Effect of UV-Absorbing Agents on Photodegradation of Tranilast in Oily Gels[1)]

Naohide HORI,*,[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] 3156 Higashi-Tamagawagakuen 3-chome, Machida, Tokyo 194–8543, Japan. Received May 28, 1999; accepted August 24, 1999

Tranilast (TL) oily gels containing UV-absorbing agents (UV absorber) were prepared, and the effect of the agents against photodegradation of TL was investigated. When 0.1% TL oily gel without UV absorber was exposed to light, TL was photochemically decomposed to the extent of 74.1% of its initial content at the end of the first hour. Although there were differences in the preventive effect on photodegradation of TL depending on the UV absorbers employed, 2-(2-benzotriazolyl)-*p*-cresol (BTPC) was the most effective absorber. The addition of UV absorbers to the oily gel did not affect the release of TL from the gel, the skin permeation, or the skin concentration of TL following topical application. UV absorbers added to TL oily gel penetrated into skin; however, their concentration in skin was similar to that following application of commercial sunscreen. These results suggest that the addition of UV absorbers to the oily gel of TL may be useful in preventing photodegradation of TL in the gel.

Key words tranilast; photodegradation; oily gel; UV absorber; skin penetration

In the development of pharmaceutical preparations, not only the release of drug from the dosage form and its absorption but also the stability of the drug is an important factor. Previously, we reported that an oily gel of tranilast (TL), *N*-(*trans*-3,4-dimethoxycinnamoyl)anthranilic acid, may be useful for the treatment of keloids and hypertrophic scars since its application leads to a high skin concentration of TL.[2)] It is well known that cinnamic acid derivatives are photochemically unstable,[3,4)] and TL has a cinnamoyl group in its molecular structure. Although the drug is relatively stable in the solid state, photodegradation such as photoisomerization and photodimerization have been observed in various solutions,[5,6)] as shown in Fig. 1. Kinetic studies on the photoreaction of TL in aqueous solution were reported in detail by Utsuki.[7)] Furthermore, Kojima *et al*.[8)] reported that photodimerization products (N-13, N-14) scarcely inhibited the homologous passive cutaneous anaphylaxis (PCA) reaction in rats and that the inhibitory effect of the *cis*-isomer of TL on PCA was about half that of TL.

Light-resistant packages consisting of various materials such as aluminum foil, colored glass, and film containing an UV-absorbing agent (UV absorber) are used to prevent photodegradation of drugs. In the case of the oily gel of TL, the drug is protected from light during the storage period, since the gel is packed in a metal ointment tube. When the gel is applied to the bare skin surface, however, TL is exposed to the light and photodegraded. It might be expected that the addition of an UV absorber to the gel would solve this problem, but there are few reports concerning such methods.[9)]

For use in the present study, we selected ethyl *p*-aminobenzoate (EPABA), 2-ethylhexyl-*p*-dimethylaminobenzoate (DABAO), 2-hydroxy-4-methoxybenzophenone (HMBP), 2-(2-benzotriazolyl)-*p*-cresol (BTPC) and 1-(4-*tert*-butylphenyl)-3-(4-methoxyphenyl) propane-1,3-dione (P1789) from among UV absorbers usually blended in commercial sunscreen products (sunscreens) for prevention of sunburn and suntan.[10)] TL oily gels containing various UV absorbers were prepared, and their effect on photostability of TL in the gel was tested. Skin penetration of the UV absorbers from the gel was examined with Yucatan micropig skin.

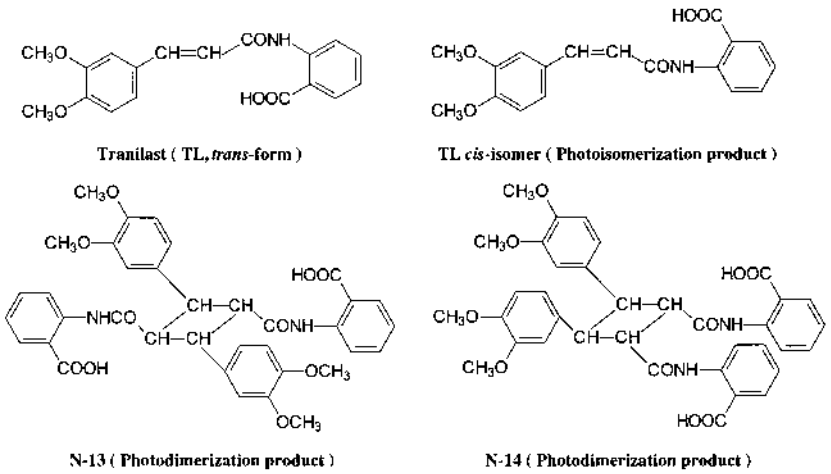

Fig. 1. Chemical Structures of Tranilast and Its Photodegradation Products

∗ To whom correspondence should be addressed.

Materials and Methods

Materials TL was synthesized by Kissei Pharmaceutical Co., Ltd. (Matsumoto, Japan). EPABA, DABAO, HMBP and BTPC were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). P1789 and hydrogenated soybean phospholipid (containing more than 80% phospholipids, of which 20% was phosphatidylcholine; Lecinol S-10) were obtained from Nikko Chemicals Co., Ltd. (Tokyo). 2-Ethylhexyl isononanoate (IOIN) was kindly supplied by Kokyu Alcohol Kogyo Co., Ltd. (Chiba, Japan). All other chemicals were of reagent grade and were used without further purification. ANESSA® 46PA+ (containing 0.11% P1789[11]; Shiseido Co., Ltd., Tokyo) was used as a commercial sunscreen. 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 of Oily Gel The preparation of oily gel (TL, 0.1 or 0.2%; UV absorber, 0 or 3.0%; hydrogenated soybean phospholipid, 15.0%; IOIN, 84.9—81.9%) was carried out according to the previous report.[2] TL, UV absorber and hydrogenated soybean phospholipid, whose water content was controlled to 0.7—0.9%, were added to IOIN 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. The tubes were then maintained at 40 °C in an air incubator for 3 d, after which they were stored at room temperature.

Light Exposure Studies (A) Photodegradation of TL: A 0.2 g of sample gel was spread over a 6.75 cm^2 (thickness: *ca.* 30 μm) area on a glass plate, and placed in a photostability testing chamber (model LT-120, Nagano Science Equipment Mfg. Co., Ltd., Osaka, Japan) equipped with six 20 W white fluorescent lamps (Type FLR20S·W/M, Matsushita Electric Industrial Co., Ltd., Osaka) at 25 °C. The distance between the sample and the light source was 30 cm, and the samples were illuminated at 3000 lx for 8 h. The detection method for light intensity was instrumental. At specified periods, a given sample was taken from the chamber and dissolved in 5 ml of methanol. After centrifugation (3000 rpm, 5 min), the supernatant was injected into an HPLC apparatus for assay of TL and its photodegradation products. In the case of the drug in solution, 1 ml of sample solution was poured into glass petri dishes (internal diameter: 30 mm) and covered with clear wrapping film made of polyvinylidene chloride (Saran Wrap®, Asahi Chemical Industry Co., Ltd, Tokyo). Then, light exposure was carried out by same procedure as used for the gel. At specified times, 0.2 ml of sample was withdrawn from the petri dishes and diluted with 5 ml of methanol. The diluted sample was then directly injected into the HPLC apparatus.

(B) Wavelength Dependency of the Photodegradation of TL: The sample solution was placed in a rectangular quartz cell and set in a grating monochrometer (model CRM-FD, Japan Spectroscopic Co., Ltd., Tokyo) equipped with a 300 W xenon lamp (Type L2479-06, Hamamatsu Photonics K. K., Hamamatsu, Japan). It was irradiated within the range of 213—497 nm; the irradiation energy was 1.5×10^8 erg/cm^2·s. After the irradiation, 0.2 ml of the sample was diluted with 5 ml of methanol, and then assayed by HPLC.

UV Absorption of TL and UV Absorbers UV absorption spectra of TL and UV absorbers were measured with a UV/VIS recording spectrophotometer (model UV-2500PC, Shimadzu Co., Kyoto, Japan).

Release and Permeation Studies The release of TL from the oily gel was measured as reported previously.[2] Skin permeation experiments on TL and UV absorbers were carried out for 30 h by the procedure described earlier.[2]

Determination of Skin Concentration The experiments for measuring the skin concentration of TL and UV absorbers were performed after the permeation studies. At 30 h after application of the gel, the skin was removed from the diffusion cell apparatus and wiped with liquid paraffin and ethanol. The stratum corneum was stripped from the epidermis using cellophane tape. The epidermis was separated from the dermis by a heat separation technique.[12] 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 sample was injected into the HPLC apparatus.

Analytical Method Concentrations of TL, its photodegradation products, and UV absorbers were determined with an HPLC system (Shimadzu model LC-10A system, Shimadzu Co., Kyoto) equipped with a spectrophotometric detector (SPD-10A). Separation was accomplished with a reversed-phase column (Inertsil ODS-2®, 150 mm×4.6 mm i.d., GL Science, Inc., Tokyo). Linear gradient elution was performed as follows: 75 : 25 methanol/0.1% phosphoric acid for 5 min, gradual change to 90 : 10 methanol/0.1% phosphoric acid for a 2 min period, 90 : 10 for 2 min, and then a gradual return over a 2 min period to the initial condition. The flow rate was 0.9 ml/min, and the eluate was detected at 320 nm.

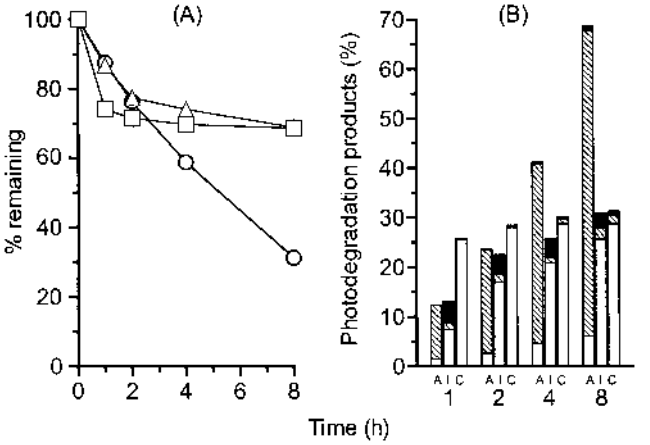

Fig. 2. Residual Content of TL (A) and Composition of Photodegradation Products (B) in Solutions and 0.1% Oily Gel under Irradiation from a Fluorescent Lamp at 3000 lx

(A) Symbols: ○, aqueous solution; △, IOIN solution; □, control gel. (B) Column and abbreviations: □, *cis*-isomer; ▨, N-13; ■, N-14; A, aqueous solution; I, IOIN solution; C, control gel.

Results

Photostability of TL In addition to the oily gel of TL, its aqueous and IOIN solutions were prepared at the concentration of 10 μg/ml to investigate the differences in the photostability of TL between the formulations. Since the solubility of TL in water is quite low, TL was dissolved in pH 7.1 isotonic phosphate buffer solution to obtain its aqueous solution. The photostability of TL in aqueous solution, IOIN solution, and 0.1% oily gel are shown in Fig. 2A. When aqueous solution was exposed to light, TL was extensively photodecomposed and reduced to 31.3% of its initial content within 8 h. With IOIN solution, although TL was degraded at the same rate as in the aqueous solution up to the first 2 h, its subsequent photodegradation proceeded slowly, and it was reduced to 68.9% of its initial content by 8 h. In the case of 0.1% TL oily gel, TL was also photodecomposed and reduced to 74.1% of its initial content after the first hour, then gradually decreased to 68.7% of its initial value by 8 h.

As shown in Fig. 2B, TL was mostly photodimerized by 8 h (*cis*-isomer, 6.2%; N-13, 61.8%; N-14, 0.7%) in aqueous solution. In contrast, it was mainly decomposed to *cis*-isomer in IOIN solution (*cis*-isomer, 25.7%; N-13, 2.3%; N-14, 3.1%) and in the oily gel (*cis*-isomer, 28.8%; N-13, 1.6%; N-14, 0.9%) by 8 h.

The effects of the various UV absorbers on the photostability of TL in the oily gel are shown in Fig. 3. As abovementioned, when 0.1% oily gel without UV absorber (control) was photoirradiated, TL was principally photoisomerized for the first 1 h, and then the level of photodimerization products increased depending on the irradiation time. The addition of EPABA or DABAO had no effect on photodegradation of TL in the oily gel. On the other hand, by the addition of 3% HMBP, P1789, or BTPC, the photodegradation of TL was depressed; and residual TL contents after 8 h of exposure increased to 76.9, 86.3, and 88.2%, respectively.

UV Absorption of TL and UV Absorber UV absorption spectra of TL and UV absorbers were measured to ex-

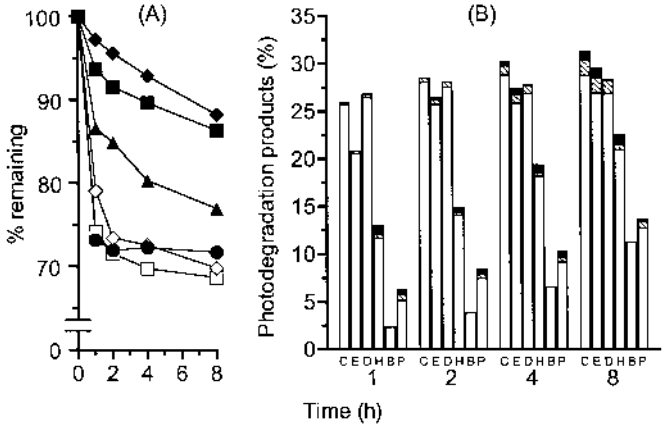

Fig. 3. Effect of UV Absorbers on Residual Content of TL (A) and on Composition of Photodegradation Products (B) in 0.1% Oily Gels under Irradiation from a Fluorescent Lamp at 3000 lx

(A) Symbols: □, control (without UV absorber); ◇, 3% EPABA; ●, 3% DABAO; ▲, 3% HMBP; ◆, 3% BTPC; ■, 3% P1789. (B) Column and abbreviations: □, *cis*-isomer; ▨, N-13; ■, N-14; C, control (without UV absorber); E, 3% EPABA; D, 3% DABAO; H, 3% HMBP; B, 3% BTPC; P, 3% P1789.

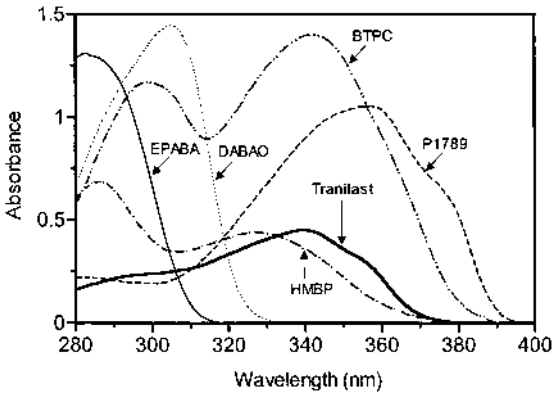

Fig. 4. UV Absorption Spectra of TL and UV Absorbers Used in This Study

Concentration of TL and UV absorbers in IOIN: 10 μg/ml of each.

amine the relationship between UV absorption of UV absorber and its effect on TL photodegradation. Since it was difficult to use the gel formulation, the measurement of UV absorption spectra was performed in IOIN (main component of the oily gel) solution at the concentration of 10 μg/ml. As shown in Fig. 4, EPABA (λ_{max}: 282 nm) and DABAO (λ_{max}: 304 nm) scarcely absorbed UV light beyond wavelengths of 330 nm. In contrast, HMBP (λ_{max}: 289, 326 nm), BTPC (λ_{max}: 297, 342 nm) and P1789 (λ_{max}: 282, 356 nm) well absorbed UV light of wavelength around 339 nm, which is the absorption maximum of TL.

Wavelength Dependency of the Photodegradation of TL In order to clarify the contribution of the wavelength of UV light to the photodegradation of TL, we investigated the effect of wavelength on the photodegradation. The experiment was carried out with TL (10 μg/ml) in IOIN solution for the same reason that the IOIN solution was used for the measurement of UV spectra. The result showed that TL was most photodegraded at the wavelengths of 260—360 nm (Fig. 5).

Skin Concentration of UV Absorbers Skin penetration of UV absorbers following dermal application of 0.1% TL

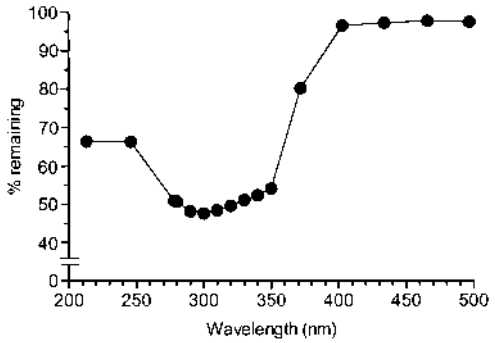

Fig. 5. Effect of Wavelength on Photostability of TL in IOIN Solution

Irradiation energy: 1.5×10^8 erg/cm^2·s.

Table 1. Skin Concentration of UV Absorbers at 30 h after Application of Various Preparations

Preparation	UV absorber	Skin concentration[a] (μg/g)		
		Stratum corneum ($\times10^3$)	Epidermis	Dermis
0.1% TL gel+3% BTPC	BTPC	301±42	111±7	31±1
0.1% TL gel+3% P1789	P1789	337±47	415±101	81±14
Commercial sunscreen (P1789 content: 0.11%)	P1789	169±37	296±65	43±9

a) Each value represents the mean±S.D. of three experiments.

gel containing 3% BTPC or P1789 was evaluated using excised Yucatan micropig skin. Both BTPC and P1789 penetrated into the skin, and were observed in the stratum corneum in extremely high concentrations (*ca*. 300 mg/g) at 30 h after application of the gel (Table 1). However, their concentration decreased in the order of stratum corneum> epidermis>dermis, and the concentration of BTPC and P1789 in the dermis was 31±1 and 81±14 μg/g, respectively, at 30 h after the application.

For comparison, skin penetration of P1789 contained in an amount of 0.11% in a commercial sunscreen product was examined, and the skin concentration was relatively the same as that obtained with the oily gel.

Discussion

TL in oily gel applied when the skin is exposed for several hours to sunlight or to the light in a room (room-light) such as that from a fluorescent lamp becomes unstable, though it is stable during storage in the tube. It is possible to avoid sunlight in daily life, whereas it is difficult to avoid room-light. Therefore, we examined the effect of several UV absorbers on photodegradation of TL in the oily gel due to exposure to light from a white fluorescent lamp for 8 h.

When an aqueous solution of TL was photoirradiated, the main photodegradation product of TL was its photodimers. In contrast, TL in IOIN solution or oily gel was mainly photoisomerized. The initial rate of photodegradation of TL in the oily gel was faster than that of IOIN solution. Although the concentration of TL in the gel was much higher than that of IOIN solution, the rate of photodegradation of TL in IOIN solutions was independent of the initial concentration of TL in the range of 1 to 100 μg/ml (data were not shown). Similar observation was reported by Utsuki[7] in his study on the pho-

todegradation of TL in aqueous solution. Furthermore, there was no difference between 0.1 and 0.2% gel with respect to the rate of photodegradation of TL (data not shown). Thus, it appears that the photodegradation of TL is different depending upon the formulations.

In the oily gel, the *cis*-isomer of TL was generated during the first 1 h, and the level did not increase much thereafter. For this reason, we believe that the *cis*/*trans* photoisomerization of TL came into equilibrium (*cis*/*trans*=3/7) by that time, as already reported.[5,7)]

TL in IOIN solution and oily gel was more stable than that in aqueous solution. Nevertheless, 30% of the TL in the oily gel was photodecomposed by light exposure at 3000 lx for 8 h. Thus, it is preferable to prevent photodegradation of TL in the gel. In general, the maximum authorized concentration of UV absorbers used in this study is approximately less than 5%, and below 10% at most.[13)] Fortunately, all of them were completely soluble in TL oily gel at the concentration of 3%. Consequently, the effect of these UV absorbers on photodegradation of TL was evaluated at 3%.

TL well absorbs a wavelength of 280—360 nm, and it was photodegraded most when exposed to light at wavelengths in this region. EPABA and DABAO did not reduce photodegradation of TL despite the fact that they absorb wavelengths of around 300 nm. On the other hand, BTPC and P1789, which well absorb a wavelength around 339 nm, prevented the photodegradation of TL. As the radiation energy of a fluorescent lamp at around 300 nm is weak,[14)] we consider that the wavelength of around 300 nm did not contribute to the photodegradation of TL in this study.

On the basis of these results, we examined the release and skin permeation of TL from oily gel containing 3% BTPC or P1789. Although the data are not shown, the addition of these absorbers to the TL oily gel did not affect TL release from the gel, skin permeation, or skin concentration following topical application.

UV absorbers might permeate the skin following topical application of formulations containing them, and it is presumed that their skin permeation would be largely affected by the vehicle formulation. However, there is little published data describing their penetration into the skin.[15—17)] The skin concentration of BTPC and P1789 following application of the oily gel containing them was similar to that obtained for the commercial sunscreen containing 0.11% of P1789. Thus, we assume that the addition of these UV absorbers to the gel is relatively safe.

With light-resistant packaging, a drug is certainly protected from light exposure. It is difficult to completely prevent photodegradation of TL, because the surface of the spread gel is exposed to light. As described already, although photodimerization products of TL have no pharmacological effect, its *cis*-isomer has about half the effect of TL against PCA reaction. By the addition of 3% BTPC, photodimerization of TL was prevented, and its photoisomerization was relatively reduced as compared with that obtained without UV absorbers.

In conclusion, we believe that the addition of UV absorbers to TL in an oily gel may be useful for the reduction of TL photodegradation.

Acknowledgments The authors wish to thank Nikko Chemicals Co., Ltd. and Kokyu Alcohol Kogyo Co., Ltd. for kindly supplying samples and information on the commercial sunscreens.

References and Notes

1) This study was presented in part at the 13th Meeting of the Japan Society of Drug Delivery System, Sapporo, July 1997.
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