Remaining FP in CFP 24 h after the Start of the Experiment under Temperature Condition C |
| Experiment | Remaining FP (\% of applied dose) | Experiment | Remaining FP (\% of applied dose) |
| In vitro penetration | 13, 19 | In vivo absorption | 53, 57 |
constants ($k_r$, $k_p$, and $k_a$) increased. (2) The value of $k_r$ was considerably larger than that of $k_p$ under all conditions, indicating that skin penetration is the rate-determining step for absorption. (3) The value of $k_a$ is close to that of $k_p$ under all conditions, reflecting a good agreement of the in vivo and in vitro experiments. (4) Lag times recognized in the in vitro experiments become shorter with a rise of temperature, which might be further evidence for increasing ease of transfer of the drug across the skin with increasing temperature. Disappearance of the lag times in the in vivo data (Fig. 5) is probably due to the blood flow in the skin. (5) The available fraction of FP ($F$) in release experiments was in the range of 0.81 to 0.94 and showed a tendency to increase with a rise of temperature. On the other hand, the values of $F$ from the in vitro penetration experiment and deconvolution data were in the ranges of 0.57 to 0.69 and 0.36 to 0.39, respectively, and showed no dependence on temperature. The difference of $F$ between these two cases was confirmed by measurement of the remaining FP in CFP at 24 h after the start of the experiments under condition C (Table III). It is considered that the release of FP from CFP might be limited depending on the experimental method employed.

Blank et al.\textsuperscript{10} studied the rate of percutaneous absorption of the alcohols from MeOH through octanol over a temperature range 5—50°C in vitro and demonstrated that their flux was an exponential function of the temperature. Further, they calculated the activation energies of the alcohols from an Arrhenius plot. In the present investigation, an Arrhenius plot was prepared of the data from the in vitro penetration experiment (Table II), to which one more point at 29°C ($k_p=0.05$ h\(^{-1}\)) was added by performing another series of experiments at that temperature. An approximately straight line was obtained and the apparent activation energy calculated from its slope was 16.7 kcal/mol. Blank et al. reported that the mean values of activation energies of polar and non-polar alcohols were 16.5 and 10.0 kcal/mol, respectively. The activation energy of FP was nearly equal to that of polar alcohols in spite of its possible lipophilicity. FP, with its complex molecular structure, might interact more strongly with the skin, resulting in a larger activation energy.

The skin accumulation of FP was not clearly related with skin temperature in the in vitro experiment (Fig. 6B). A tendency for decreasing accumulation with increasing temperature was, however, recognized in the in vivo experiment (Fig. 6A), and this might be due to enhancement of drug clearance resulting from an increase of blood flow.

A suppressive effect of CFP on the swelling edema in the foot of rats was observed at normal temperature (condition C) and especially on cooling (conditions A and B). However, the effect was not apparent under condition D (Fig. 7) where the plasma levels of FP were the highest. Thus, the skin temperature has a greater influence than the absorbed amount of FP. It is well-known that cooling itself has an anti-inflammatory effect. In fact, CFP containing no FP showed an appreciable effect simply due to the cooling action (Fig. 8), but CFP was more effective when it contained FP, as seen in conditions B and C (compare the corresponding data in Figs. 7 and 8).

In conclusion, it is suggested that the transdermal absorption of FP from CFP increased with a rise of skin surface temperature, and both the concentration of FP absorbed and topical cooling are important factors in the use of CFP for topical inflammation. On the other hand, the increase of the drug absorption that occurs on warming might be advantageous when a systemic effect of FP is required after topical application.

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References

1) R. Brandau and B. H. Lippold, "Dermal and Transdermal Absorption," Wissenschaftliche Verlagsgesellschaft


