In Vitro Release of Tranilast from Oily Gels and Penetration of the Drug into Yucatan Micropig Skin

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For the transdermal delivery of tranilast (TL), a drug used for the treatment of skin diseases such as keloids and hypertrophic scars, its oily gels were prepared; its in vitro release and penetration into Yucatan micropig skin were evaluated. In the gels that consisted of hydrogenated soybean phospholipids (HSL) and octyl isononanoate (IOIN), a fatty-acid ester, the release of TL from the gels was proportional to the drug content, and the extent of TL release up to 6 h from them was approximately 70% of the amount of applied TL. On the other hand, with the gels consisting of HSL and isocetyl isostearate (ICIS), the release of TL from the gels was about half of that from IOIN gels, even at the same drug concentration. When oily gels were used, the TL skin concentration was rapidly increased compared with the level obtained with suspensions. With 0.1% IOIN gel, a high concentration of TL (ca. 160 μg/g) in the dermis was obtained and continued until at least 48 h. These results suggest that oily gels may be useful for the topical application of TL.

Key words tranilast; skin penetration; oily gel; fatty-acid ester; hydrogenated soybean phospholipid; Yucatan micropig skin

Tranilast (TL), N-(3,4-dimethoxyaminomethyl)anthranilic acid, is an antiallergic agent. Although originally developed for the treatment of bronchial asthma,12 it has also been used orally for atopic dermatitis,13 keloids and hypertrophic scars14–16 in recent years. The skin concentration of the drug is more significant than the blood concentration in the treatment of skin diseases such as keloids. When TL was administered orally to rats, its skin concentration was only about 10–15% of its plasma concentration.5 Shigeki et al.27 reported, in a study on iontophoretic delivery, that the transdermal delivery of TL was an effective administration route for selective distribution into restricted skin tissues as compared with oral administration. Consequently, its topical administration is more efficient for treatment of the above-mentioned diseases. However, topical applications of TL are not available commercially. Therefore, we attempted to deliver TL into skin tissue transdermally.

Selection of the vehicle is important in the development of topical formulations such as creams and ointments, because the vehicle plays an important role in the skin permeation of a drug. Vehicles for transdermal delivery can be roughly divided into aqueous and oleaginous bases. In terms of the solubility and stability of TL in the vehicle, oleaginous bases are preferred. Although white petrolatum (WP) and plasti base8 have been used as topical oleaginous bases, they are not adequate as a vehicle because of the low solubility and poor skin permeability of the drugs contained in them.3,4

We reported earlier that hydrogenated soybean phospholipids (HSL) changed liquid paraffin (LP) or fatty-acid esters into an oily gel,10,11 and that the skin permeability of several anti-inflammatory drugs was improved by the use of these oily gels.11,12

In the present study, we selected octyl isononanoate (IOIN) and isocetyl isostearate (ICIS) as fatty-acid esters, and evaluated the in vitro release of TL from the oily gels consisting of HSL and these esters. Furthermore, the skin penetration of TL was examined by use of dorsal skin excised from Yucatan micropigs.14–16

MATERIALS AND METHODS

Materials TL was synthesized by Kissei Pharmaceutical Co., Ltd. (Matsumoto, Japan). HSL (containing more than 80% phospholipids, of which 20% was phosphatidylycholine; Lecinol S-10) and ICIS were obtained from Nikko Chemicals Co., Ltd. (Tokyo, Japan). IOIN was kindly supplied by Kokyu Alcohol Kogyo Co., Ltd. (Chiba, Japan). LP and WP were JP XII grade. All other chemicals were of reagent grade and were used without further purification. Skin samples, excised from Yucatan micropig (female, 5 months of age), were purchased from Charles River Japan, Inc. (Yokohama, Japan) in the frozen state at −80 °C.

Preparation Oily Gel: TL and HSL, whose water content was controlled to 0.7–0.9%, were added to IOIN or ICIS in a flask, capped tightly, and then heated at 95 °C in a water bath with stirring until a homogenous solution was obtained. The solution was packed into metal ointment tubes and cooled to 20 °C in water for 30 min. They were then maintained at 40 °C in an air incubator for 3 d, then stored at room temperature.11

Suspension: TL was added to IOIN or ICIS in a flask and shaken overnight in a water incubator at 37 °C.

WP Ointment: TL was mixed with WP on an ointment plate.

The composition of each formulation used in this study is shown in Table 1.

Determination of Consistency and Solubility The consistency of the gels used in this study and the solubility of TL in LP, IOIN and ICIS were measured as previously described.12

Release Studies The release of TL from a gel was measured in a modified Franz-type diffusion cell apparatus. The effective area available for release was 1.1 cm². The receptor compartment was filled with pH 7.1 isotonic phosphate buffer solution (PBS, ca. 16 ml), which was kept at 37 °C and stirred with a magnetic stirrer at 600 rpm. Approximately 0.1 g of oily gel was spread on a membrane filter (cellulose

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Table 1. Composition of Formulations Used in This Study

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (%)</th>
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<th>Composition (%)</th>
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<tbody>
<tr>
<td></td>
<td>IOIN</td>
<td>WL</td>
<td>ICIS</td>
</tr>
<tr>
<td></td>
<td>Gel</td>
<td>0.1% 0.2% 0.3% 0.4%</td>
<td>Gel 0.1% 0.2%</td>
</tr>
<tr>
<td>TL</td>
<td>0.1 0.2 0.3 0.4</td>
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<td>0.2</td>
</tr>
<tr>
<td>HSL</td>
<td>15.0 15.0 15.0 15.0</td>
<td>15.0 15.0</td>
<td>15.0</td>
</tr>
<tr>
<td>IOIN</td>
<td>84.9 84.8 84.7 84.6</td>
<td>84.9 84.8 99.8</td>
<td>84.9 84.8 99.8</td>
</tr>
<tr>
<td>ICIS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP</td>
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Permeation Studies Skin permeation was measured in a similar cell apparatus as that used for the release test. Prior to the experiment, the skin sample was thawed at room temperature for approximately 30 min. The remaining fatty layers were carefully removed with scissors and a mesh-type flat file, and the skin sample was then cut into sections of ca. 2 cm x 2 cm. The receptor compartment was filled with pH 7.1 isotonic PBS (ca. 7 ml) containing 0.01% kanamycin, which was kept at 37°C and stirred with a magnetic stirrer at 600 rpm. Approximately 0.1 g of preparation was spread on the cornual side of the skin, and the skin was then mounted on the cell. In the case of suspensions, a 0.5 ml aliquot was poured onto the skin after the skin was placed into the apparatus. Each experiment was carried out for 48 h using the same procedure as the release test.

Determination of Skin Concentration The experiments for measuring the skin concentration of TL were conducted in a manner similar to the permeation studies. At 3, 6, 12, 18, 24 and 48 h after application of the formulations, the skin was removed from the diffusion cell apparatus and wiped with LP and ethanol. The epidermis, consisting of the stratum corneum and viable epidermis, was separated from the dermis by a heat separation technique. The separated tissues were then minced with scissors and homogenized with an Omni Homogenizer (Omni International, Inc., Gainesville, VA, U.S.A.) following the addition of methanol. After centrifugation, the supernatant of each was injected into an HPLC apparatus.

Analytical Method Concentrations of TL were determined with an HPLC system (Shimadzu model LC-10A system, Shimadzu Co., Kyoto, Japan) equipped with a spectrophotometric detector (SPD-10A). The HPLC analysis was performed using a mixture of methanol and 0.1% phosphoric acid (75:25) as the mobile phase and a flow rate of 1.0 ml/min on a reversed-phase column (Inertsil ODS-2, 150 mm x 4.6 mm i.d., GL Science, Inc., Tokyo, Japan) with UV detection at 320 nm.

RESULTS

Release Studies When IOIN was used, the TL oily gel could only be obtained at a TL content up to 0.4% because the drug was not completely dissolved in the vehicle at any higher concentration. Similarly, the oily gel could only be obtained up to 0.2% with ICIS. Thus, the release studies of TL gels were carried out in these concentration ranges. Regardless of the drug content, the consistency of IOIN and ICIS gels was ca. 0.8 and ca. 1.6 kg, respectively.

The release profiles of TL from the oily gels consisting of HSL and IOIN (IOIN gel) are shown in Fig. 1. The release of TL from IOIN gels demonstrated linearity versus square root of time, and approximately 70% of the amount of TL applied was released by 6 h at each drug concentration. On the other hand, TL was scarcely released from WP ointment used for comparison with the oily gels (data not shown).

As shown in Fig. 2, the release rate of TL from the IOIN gel increased depending on the TL content, which ranged from 0.1 to 0.4%. In the case of the oily gels containing ICIS as the fatty-acid ester (ICIS gel), TL was released in a manner similar to that from the IOIN gel. However, the release rate of TL was about half that from the IOIN gel at the same TL concentration.

Effect of Storage on the Release of TL TL oily gel was stored at room temperature in order to evaluate its stability as a dosage form. Figure 3 shows changes in the rate of TL release from the oily gel with time. The rate of TL release from 0.1—0.3% IOIN gel did change, whereas that from 0.4% IOIN gel decreased for the first 2 weeks. On the other hand, the rate of TL release from 0.1 and 0.2% ICIS gels did not change for 3 months.
Permeation Studies Profiles of TL permeation from suspensions and gels through Yucatan micropig skin are shown in Fig. 4. When the IOIN suspension was applied, the permeation rate of TL was $0.69 \pm 0.14 \mu g/cm^2/h$. On the other hand, with 0.1% IOIN gel, the permeation rate of TL was enhanced about two-fold ($1.56 \pm 0.12 \mu g/cm^2/h$) in comparison to that of the suspension form, and the lag time of the permeation was reduced. The permeation of TL from ICIS preparations was lower than that from IOIN preparations.

Skin Concentration As shown in Fig. 5, with 0.1% IOIN gel, the concentration of TL in epidermal and dermal tissues rapidly increased compared with their level obtained with the IOIN suspension. The concentration of TL in the epidermis obtained with IOIN gel increased to 2350 $\mu g/g$ at 18 h after application of the gel; however, it then decreased. The TL concentration in the dermis obtained with 0.1% IOIN gel was about two-fold higher than that with the suspension. In the case of ICIS, the TL skin concentration with 0.1% gel was rapidly increased compared with that obtained with the suspension, as occurred with IOIN (Fig. 6). With respect to the TL concentration in the epidermis, for both the gel and the suspension, there was no difference between IOIN and ICIS. In the dermis, however, the TL concentration achieved with the IOIN suspension was higher than that with the ICIS suspension, and the TL concentration with IOIN gel was higher than that with ICIS gel.

DISCUSSION

In a previous study, we selected 20 fatty-acid esters from cosmetic ingredients and reported that several of these esters were more useful than LP as a vehicle for absorption-type
ointments, with respect to drug solubility, permeability and a pleasant feeling after their application. For example, IOIN caused the high skin permeation of indomethacin (IM) and a good feeling after application to the skin, and ICIS was safer than IOIN for the skin, though the drug permeation with it was lower than that with IOIN. On the basis of these results, IOIN and ICIS were selected for use in this study.

The solubility of TL in LP was quite low (20 μg/ml at 37°C), whereas its solubility in IOIN and ICIS was much higher (IOIN, 240 μg/ml; ICIS, 76 μg/ml at 37°C). Further, the TL solubility became even higher when HSL was added, and we were able to obtain oily gels with a TL content of up to 0.4% or 0.2% by using IOIN or ICIS, respectively, with HSL.

In the case of a solution-type ointment, the release rate is proportional to the drug concentration. Since the release of TL from the oily gels was proportional to the drug content in this study also, we considered that the TL dissolved completely. On the other hand, TL was scarcely released from WP ointment because its solubility was low and a large portion of it existed in the crystal form in WP.

The rate of TL release from ICIS gels was about half that from IOIN gels at the same TL concentration. As the consistency of ICIS gels was about two-fold greater than that of IOIN gels, the diffusion of TL in ICIS gels may have been suppressed compared with that in IOIN gels.

With the 0.4% IOIN gel, the release rate of TL decreased for the first 2 weeks. It seems that TL is supersaturated in the oily gel and recrystallized with time, as occurs with IM. In the case of ICIS gel, however, the release rate of TL did not change, even at the concentration of 0.2%, which was the upper limit of solubility in the ICIS gel. It has been reported that drug diffusion may be an important factor in the control of crystallization in viscous systems. As mentioned already, the diffusion rate of TL in ICIS gels is possibly low. Thus, ICIS gels might be a stable supersaturated system compared with IOIN gels.

In general, skin permeation depends on a drug's formulation. Although the data were not shown, TL permeation from WP ointment through the skin could not be determined within 48 h, because it was scarcely dissolved in WP and was not released from the formulation. In contrast, the skin permeation of TL was observed with IOIN and ICIS suspensions, and its permeation rates from gels were higher than those from suspensions for both IOIN and ICIS.

In both epidermal and dermal compartments, the TL concentration increased more rapidly with oily gels than with suspensions. More than 80% of applied TL was found in the skin or in the receptor phase after the application of IOIN gel for 18 h; however, the TL concentration in the epidermis decreased after its application for 18 h. The TL concentration in the epidermis tended to be higher with the gel than with the suspension, and also tended to be higher with IOIN than with ICIS, but there was no significant difference. The TL concentration in the dermis was 1/20th of that in the epidermis, and increased in the order of ICIS suspension<IOIN suspension=ICIS gel<IOIN gel; a difference between IOIN and ICIS was observed. Therefore, the permeation rate of TL seems to be correlated with its concentration in the dermis.

Since keloid is principally a disease of the dermis, the drug concentration in dermal tissue is important in its treatment. Although a relatively high concentration of TL (>1000 μg/g) was observed in the epidermis by use of these preparations, TL concentrations in the dermis were about 1/20th. It has been reported that, depending on its concentration, TL inhibits collagen synthesis by keloid fibroblasts in vitro in the range of 1 to 100 μg/g. Thus, we consider that the TL concentration in the dermis achieved by the use of these formulations is sufficient for the treatment of keloids. Especially, with the 0.1% IOIN gel, a high dermal concentration of TL (ca. 160 μg/g) was obtained and continued for at least 48 h.

In conclusion, oily gels are considered to be superior to suspensions in view of the rapid increase and higher concentration of the drug in dermis, and they are convenient to use. Thus, the topical application of TL oily gels appears useful for the treatment of skin diseases such as keloids and hypertrophic scars.

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REFERENCES AND NOTES

1) This study was presented in part at the 12th Meeting of the Japan Society of Drug Delivery System, Kyoto, July 1996.