Effect of Tranilast Oily Gel on Carrageenin-Induced Granulation in Rats

Naohide Horii, a Makiko Fujii, b Kazuhiko Ikegami, a Den-ichi Momose, a Noriyasu Saito, a and Mitsuo Matsumoto b

Pharmaceutical Research Laboratories, Kissei Pharmaceutical Co., Ltd., a 4365-1 Kashiwabara, Hotaka-machi, Nagano 399-8304, Japan, and Department of Pharmaceutics, Showa College of Pharmaceutical Sciences, b 3–3156 Higashitamazawagakuen, Machida, Tokyo 194–8543, Japan. Received July 8, 1999; accepted September 14, 1999

Tranilast (TL) oily gels consisting of hydrogenated soybean phospholipid and fatty-acid ester were prepared, and the inhibitory effect of the gels on the growth of granulation tissue were evaluated in a carrageenin-induced rat granulation model. By the application of 0.1 and 0.2% TL oily gel, the weight of granulation tissue was significantly reduced to 64 and 55%, respectively, of control value. Furthermore, these gels reduced their respective hydroxyproline content to 64 and 51% of the control. On the other hand, the inhibitory effect of 10% TL ointments, which are clinically used for the treatment of keloids and hypertrophic scars as hospital preparations, was much lower than that of the oily gels. In addition, the application of 0.1 and 0.2% oily gel led to high concentration (0.1% gel, 168±18 µg/g; 0.2% gel, 221±16 µg/g) of TL in the dermis as compared with the 10% TL ointments.

These results suggest that TL oily gels may be a useful topical formulation for the treatment of keloids and hypertrophic scars.

Key words tranilast; oily gel; keloid; hypertrophic scar; granulation tissue

Keloids and hypertrophic scars are clinically intractable diseases that show symptoms such as disfigurement, itching, and pain. 1,2 Although their pathogenesis has not yet been determined, keloids and hypertrophic scars are believed to be conditions characterized by abnormal proliferation of fibroblasts and excessive collagen accumulation during the granulation period in the process of healing of injuries. 3,4 As treatment for keloids and hypertrophic scars, several therapeutic methods such as topical application of steroids, 5 of adhesive zinc tape, 6 and of silicone gels 7,8 have been reported.

Tranilast (TL) is an anti-allergic drug originally used for the treatment of bronchial asthma. Recently, it has also been used orally for the treatment of keloids and hypertrophic scars. 8 9 It seems that topical application of TL is more efficient for treatment of the above-mentioned skin diseases than oral administration, because the skin concentration of the drug is more significant than the blood concentration in the treatment of such diseases. In fact, Murakami et al. 9 have already reported that the topical application of TL solution might be a more effective medication than its oral administration for the treatment of keloids and hypertrophic scars. Furthermore, it has also been reported that iontophoretic transdermal delivery of TL is more beneficial than TL given orally for these kind of lesions. 10 11 However, topical formulations of TL are not yet commercially available, with the exception of ointments 12 15 which are prepared individually in the hospital pharmacy (hospital preparations). Thus, the development of more effective and convenient TL topical formulations has been awaited.

We earlier reported that TL oily gels, which consist of hydrogenated soybean phospholipid (HSL) and 2-ethylhexyl isononanoate (IOIN), lead to a high skin concentration of TL in vitro. 16 Since TL was supersaturated in the oily gels, the drug easily partitioned into the skin. The supersaturated system was stable at a TL content up to 0.3%. We also reported that the enhancing effect of IOIN on the partition of TL into the skin was higher than that of isocetyl isostearate. However, the irritation of IOIN on the skin has not been evaluated.

In the present study, we investigated the effect of TL oily gel on the growth of granulation tissue in the carrageenin-induced rat granulation model, 17 which is a useful model to evaluate the effect of drug on keloids and hypertrophic scars. Moreover, the effect of the oily gel was compared with that of 10% TL ointments clinically used as hospital preparations.

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) was obtained from Nikko Chemicals Co., Ltd. (Tokyo, Japan). IOIN was kindly supplied by Kokyu Alchol Kogyo Co., Ltd. (Chiba, Japan). κ-Carrageenin (Picnin-B 8 ) was purchased from Zushikagaku Laboratory Inc. (Kanagawa, Japan). Liquid paraffin (LP), hydrophilic ointment, and absorptive ointment were JP XIII grade. All other chemicals were of reagent grade and were used without further purification. Mini-osmotic pumps (Alzet 6 , model 2002) were obtained from Alza Corp. (Palo Alto, CA, U.S.A.). Skin samples, excised from Yucatan micropigs (female, 5 months of age), were purchased in the frozen state at −80°C from Charles River Japan Inc. (Yokohama, Japan).

Animals Male Wistar rats (Japan SLC Inc., Shizuoka, Japan) weighing approximately 220g were used. Rats were housed with 12-h light-dark cycle. Food and water were freely available.

Preparation of Topical Formulations Oily Gels: The preparation of oily gels was carried out as described previously. 18 Briefly, TL and HSL (water content, 0.7—0.9%) were added to IOIN in a flask, capped tightly, and heated at 95°C in a water bath with stirring until the solution became homogenous. The solution was packed into metal ointment tubes and cooled to 20°C in a water bath for 30 min. The tubes were then heated at 40°C in an air incubator for 3 d, and stored at room temperature before use.
Ointment 1 (HO): TL was dissolved in 1% sodium bicarbonate aqueous solution, and homogeneously mixed with hydrophilic ointment on an ointment plate.\(^{(13)}\)

Ointment 2 (AO): TL was added to LP, impasted, and homogeneously mixed with absorptive ointment.\(^{(15)}\)

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

**Carrageenin-Induced Granulation Model**\(^{(17)}\) The hair on the dorsal skin of rats was removed with an electric razor. The caudal side of the dorsal skin was then incised under ether anesthesia, and a mini-osmotic pump (length, 3.0 cm; diameter, 0.7 cm; pumping rate, 0.5 μl/h for 14 d) was placed in a subcutaneous pouch with the exit port at the cranial end of the pouch. The incision in the skin was closed by suturing. The rats with an implanted osmotic pump were divided into six groups, each group consisting of five rats, and designated as Groups 1 (0.1% gel), 2 (0.2% gel), 3 (AO), 4 (HO), 5 (0% gel), 6 (control). Each rat was caged individually and equipped with a cuaguent to prevent it from licking TL formulations applied to the skin.

Starting the day after the implantation, Groups 1—4 were treated by application of a respective 0.2 g of TL formulation to the skin over the implanted pump once a day for 14 d.

Group 5 was applied the oily gel without TL (0% gel), and Group 6 was not treated medicinally (control).

After the treatment for 14 d, the rats were sacrificed by the lethal inhalation of ether.

The treated skin was wiped with ethanol and LP or distilled water to remove excess formulations, and excised to determine the skin concentration of TL. Then, the granulation tissues formed around the pump were carefully removed from the pump and weighed.\(^{(19)}\)

**Skin Penetration of TL from Topical Formulations** *In vitro* skin penetration of TL from the topical formulations was determined using Yucatan micropig skin in accordance with a previous report.\(^{(18)}\)

**Analytical Method** TL: To determine the concentration of TL in the granulation tissue and skin excised from rat, we removed these tissues from the animals, minced them with scissors and homogenized them with an Omni Homogenizer\(^{(9)}\) (Omni International, Inc., Gainsville, VA, U.S.A.) following the addition of methanol. After centrifugation, the supernatant of each was injected into an HPLC apparatus (Shimadzu model LC-10A system, Shimadzu Co., Kyoto, Japan) equipped with a spectrophotometric detector (SPD-10A). The HPLC analysis was performed with a mixture of methanol and 0.1% phosphoric acid (75:25) as the mobile phase and at a flow rate of 1.0 ml/min on a reversed-phase column (Inertsil ODS-2\(^{(5)}\), 150 mm×4.6 mm i.d., GL Science, Inc., Tokyo) with UV detection at 320 nm. Although the quantitative determination of TL was performed by HPLC without clean-up procedure, it was assayed with high sensitivity, and the recovery of the drug was about 93%.

Hydroxyproline: For the determination of hydroxyproline content in the granulation tissue, the tissue was treated by the method of Nagatani et al.,\(^{(20)}\) which could determine hydroxyproline with high sensitivity and reproducibility. Concentration of hydroxyproline in sample solutions was determined spectrophotometrically (model UV-240, Shimadzu Co., Kyoto) at two wavelengths, 650 and 560 nm.

**Statistical Analysis** For statistical evaluation of results, the one-way analysis of variance (ANOVA) and Dunnett’s test were used. A p-value smaller than 0.05 was considered as significant.

**RESULTS**

**Effect of Formulations on the Growth of Granulation Tissue** As shown in Fig. 1, significant granulation tissue formation (weight, 438±45 mg) was observed in the rats of the control group. In the group treated with 0% oily gel, the granulation was not suppressed, while the application of 0.1 and 0.2% gels significantly reduced the granulation to 64 and 55%, respectively, of the control value. Although AO also depressed granulation tissue formation, its effect was lower than that of the TL gels. When HO was applied, there was no significant difference from the control in the inhibitory effect on proliferation of granulation tissue.

**Effect on Hydroxyproline Content in Granulation Tissue** The amount of hydroxyproline in granulation tissue was 2.07±0.31 mg in the control (Fig. 2). Application of the 0% gel had no effect on the hydroxyproline content, as in the case of granulation tissue weight, whereas 0.1 and 0.2% gel significantly reduced the hydroxyproline content to a respective 64 and 51% of the control. Although the use of AO and HO tended to decrease the hydroxyproline content in the granulation tissue, no significant difference was observed between these formulations and the control.

**Concentration of TL in Granulation Tissue and Skin from Treated Rats** Table 2 shows the concentration of TL in the granulation tissue and skin after successive treatment
with TL formulations for 14 d. The TL concentration in granulation tissue and skin increased in the order of HO<AO<0.1% gel<0.2% gel. The granulation tissue concentration of TL was about one-half that in the skin for all formulations, and the highest concentration of TL was observed in the skin treated with the 0.2% gel.

**Skin Penetration of TL from Topical Formulations** In the carrageeinen-induced model, the skin concentration of TL was determined using whole skin since it is difficult to separate the dermis from the epidermis in rat skin. The drug concentration in the dermis is important for the treatment of keloids and hypertrophic scars. Therefore, in vitro skin penetration of TL into the dermis from various formulations was examined using excised Yucatan micropig skin.

As shown in Fig. 3, a single application of 0.1 g of oily gel to the skin led to a high concentration of TL (0.1% gel, 1525±102 μg/g; 0.2% gel, 2844±191 μg/g) in the epidermis at 48 h after the application. The concentration of TL in the dermis was remarkably lower than that in the epidermis; however, the concentration was still much higher (0.1% gel, 168±18 μg/g; 0.2% gel, 221±16 μg/g) than that of AO and HO. With the oily gels, the amount of TL penetrating the epidermis and the dermis was respectively ca. 15 and 50% of applied dose, and considerably higher than that (below 0.2%) of AO and HO.

**DISCUSSION**

Since keloids and hypertrophic scars appear to be diseases peculiar to human, relevant animal models reflecting these disease conditions have not yet been reported. There are some reports on a model utilizing human keloid tissues implanted into athymic mice. Unfortunately, we could not obtain human keloid tissues in this study.

The etiology of these lesions is not yet known, but keloids and hypertrophic scars are thought to be conditions in which the granulation period, accompanied with proliferation of fibroblasts and collagen synthesis, is prolonged. Therefore, we have evaluated the pharmacological effect of TL oily gel on these lesions using the carrageeinen-induced granulation model, which displays the pathological change mentioned above.

When TL oily gels were used in the carrageeinen-induced granulation model, the weight and hydroxyproline content of granulation tissue were evidently decreased as compared with those of the control. Especially, with 0.2 g of 0.2% gel (dose: ca. 1.6 mg/kg), they were decreased to 55 and 51%, respectively, of control values. As hydroxyproline is a specific marker of collagen, its decrease indicates the inhibition of collagen synthesis. In a similar model, Suzawa et al. reported that TL (200 mg/kg/d) administered orally for 14 d decreased the weight and hydroxyproline content of granulation tissue to approximately 74 and 67%, respectively, of the control. In addition, the TL concentration in the granulation tissue and skin following topical application of the oily gels was higher than that (granulation tissue, 25.4±1.6 μg/g; skin, 23.7±1.7 μg/g) obtained by oral administration (200 mg/kg/d) in another rat model. Consequently, it appears that the topical application of TL oily gel is more effective than its oral administration.

Waseda reported that the TL concentration in keloid tissue in humans following oral administration (300 mg/d) for 3 and 14 d was 10.44±1.80 and 10.12±2.04 μg/g, respectively. Nishihira et al. indicated that the application of 10% ointment (HO) was effective against symptoms such as pain, itching, and redness in patients with keloids and healed thermal burn scar. However, skin concentration of TL in this case (3.3±0.7 μg/g) was lower than that by oral administration (300 mg/d). In our study also, the effect of AO and HO on the carrageeinen-induced granulation model and skin concentration of TL following their application was less than that of the oily gels. A large amount of TL exists in crystal form in 10% ointments, AO and HO, whereas it is completely dissolved and supersaturated in the oily gel. Thus, skin penetration of the drug from AO and HO may be low. TL skin penetration from AO was slightly higher than that from HO. This phenomenon agrees well with the report that the skin absorp-
tion of indomethacin from an absorptive ointment base was higher than that from a hydrophilic ointment base.\textsuperscript{26} Although it is not clear in detail, we assume that an absorptive ointment, which consists of an aqueous inner phase and an oily outer phase, is preferable for these drugs in comparison with a hydrophilic ointment, because TL is a lipopholic drug as indomethacin.

Isaji et al.\textsuperscript{20} reported that TL inhibited collagen accumulation in carrageenin-induced granulation tissue without affecting the accumulation of non-collagenous proteins. Moreover, it was reported that TL decreased the weight of the keloid tissue in a keloid tissue-implanted model and inhibited the collagen synthesis in keloid fibroblasts at the concentration of 1—100 μg/g.\textsuperscript{29} Since fibroblast proliferation and collagen synthesis are stimulated by chemical mediators released from various inflammatory cells such as mast cells,\textsuperscript{1—4} TL, which suppresses the release of chemical mediators from inflammatory cells,\textsuperscript{20} is considered to inhibit the proliferation of fibroblasts and collagen synthesis.

When 0.1 and 0.2% gels were applied to the model rats, the TL concentration in the granulation tissue was 83.4±7.1 and 144±12 μg/g, respectively. In this model, most of the TL was delivered to the subcutaneous granulation tissue through the full-thickness skin. However, keloid principally occurs in the dermis.\textsuperscript{1,3} The application of the oily gels led to high concentration of TL (0.1% gel, 168±18 μg/g; 0.2% gel, 221±16 μg/g) in the dermis in the skin penetration study. Thus, we expect that TL in oily gel would be effectively delivered to keloid tissue in practice.

In conclusion, it is necessary to ascertain the safety of the oily gels for clinical use. Further, since some problems such as photoactivity of TL remain to be solved, the pharmaceutical development of TL oily gel is difficult. In terms of its effectiveness, however, we anticipate that topical application of TL oily gel might be useful for the treatment of keloids and hypertrophic scars.

Acknowledgments The authors wish to thank Nikko Chemicals Co., Ltd. and Kokyu Alcohol Kogyo Co., Ltd. for kindly supplying samples. They are also grateful to Mr. Kiyoshi Ichikawa for technical assistance.

REFERENCES