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Application of Calcium Thioglycolate to Improve Transdermal Delivery of Theophylline in Rats

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To improve drug penetration through the skin, we studied the effect of calcium thioglycolate (Ca-TGA) on the percutaneous absorption of theophylline in comparison with the promoting effects of dimethylsulfoxide (DMSO) and polyoxyethylenelaurylether (BL-9EX). Plasma theophylline concentrations were determined following dermal application of aminophylline to male rats *in vivo*. The use of aqueous 4 w/v% Ca-TGA gave a plasma concentration about 40 times as high as that of the control, whereas 80 v/v% DMSO and 20 w/v% BL-9EX gave concentrations about 9 times as high as that of the control at most. To obtain a marked promoting effect with Ca-TGA, a pretreatment time of more than 10 min was needed. When Ca-TGA was removed, the promoting effect gradually disappeared and the control value was reached at 48 h. Furthermore, when pretreatments with Ca-TGA and BL-9EX were combined, the transdermal delivery of theophylline was promoted more than by each treatment alone.

Keywords——transdermal delivery system; dimethylsulfoxide; absorption promoter; calcium thioglycolate; theophylline; aminophylline; systemic effect

Recently, many pharmacokinetic studies on the dermal delivery of drugs have been carried out and the concept of the transdermal delivery system was proposed.[1-3] In the field of dermatology, topical dosage forms are generally used for the cure of skin diseases. However, new topical dosage forms can be applied to elicit systemic effects of drugs such as the parasympatholytic effect of scopolamine for motion sickness,[4,5] and nitroglycerin delivery for angina pectoris.[6,7] The advantage of these dosage forms is to minimize the side effects of the drugs.

However, it is well known that the skin itself has a barrier function to prevent the entrance of the majority of drugs.[8] Therefore, maximizing drug penetration into the skin has been a key area of pharmaceutical research for topically applied drugs. The hope is that some agent would be found to safely reduce or abolish the barrier function of the skin. Although there are numerous substances that have been shown to act on the skin to increase its permeability, their effects were not remarkable, with the exception of dimethylsulfoxide (DMSO).[9,10]

The pharmacological mechanism and pharmacokinetics of theophylline have been studied by many investigators[11,12] and it has been shown that the therapeutic plasma level of theophylline ranges from 10 to 20 μg/ml.[13] However, conventional oral dosage form and injection can produce side effects. Thus, a new parenteral dosage form having less side effects that the above dosage forms is desirable.

In the present study, in order to achieve a blood concentration of theophylline above the minimum therapeutic level, the possible utility of calcium thioglycolate (Ca-TGA) was examined as an absorption promoter for the topical administration of aminophylline

(theophylline ethylenediamine). Ca–TGA has been widely used as a depilatory[14] and has been applied to the skin at concentrations ranging from 2 to 10%. The mechanism of action of this material has been considered to involve the reduction of cystine linkages, thus weakening the keratin structure, but its effect on the skin permeability of drugs has not been investigated. A fundamental study on the mechanism of action in male rats is reported here.

Experimental

Materials——Aminophylline, theophylline and β-hydroxyethyltheophylline were obtained from Sigma Co., Ltd. Ca–TGA was obtained from Tokyo Chemical Industry Co., Ltd. Polyoxyethylenelaurylether (BL-9EX) was supplied by Nikko Chemicals Co., Ltd. All other chemicals were reagent-grade products obtained commercially.

Preparation of Test Solution——A 1 w/v% aminophylline solution and Ca–TGA (1, 2 and 4 w/v%) solutions were prepared by dissolving the compounds in distilled water. DMSO was diluted to 80 v/v% by the addition of distilled water. BL-9EX solutions (5, 10 and 20 w/v%) were prepared by dissolving BL-9EX in 1 w/v% aminophylline solution.

Analytical Method——A 15 ml centrifuge tube containing 200 μl of rat plasma, 20 μl of acetic acid (99.0%) and 5 ml of chloroform was placed on a reciprocating shaker for 20 min. The aqueous and organic phases were separated by centrifugation (1500 rpm, 10 min). Then, 3 ml of the organic phase was removed with a Pasteur pipette and placed in a 15 ml centrifuge tube. The organic phase was evaporated at room temperature under a vacuum. The residue was dissolved in 100 μl of methanol containing β-hydroxyethyltheophylline (6.8 μg/ml) as an internal standard, and 20 μl of the resulting mixture was injected into a chromatograph. Analysis was performed using a Shimadzu model LC-3A pump (Kyoto, Japan) and Model SPD-2A ultraviolet (UV) absorbance detector. The column packing material consisted of porous silica having an average particle size of 5 microns and a chemically bonded, monomolecular layer of octadecyltrichlorosilane (Cosmosil® $5C_{18}$, Nakarai Chemical Co., Ltd., Kyoto, Japan). The mobile phase, acetonitrile–distilled water (7:93), was prepared fresh daily. The flow rate was 2.0 ml/min and the pressure was approximately 80 kg/cm^2. Detection was achieved by UV absorption measurement at 273 nm. The detector signal was processed and recorded using a Shimadzu Model C-R1A reporting integrator.

Procedure for Animal Experiment——Male Wistar rats weighing between 180 and 200 g were used. The hair of the abdominal parts was carefully removed with electric clippers and an electric razor without breaking the skin one day before the experiment. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital, 32 mg/kg of body weight. A glass chamber (inside diameter = 3.2 cm) was fixed on the surface of the hairless abdomen with surgical tissue cement (Aron Alpha®, Toa Gosei Chemical Co., Ltd., Tokyo, Japan). The body temperature of rats was maintained at 37 °C by heating with a lamp over the animals.

1) Percutaneous Absorption of Aminophylline (Control Experiment): After 10 ml of 1 w/v% aminophylline solution had been placed in the glass chamber, 0.5 ml blood samples were collected at 1, 2, 3, 4 and 5 h through polyethylene tubing cannulated into the carotid artery. The blood samples were centrifuged at 12000 rpm for 2 min (Eppendorf micro centrifuge, Model MC-15A). The resulting plasma was used for theophylline content measurement by the high-performance liquid chromatography (HPLC) method.

2) Effect of BL-9EX Addition on Percutaneous Absorption: Ten ml of 1 w/v% aminophylline solution containing BL-9EX (5, 10 or 20 w/v%) was placed in the glass chamber and blood samples were collected for 5 h as described for the control experiment.

3) Effect of Pretreatment with 80 v/v% DMSO on Percutaneous Absorption: Ten ml of 80 v/v% DMSO solution was placed in the glass chamber. After 30 min, DMSO solution was removed and the inside of the glass chamber was washed several times with distilled water. Then 10 ml of 1 w/v% aminophylline solution was placed in the glass chamber and blood samples were collected for 4 h as described for the control experiment.

4) Effect of Pretreatment with Ca–TGA on Percutaneous Absorption: Ten ml of Ca–TGA (1, 2 or 4 w/v%) solution was placed in the glass chamber. At the end of the pretreatment with Ca–TGA (5, 10, 15 or 30 min), Ca–TGA (1, 2 or 4 w/v%) solution was removed and the inside of the glass chamber was washed several times with distilled water. Then 10 ml of 1 w/v% aminophylline solution was placed in the glass chamber and blood samples were collected for 5 h as described for the control experiment.

5) Effect of Both Pretreatment with Ca–TGA and the Presence of BL-9EX on Percutaneous Absorption: Ten ml 4 w/v% Ca–TGA solution was placed in the glass chamber and pretreatment was carried out for 30 min. The 4 w/v% Ca–TGA solution was removed and the inside of the glass chamber was washed several times with distilled water. Then 10 ml of 1 w/v% aminophylline solution containing 5 w/v% of BL-9EX was placed in the glass chamber and blood samples were collected for 5 h as described for the control experiment.

6) Duration of the Absorption Promoting Effect of Ca–TGA: Ten ml of 4 w/v% Ca–TGA solution was placed in the glass chamber and pretreatment was carried out for 10 min. Then 10 ml of 1 w/v% aminophylline solution was placed in the glass chamber at 0, 2, 4, 6, 12, 24 or 48 h after the end of the pretreatment and blood samples were

collected at appropriate times after administration of aminophylline.

Results

Effects of BL-9EX and DMSO on Percutaneous Absorption of Theophylline

As a control experiment, 10 ml of 1 w/v% aminophylline solution was placed in the glass chamber on the rat abdomen. The plasma theophylline concentration–time curves obtained are shown in Fig. 1, which indicates that plasma theophylline concentration at 5 h after the percutaneous dose of aminophylline was less than 1 μg/ml. When BL-9EX was added to the glass chamber at various concentrations (5, 10 and 20 w/v%), the plasma theophylline concentrations at 5 h after the dermal administration were increased 4.0, 8.0 and 8.0 times at 5, 10 and 20 w/v% of BL-9EX, respectively, as compared to the control. There was no significant difference between 10 and 20 w/v% of BL-9EX. Therefore, the promoting effect of BL-9EX was suggested to reach a plateau at 10 w/v% concentration.

DMSO has been reported to be one of the most effective absorption promoters for topically applied drugs. Therefore, we investigated the effect of pretreatment with 80 v/v% DMSO, the concentration found to be most effective.[15] Thus, 10 ml of 80 v/v% DMSO solution was used in pretreatment for 30 min, and then 1 w/v% aminophylline solution was placed in the glass chamber. Figure 2 shows the plasma theophylline concentration–time curve after pretreatment with DMSO solution. The plasma theophylline concentration rose rapidly after dermal dosing of aminophylline, and the plasma theophylline concentration at 4 h after the dosing was 8.5 times higher than the control.

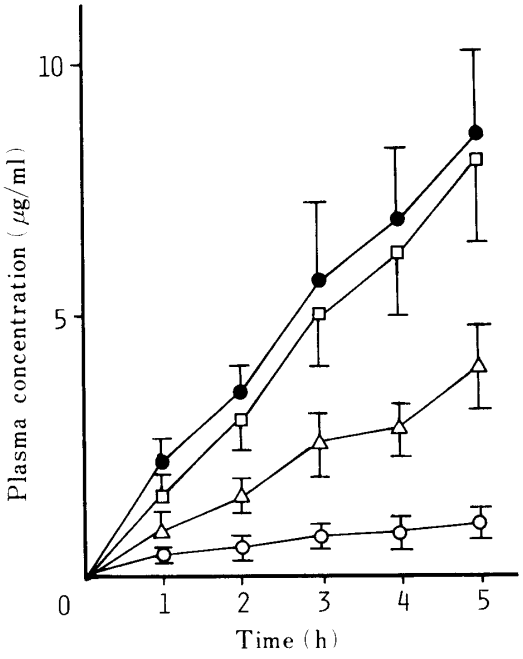

Fig. 1. Effect of BL-9EX on Plasma Theophylline Concentration–Time Curves Following Percutaneous Administration of Aminophylline in Rats

Each point represents the mean ± S.E. ($n=5$ or 6).
○: control.
△: 5 w/v% BL-9EX added to the chamber.
□: 10 w/v% BL-9EX added to the chamber.
●: 20 w/v% BL-9EX added to the chamber.

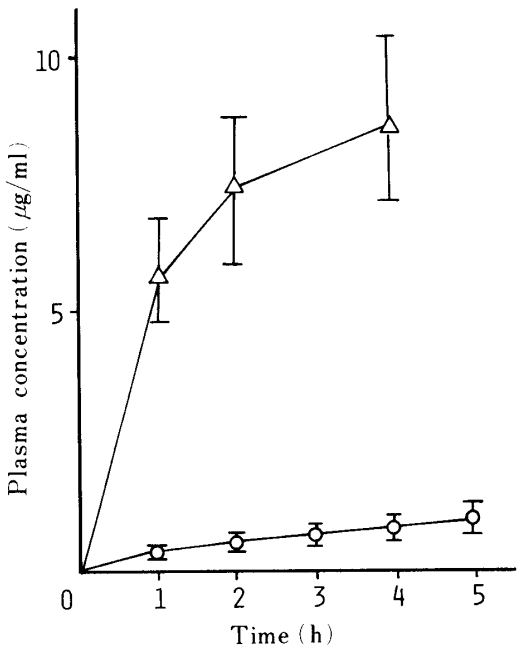

Fig. 2. Effect of Pretreatment with DMSO on Plasma Theophylline Concentration–Time Curves Following Percutaneous Administration of Aminophylline in Rats

Each point represents the mean ± S.E. ($n=5$).
○: control.
△: pretreatment with 80 v/v% DMSO for 30 min.

Effect of Ca–TGA on Percutaneous Absorption of Theophylline

Ca–TGA was chosen as a new absorption promoter for the percutaneous absorption of theophylline. The rat skin was also pretreated for 30 min with appropriate concentrations of Ca–TGA solution. Figure 3 shows the plasma theophylline concentration–time curves after pretreatment with Ca–TGA. When lower concentrations of Ca–TGA solution (1 and 2 w/v%) were used, plasma concentrations were increased by 1.7 and 3.9 times, respectively, as compared to the control. When the concentration of Ca–TGA solution was increased to 4 w/v%, however, the increase of plasma theophylline concentration at 5 h was about 40 times as compared with the control. This excellent result with Ca–TGA is considered to be due to the promoting effect of this agent on the skin. In the case of BL-9EX or DMSO, the plasma theophylline concentrations at 5 h after dermal administration of aminophylline were less than 10 μg/ml. The above results suggest that the promoting effect of Ca–TGA was greater than that of DMSO or BL-9EX.

Figure 4 shows the effect of pretreatment time with 4 w/v% of Ca–TGA on the percutaneous absorption of theophylline. When the pretreatment time was 5 min, plasma theophylline concentration was considerably increased over the control. In the case of longer periods of pretreatment (10 and 15 min), however, plasma theophylline concentrations were remarkably increased by 28.2 and 30.4 times, respectively, as compared to the control. No significant difference was detected in the absorption promoting effects of Ca–TGA at the pretreatment times of 10, 15 and 30 min.

Combination Effect of Ca–TGA and BL-9EX on Percutaneous Absorption of Theophylline

The above results suggest that BL-9EX, DMSO and Ca–TGA all have promoting effects

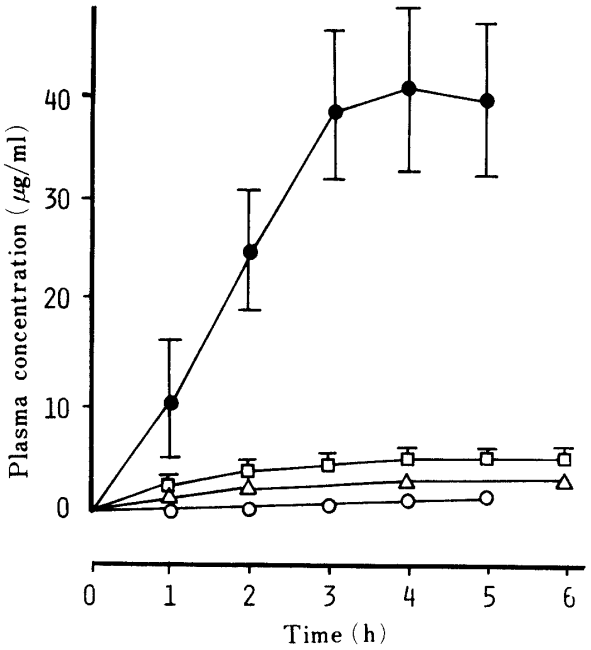

Fig. 3. Effect of Pretreatment with Ca–TGA on Plasma Theophylline Concentration–Time Curves Following Percutaneous Administration of Aminophylline in Rats

Each point represents the mean ± S.E. (n = 5 to 10).
○: control.
△: pretreatment with 1 w/v% Ca–TGA for 30 min.
□: pretreatment with 2 w/v% Ca–TGA for 30 min.
●: pretreatment with 4 w/v% Ca–TGA for 30 min.

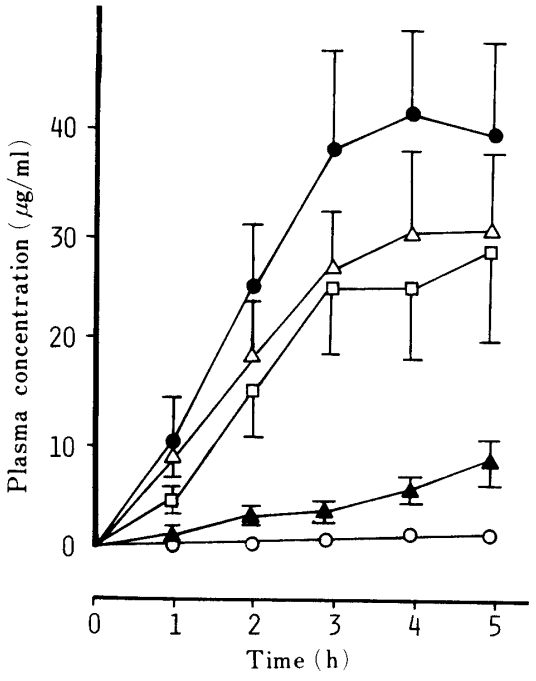

Fig. 4. Effect of the Duration of Pretreatment on Plasma Theophylline Concentration–Time Curves Following Percutaneous Administration of Aminophylline in Rats

Each point represents the mean ± S.E. (n = 5 to 9).
○: control.
▲: pretreatment with 4 w/v% Ca–TGA for 5 min.
□: pretreatment with 4 w/v% Ca–TGA for 10 min.
△: pretreatment with 4 w/v% Ca–TGA for 15 min.
●: pretreatment with 4 w/v% Ca–TGA for 30 min.

on percutaneous absorption of theophylline. Thus, the combined effect of Ca–TGA and BL-9EX was examined (Fig. 5). When the administration of 5 w/v% BL-9EX was carried out after pretreatment with 1 w/v% Ca–TGA, plasma theophylline concentration was increased from 4 h after dosing. Furthermore, in the case of pretreatment with 4 w/v% Ca–TGA followed by 5 w/v% BL-9EX administration, the plasma theophylline level was most increased, and this increase appeared from 1 h after dosing.

Duration of Promoting Effect of Ca–TGA

Figure 6 shows the result of an experiment to examine the duration of the promoting effect of Ca–TGA. The interval between the end of pretreatment and drug administration was varied from 0 to 48 h. With a 2 h interval, the plasma theophylline concentration was already reduced to a large extent as compared to that at 0 h interval. There was no significant

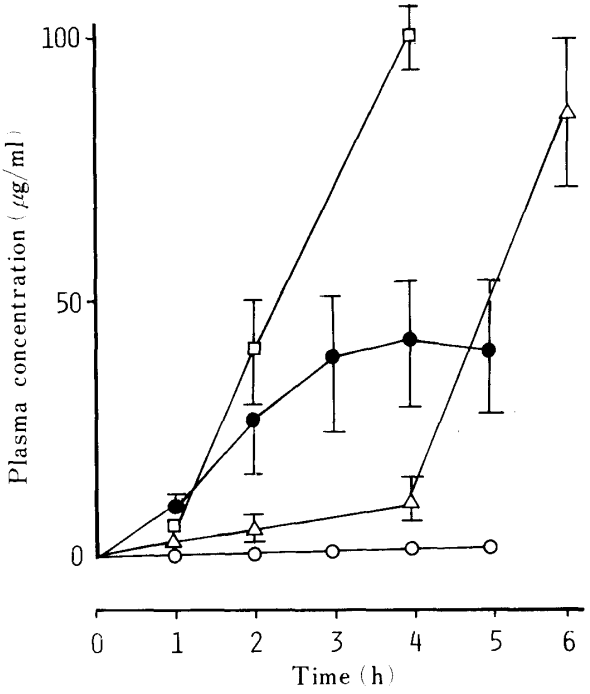

Fig. 5. Effects of Ca–TGA and BL-9EX on Plasma Theophylline Concentration–Time Curves

Each point represents the mean ± S.E. (n=4 to 6).
○: control.
△: pretreatment with 1 w/v% Ca–TGA for 30 min and 5 w/v% BL-9EX added to the chamber.
●: pretreatment with 4 w/v% Ca–TGA for 30 min.
□: pretreatment with 4 w/v% Ca–TGA for 30 min and 5 w/v% BL-9EX added to the chamber.

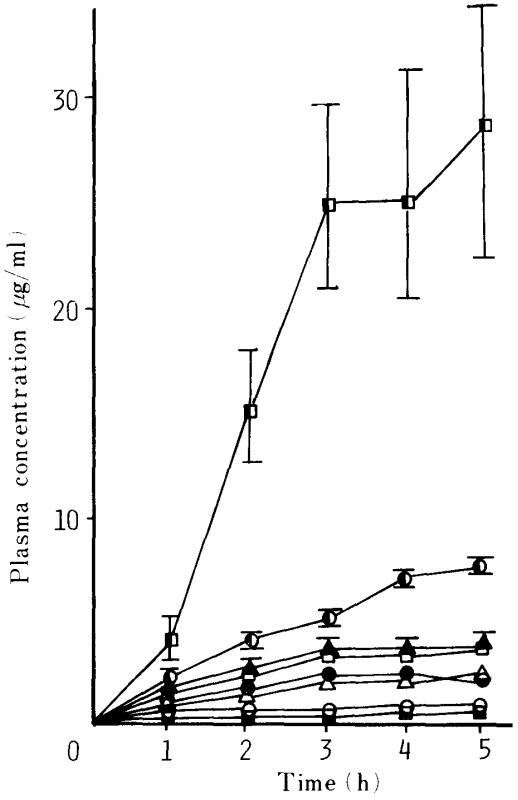

Fig. 6. Effect of Interval after Pretreatment with Ca–TGA on Plasma Theophylline Concentration–Time Curves

Each point represents the mean ± S.E. (n=4 to 6). Each point without a bar had a coefficient variation of less than 10%.
○: control.
◧: aminophylline was administered immediately after pretreatment with 4 w/v% Ca–TGA for 10 min.
◐: aminophylline was administered at 2 h after pretreatment with 4 w/v% Ca–TGA for 10 min.
▲: aminophylline was administered at 4 h after pretreatment with 4 w/v% Ca–TGA for 10 min.
●: aminophylline was administered at 6 h after pretreatment with 4 w/v% Ca–TGA for 10 min.
△: aminophylline was administered at 12 h after pretreatment with 4 w/v% Ca–TGA for 10 min.
□: aminophylline was administered at 24 h after pretreatment with 4 w/v% Ca–TGA for 10 min.
■: aminophylline was administered at 48 h after pretreatment with 4 w/v% Ca–TGA for 10 min.

difference among plasma concentrations obtained with intervals of 4 h to 48 h and the control. These results suggest that the remarkable promoting effect of Ca–TGA on the skin permeability is not sustained for a long period.

Discussion

There are numerous reports describing specific vehicle effects on the permeability of the skin. The ultimate aim of such research is to find a safe, nontoxic substance that can be used to temporarily abolish the barrier function of the skin so that the penetration of topically applied drugs can be enhanced significantly without causing skin damage or irritation.

DMSO is the most widely studied absorption promoter.[16] It has been shown in numerous *in vitro* and *in vivo* experiments to enhance the penetration of many drugs. However, an adverse effect of DMSO, a lens change, was observed primarily in dogs, following topical and oral administration of DMSO. The use of this substance has been limited in humans to the treatment of interstitial cystitis by intravesicular instillation. Some surface-active agents can also accelerate and increase transdermal permeation,[17] but their activity is not great.

We attempted to study Ca–TGA as a new absorption promoter; it has been used on human skin at concentrations from 2 to 10 w/v%, and skin irritation is within tolerable limits.[14] Theophylline was also chosen as a drug with rather poor absorption through the skin,[18] so that an improved topical delivery system is desirable.[19] The promoting effect of Ca–TGA on the dermal absorption of theophylline was greatest at 4 w/v% concentration (Fig. 3). The degree of promoting effect by this agent was much larger than that of DMSO or BL-9EX (a nonionic surface-active agent). However, from the pretreatment time experiment (Fig. 4), it is clear that more than 10 min is required to obtain this remarkable effect.

On the other hand, when Ca–TGA was removed from the skin, the promoting effect fell quite rapidly to the control level (Fig. 6). This rapid disappearance is of the viewpoint of safety in clinical therapy.

Furthermore, it is interesting that when pretreatment was carried out with Ca–TGA in conjunction with BL-9EX, the dermal absorption of theophylline was accelerated more than by each treatment alone (Fig. 5). This combination method should be suitable for very greatly enhancing the skin permeability. The plasma theophylline level was increased to above the systemic toxic level under such conditions. If such a system were to be used practically, it would be necessary to use a formulation design involving controlled release and to use a reduced dose.

The mechanism of action of Ca–TGA is considered to involve reduction of cystine linkages in the hair cortex by the thioglycolate ion, thus weakening the keratin structure.[14] Most of the attack occurs at the hair root, and therefore, the mechanism of the promoting effect of Ca–TGA might be related to weakening of the keratin structure at this point on the skin. On the other hand, the promoting action of a surface-active agent such as BL-9EX is assumed to be due to improved wetting of the skin, enhancing the distribution of drugs. It is, therefore, likely that the marked combined effect of Ca–TGA and BL-9EX involves two different mechanisms of action. Further studies on the mechanisms of action are required, and are in progress in our laboratory.

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