In vitro Studies of Mucoadhesiveness and Release of Nicotinamide Oral Gels Prepared from Bioadhesive Polymers

Sarunyoo Songkro, Naranut Rajatasereekul, and Nipapat Cheewasrirungrueng

Abstract—The aim of the present study was to evaluate the mucoadhesiveness and the release of nicotinamide gel formulations using in vitro methods. An agar plate technique was used to investigate the adhesiveness of the gels whereas a diffusion apparatus was employed to determine the release of nicotinamide from the gels. In this respect, 10% w/w nicotinamide gels containing bioadhesive polymers: Carbopol 934P (0.5-2% w/w), hydroxypropylmethyl cellulose (HPMC) (4-10% w/w), sodium carboxymethyl cellulose (SCMC) (4-6% w/w) and methylcellulose 4000 (MC) (3-5% w/w) were prepared. The gel formulations had pH values in the range of 7.14 - 8.17, which were considered appropriate to oral mucosa application. In general, the rank order of pH values appeared to be SCMC > MC4000 > HPMC > Carbopol 934P. Types and concentrations of polymers used somewhat affected the adhesiveness. It was found that anionic polymers (Carbopol 934 and SCMC) adhered more firmly to the agar plate than the neutral polymers (HPMC and MC 4000). The formulation containing 0.5% Carbopol 934P (F1) showed the highest release rate. With the exception of the formulation F1, the neutral polymers tended to give higher relate rates than the anionic polymers. For oral tissue treatment, the optimum has to be balanced between the residence time (adhesiveness) of the formulations and the release rate of the drug. The formulations containing the anionic polymers: Carbopol 934P or SCMC possessed suitable physical properties (appearance, pH and viscosity). In addition, for anionic polymer formulations, justifiable mucoadhesive properties and reasonable release rates of nicotinamide were achieved. Accordingly, these gel formulations may be applied for the treatment of oral mucosal lesions.

Keywords—Nicotinamide, bioadhesive polymer, mucoadhesiveness, release rate, gel.

I. INTRODUCTION

Oral mucosa is composed of three layers: stratified squamous epithelium, lamina propria and submucosa [1]. Generally, topical corticosteroids have been used for the treatment of oral mucosal lesions (i.e. inflammatory, atropic and ulcerative conditions) [2]. For example, 1% trimacinolone acetonide in dental paste (Kenalog in Orabase®), which has anti-inflammatory, antipruritis and antiallergic action, is useful in temporarily relieving symptoms associated with oral inflammatory lesions and ulcerative lesions resulting from trauma. However, topical triamcinolone acetonide, like other topical corticosteroids, may have adverse systemic effects and/or adverse local effects. Accidental intakes a large amount of corticosteroids or long-term use of these drugs may elicit numerous systemic side effects such as hyperglicemia, glaucoma, proximal myopathy and adrenal insufficiency [3-4]. Moreover, oral candidosis can occur when topical corticosteroids have been administered [2]. As it contains a corticosteroid, Kenalog in Orabase® is contraindicated in patients who have fungal, viral or bacteria infections in mouth or throat. In addition, a sticky unpleasant feeling has been reported when the preparation is applied to the oral mucosa [5]. As a result, alternative substances, such as nicotinamide, have been explored. Nicotinamide is an amide form of niacin. The most common synonyms for nicotinamide are vitamin B3 and niacinamid. It is a white crystalline powder or colourless crystals. The solubility of nicotinamide in water is 1: 1.5 [3]. The molecular formula of nicotinamide is C6H6N2O and the chemical structure consists of a pyridine ring with an amide group as shown in Fig. 1. Nicotinamide is an essential component of two nucleotide coenzymes namely nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, which are involved in many biological reduction and oxidation reactions [3, 6].

Fig. 1 Chemical structure of nicotinamide

Up until now, nicotinamide has been administered by mouth, by injection and by topical route [3]. Importantly, oral nicotinamide is considered an alternative approach to oral steroids for the treatment of inflammatory skin conditions [7]. The incidence of nicotinamide toxicity after oral administration appears quite low. It is exceptional safe at pharmacologic doses [7]. However, administration of more
than 3,000 mg/day of nicotinamide has resulted in liver toxicity and gastrointestinal side effects [8, 9].

For topical application, it has been reported that nicotinamide exhibits several effects on the skin including anti-aging, anti-inflammatory and depigmenting effects [6, 10-11]. Nicotinamide has been used in the treatment of some dermatological diseases [12]. For example, a 4% nicotinamide gel is used to cure mild and moderate inflammatory acne [3]. In addition, topical nicotinamide is capable of accelerating skin and mucosal wound healings [13]. The amount of nicotinamide administered topically may vary from 1 to 25% as suggested by Kull and Voelker [13]. With respect to the formulation development, nicotinamide is easily formulated since it is water soluble and stable in the presence of oxygen and light [6]. In the case of adverse effects, topical nicotinamide is considered safe [6-7]. Nevertheless, it may induce skin irritation responses such as dryness, erythema, burning and stinging in some patients. There is no information whether nicotinamide has potential to irritate oral mucosa or not.

The benefits of topical nicotinamide, especially anti-inflammatory action and healing property, with exceptionally low adverse effects led us to formulate nicotinamide in topical pharmaceutical bases for the potential treatment of oral inflammatory lesions. For topical oral administration, it is generally recognized that profuse salivary flow and the mobility of oral organs can easily remove the formulations from the site of application [14]. Of all topical formulations available, gel base, which is likely to stay on the mucosal surface, seems to be the most suitable vehicle for local drug delivery to the oral cavity tissues. To increase an adherence between the bases and oral tissues, polymers with bioadhesive properties were selected as gelling agents [14-15]. Among bioadhesive polymers, polyacrylic acid, hydroxypropyl methylcellulose, sodium carboxymethyl cellulose and methylcellulose were of particular interest. With the exception of polyacrylic acid polymer, these polymers are cellulose derivatives. They are water soluble, capable of swelling in aqueous media and able to form hydrogels which have been used as vehicles for various types of drug delivery applications [1, 15-19].

The objective of this study was to formulate nicotinamide in adhesive oral gels and to evaluate the effectiveness of the formulations by measurement of in vitro mucoadhesive property of the gels using a simple agar plate technique [20] and by determination of in vitro release of nicotinamide from the gels using diffusion apparatus. Effects of types and concentrations of gelling agents on the mucoadhesiveness and the release of nicotinamide from oral gels were also studied.

II. MATERIALS AND METHODS

A. Materials

Nicotinamide, polyacrylic acid polymer (Carbopol 934P), hydroxypropyl methylcellulose (HPMC, 1500 cps), sodium carboxymethyl cellulose (SCMC, high viscosity grade) and methylcellulose (MC 4000 cps) were purchased from P.C. Drug Center (Thailand). Sodium dihydrogen orthophosphate dihydrate, disodium hydrogen orthophosphate anhydrous, sodium hydroxide and sodium chloride were supplied by Ajax Finechem (Australia). Glycerol, propylene glycol, methyl paraben and propyl paraben were obtained from P.C. Drug Center (Thailand). Cellulose acetate membrane (Spectra/Por®3) was obtained from Spectrum Laboratories, Inc. (US & Canada).

B. Methods

Preparation of Nicotinamide Gels

The concentration of nicotinamide used in the current study was 10% w/w [13]. In order to obtain gel formulations with mucoadhesive properties, several different bioadhesive polymers were tested as gelling agents. These polymers were Carbopol 934P, HPMC, SCMC (high viscosity) and MC 4000 in various concentration ranges as recommended by Klech [21]. The other compositions of nicotinamide gels were glycerol (10% v/w), orange flavor (0.18% w/w), paraben concentrate (1% v/w) and purified water to make 100% w/w. All formulations were prepared at room temperature by hydrating the gelling agents in water and stirring until clear gels had formed. In the case of Carbopol 934P gel, the powder was dispersed in some glycerol and water. The mixture was allowed to stand for air bubbles to separate and then gelled by neutralization with 10% w/v NaOH solution. HPMC and MC gels were prepared by dispersing each powder in hot water, followed by the addition of cold water to dissolve the gelling agents. SCMC gel was prepared by dispersing the powder of SCMC in some glycerol, and then adding water to hydrate the gelling agent. After the full swelling was obtained, nicotinamide previously dissolved in the remaining water was incorporated into the gel bases. Finally, the other components were added with continuous stirring. The formulations were then kept in tight containers and stored in a cool place for 24 hours prior to use.

pH measurement

The pH of the formulations was determined by digital pH meter (model 410A, Orion, USA). The pH was determined at room temperature (32 ±1°C).

Viscosity measurement

The viscosity measurements were carried out on a bob/cup Brookfield Synchro-Lectric viscometer (model RV, Brookfield, USA). The viscosity was determined at room temperature (32 ±1°C). The gel formulation was placed in the sample cup and allowed to stand until it reached room temperature before the viscosity was determined.

In vitro mucoadhesive measurement

The experiment was performed following the published guideline by Nakamura et al. [20] with slight modification. An agar plate which contained agar at 1.5% w/v was prepared by weighing 1.5 g of agar powder, then adding pH 7.2 phosphate
buffer into the agar, and finally gently heating a mixture with a water-bath. The hot solution was poured into the plate with diameter of 9 cm. The agar was then allowed to cool to a setting point. An equal amount of each sample was placed onto the center of the agar plate and a circle with a diameter of 1 cm was made as shown in Fig. 2. The longest movement distance of the sample (L) at room temperature was determined by slanting the plate at 30°. At 1, 2, 3 and 4 h, the movement distance of the sample was measured and recorded.

![Diagram](image.png)

**Fig. 2 Experimental setup for in vitro mucoadhesive measurement of nicotinamide gels**

**In vitro release study**

The apparatus used to study the in vitro release of nicotinamide from gels was composed of a cylindrical glass donor cell (internal diameter: 1.8 cm; height: 1.0 cm) as a donor compartment and a double-jacketed beaker (internal diameter: 4.5 cm; height: 6.0 cm) as a receptor compartment. Receptor fluid of the system was pH 7.4 isotonic phosphate buffer (IPB) [22]. A cellulose acetate membrane with a molecular weight cut off point of 3,500 (Spectra/Por®) was used as a semipermeable membrane separating the donor compartment from the receptor compartment. The double-jacketed beaker was filled with the degassed receptor fluid (80 ml) and maintained at 37.0 ± 0.5 °C by a circulating water bath. The donor cell was filled with an accurate weight of the test sample. Then, the fully hydrated cellulose acetate membrane was placed on top of the sample, giving a diffusion area of 2.54 cm². A rubber band was used to secure the membrane. With the aid of a stirring rod, the donor cell was immersed in the receptor fluid. Throughout the experimental period, the receptor fluid was kept homogeneous using a magnetic stirring bar. At suitable time intervals (0.5, 1, 2, 3, 4, 5 and 6 h), 2 ml of sample was withdrawn from a middle region of the receptor compartment and immediately replaced with the same volume of fresh degassed IPB. The sample was analyzed spectrophotometrically at the maximum absorption wavelength of 262 nm using a UV-visible spectrophotometer (Spectronic Genesys 5, Thermo Fisher Scientific, USA). Nicotinamide standard solutions (10-40 μg/ml) were prepared with IPB. Blank gel samples were also run at the same time in order to check for any interference.

**Data analysis**

All experiments were replicated at three times. All data were calculated and presented as mean ± SD. The percent cumulative amount of nicotinamide released per unit area was plotted against the square root of time, giving the in vitro release profile of nicotinamide. The release rates of nicotinamide from the gels were analyzed based on the simplified Higuchi equation [23]:

\[ Q = 2C_0 \sqrt{Dt} \]

where \( Q \) is the amount of nicotinamide released into the receptor phase per unit area at time \( t \), \( C_0 \) is the initial concentration of nicotinamide in the gel, \( D \) is the effective diffusion coefficient of nicotinamide in the gel.

**Statistical Analysis**

One-way ANOVA was used to analyze the obtained data. The level of significant difference was set at \( p < 0.05 \). A Tukey’s comparison test was used if the ANOVA indicated that there was a significant difference. Minitab release version 14 (Minitab Inc., State College, PA, USA) was used for statistical analysis.

**III. RESULTS AND DISCUSSION**

Nicotinamide was completely dissolved in the gel formulations (F1-F12). The clear gels were obtained in all formulations with the exception of the formulations F4-F6 which contained HPMC as gelling agent.

**Viscosity and pH of nicotinamide gels**

The pH and viscosity values of the gel formulations were in the range of 7.14-8.17 and 2.19 × 10⁴ -256 × 10⁴ cps, respectively (Table I). The effects of concentrations and types of polymers on pH and viscosity were examined.

When each type of polymer was considered, there was no significant influence of the polymer concentration on the pH of the formulations in the case of Carbopol 934P and HPMC. However, the pH value of 4% SCMC (F7) was significantly different to those of 5% and 6% (F8-F9) (p<0.05, One-Way ANOVA).

In the case of MC4000, significant difference was also observed between the formulation F10 and the formulation F12 as well as the formulation F11 and the formulation F12 (p<0.05, One-Way ANOVA). With respect to the types of polymers, the rank order of pH values appeared to be SCMC > MC4000 > HPMC > Carbopol 934P. The highest pH value was observed in the formulation F9 which contained SCMC whereas the lowest value was found in the formulation F3 which had 2% Carbopol 934P as gelling agent. The pH values of the formulations containing Carbopol 934P (F1-F3) were significantly lower than those of the formulations containing SCMC (F7-F9) (p<0.05, One-Way ANOVA).
The normal range of oral mucosal pH is reported to be between 6.2-7.4 [24]. Therefore, the effects of the formulations (F1-F12) on the pH of oral tissues should be considered. It was found that the pH values of the formulations containing SCMC (F7-F9) were somewhat higher than that of the oral tissues. These SCMC formulations may transitory increase the pH of the oral mucosa. It was notable that the pH values of all the formulations (pH 7.14 - 8.17) were not acidic, and thereby not causing any damage to the hard (enamel and dentin) and soft oral tissues. Furthermore, the three buffer systems of the salivary system are able to maintain a non-harmful pH (6.0-7.5) in the oral cavity [24]. Thus, it may be assumed that all the formulations are applicable for oral mucosal treatment.

Based on the results, the viscosity of the formulations was found to be influenced by the concentrations of the polymers used. In each type of polymer, the viscosity of the formulations significantly increased (p < 0.05) with the increase of the polymer concentrations. For example, the viscosity of the formulation F6 which contained 10% HPMC was significantly higher than that of the formulation F4 and the formulation F5 which contained the lower concentrations of such polymer. In addition, there were significant differences in viscosity among the gel formulations containing different types of polymers (F1-F12) (p<0.05, One-Way ANOVA). It may be concluded that polymer type also affects the viscosity of the formulations. However, it should be cautioned that these polymers were not used at the same concentration ranges.

In vitro mucoadhesive measurement

The movement of the gel formulations on the agar plate is listed in Table II. At 1 h, the movement distances of the seven formulations (F1, F4-F6, F10-F12) were already longer than 4 cm. Therefore, it was not necessary to measure the distances of these formulations in the following hours. Nevertheless, the duration for a 4 cm- movement distance of these formulations is detailed in Table III. It was found that the formulation F1 which contained 0.5% Carbopol 934P showed the shortest movement time on the agar plate whereas the formulation F12 which contained MC4000 exhibited the longest movement time.

| TABLE I
| PH AND VISCOSITY OF NICOTINAMIDE GEL FORMULATIONS (MEAN ± SD, N=3) |
|---|---|---|---|---|
| Formula- | Polymer | Polymer | pH | Viscosity (10⁴) |
| tion code | type | concentration | (μm) | (cps) |
| F1 | Carboxyl 934P | 0.5 | 7.26 ± 0.17 | 2.19 ± 0.15 |
| F2 | 1.0 | 7.18 ± 0.24 | 6.70 ± 0.01 |
| F3 | 2.0 | 7.14 ± 0.20 | 19.53 ± 0.31 |
| F4 | HPMC | 4.0 | 7.46 ± 0.05 | 3.63 ± 0.05 |
| F5 | 7.0 | 7.52 ± 0.14 | 103.98 ± 2.88 |
| F6 | 10.0 | 7.35 ± 0.08 | 231.78 ± 13.04 |
| F7 | 4.0 | 7.85 ± 0.05 | 106.22 ± 6.73 |
| F8 | 5.0 | 8.04 ± 0.09 | 153.87 ± 4.20 |
| F9 | 6.0 | 8.17 ± 0.01 | 256.00 ± 5.81 |
| F10 | 3.0 | 7.46 ± 0.01 | 7.25 ± 0.17 |
| F11 | MC 4000 | 4.0 | 7.52 ± 0.03 | 8.35 ± 0.28 |
| F12 | 5.0 | 7.63 ± 0.04 | 11.89 ± 0.81 |

In the current study, the movement distance was used to determine the adhesive properties of the polymers/formulations on the agar plate. The shorter the movement distance, the better adhesion between the polymer and the agar plate was obtained. With the exception of the formulations F8 and F9, there was a movement of all formulations on the agar plate at 1 h (Table II). It was found that the movement distances of the formulations F7-F9 increased with increasing time. Whereas, the movement distances of the formulations F2 and F3 did not increase after three hours of experiment. The movement distance of the formulation appeared to be inversely related to the polymer concentration. The results suggest that the increase in polymer concentration will provide better adhesion and hence longer residence time. There is a possibility that the adhesiveness of the polymers is influenced by charge of polymers used.
Anionic polymers which were Carbopol 934P and SCMC (F2, F3, F7-F9) adhered more firmly to the agar plate than the neutral polymers (HPMC and MC 4000) did. This is possibly due to the electrostatic interaction between negative charge from the anionic polymers and positive charge from the agar [20]. Furthermore, there may be hydrogen bond formation between the hydrophilic functional groups of the polymers and the corresponding functional groups of the agar. Apart from the charge of polymers, viscosities of the gel formulations tended to affect the residence time of the polymers on the agar plate. Again, the effect of viscosity was more obvious in the case of anionic polymers. For neutral polymers, HPMC and MC 4000, the formulation with higher viscosity tended to give longer residence time (see Table I and Table III).

**In vitro release study**

The amount of nicotinamide released from the formulations was expressed as the percent cumulative release per unit area of application (Fig. 3, a-d).

The *in vitro* release profiles of nicotinamide generated linear relationship between the amount released per surface area and the square-root of time. The results showed the values of coefficients of determination ($R^2$) over 0.99 for all formulations, indicating that the release kinetics of nicotinamide from the gels followed the Higuchi diffusion model. Also, this suggests that the synthetic membrane does not provide any significant effects in the release of nicotinamide from the gels. The gel formulations themselves controlled the release of the active substance.

It is generally recognized that if the active substance is held firmly by the vehicle, the release of the active substance is slow. In the case of Carbopol 934P (Fig. 3, a), the formulation F1 which contained the lowest concentration of Carbopol 934P (0.5%) showed the highest release of nicotinamide in comparison with the formulations F2 and F3 which contained the higher concentrations (1% and 2%). As the release of the active substance generally occurred through the spaces or
channels within the hydrogel network [1], it was likely that at higher polymer concentrations, nicotinamide was trapped in smaller polymer cells. Consequently, it was structured by its close proximity to those polymer molecules, resulting in an increase in the diffusional resistance [25]. In addition, the increase in polymer concentration could increase the density of chain structure, thereby limiting the movement area of nicotinamide [26]. Nevertheless, further increasing the concentration of Carbopol 934P from 1% to 2% did not affect the release of nicotinamide from the formulations. The similar findings were also observed in the case of HPMC (Fig. 3, b). It was found that increasing concentrations of HPMC from 4% to 10% decreased the percents of nicotinamide released from the formulations (F4-F6). It was likely that the release of nicotinamide from the formulations was affected by the concentrations of such polymers. Unlike Carbopol 934P and HPMC, the release of nicotinamide from the formulations containing SCMC (F7-F9) (Fig. 3, c) and MC 4000 (F10-F12) (Fig. 3, d) was not influenced by the range of concentrations investigated in the current study.

The cumulative amount of nicotinamide released at 6 h and the values of the release rates of all formulations are summarized in Table IV. The release rates were obtained from the slopes of the in vitro release profiles as shown in Fig. 3. In the case of Carbopol 934P, the formulation F1 exhibited the highest cumulative amount of nicotinamide released at 6 h in comparison with the formulations F2 and F3 (p<0.05, One-Way ANOVA). There was no significant difference in the amount of nicotinamide released between the formulations F2 and F3. It was obvious that as the polymer concentration increased from 0.5 to 2%, the cumulative amount of nicotinamide significantly decreased. However, further increasing the concentration of Carbopol 934P from 1 to 2% did not significantly alter the amount of nicotinamide released. For HPMC, the cumulative amount released of nicotinamide from the formulations F4 (4%) and F5 (7%) was significantly superior to that from the formulation F6 (10%) (p<0.05, One-Way ANOVA). No significant difference in the cumulative released was observed between the formulation containing either 4% or 5% HPMC. In the case of the formulations containing SCMC (F7-F9) or MC 4000 (F10-F12), the cumulative amount of nicotinamide released from the formulations was about the same.

According to Table IV, the formulation F1, which contained 0.5% Carbopol 934P, seemed to give the highest release rate of nicotinamide among the formulations tested. Statistical differences in release rates were observed between formulation F1 and the four formulations F6-F9 (p<0.05, One-Way ANOVA). However, there was no significant difference in release rate of formulation F1 and the release rates of the other seven formulations (F2, F3, F4, F5, F10, F11, F12).

The formulation F1 also exhibited the highest amount of nicotinamide released at 6 h (see Table IV).

### Table IV

| Formulation code | Cumulative amount of nicotinamide released at 6 h (%/cm²)  
<table>
<thead>
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<tr>
<td></td>
<td>(mean ± SD, n=3)</td>
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<tr>
<td>F1</td>
<td>14.7914 ± 1.7429</td>
</tr>
<tr>
<td>F2</td>
<td>11.8518 ± 0.2580</td>
</tr>
<tr>
<td>F3</td>
<td>11.9527 ± 0.5869</td>
</tr>
<tr>
<td>F4</td>
<td>13.2876 ± 0.4997</td>
</tr>
<tr>
<td>F5</td>
<td>12.9878 ± 0.4269</td>
</tr>
<tr>
<td>F6</td>
<td>10.9177 ± 0.7277</td>
</tr>
<tr>
<td>F7</td>
<td>11.1192 ± 1.3150</td>
</tr>
<tr>
<td>F8</td>
<td>10.9405 ± 0.2083</td>
</tr>
<tr>
<td>F9</td>
<td>10.7681 ± 1.5512</td>
</tr>
<tr>
<td>F10</td>
<td>12.7793 ± 0.2931</td>
</tr>
<tr>
<td>F11</td>
<td>12.8731 ± 0.4373</td>
</tr>
<tr>
<td>F12</td>
<td>12.6505 ± 1.3152</td>
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* release rate of formulation F1 was significantly higher than that of formulations F6-F9 (p<0.05)

**Relationship between viscosity/pH and release rate of nicotinamide**

As seen from Fig. 4, there was rather strong linear correlation between log viscosity of the formulations and the release rates of nicotinamide from the gels (R² = 0.7289). This result indicated that the viscosities of the formulations had an effect to the release rates of nicotinamide from the gels. In general, a decrease in viscosity of the formulation would provide a better release rate. There are several factors influencing the release of active substances from the vehicle including viscosity. The inverse relation between viscosity and drug release has been shown in several investigations.
According to Fig. 5, no linear correlation exhibited between pH of the formulation and the release rates. The relationship between pH and release rates of nicotinamide gels, Key: F1, 0.5% Carbopol 934P; F2, 1% Carbopol 934P; F3, 2% Carbopol 934P; F4, 4% HPMC; F5, 7% HPMC; F6, 10% HPMC; F7, 4% SCMC; F8, 5% SCMC; F9, 6% SCMC; F10, 3% MC 4000; F11, 4% MC 4000; F12, 5% MC 4000

IV. CONCLUSION

Based on the current investigation, it appeared that the proper nicotinamide gels, which intended to use for oral mucosa, could be achieved with anionic polymers. The effectiveness of the formulations was evaluated by the in vitro mucoadhesive study as well as the in vitro release through synthetic membrane. Although these in vitro tests are less meaningful than the in vivo methods, they can be used for preliminary screening of the potential formulations. It must be pointed out that the in vitro/in vivo permeations through oral mucosa and a clinical trial should be further investigated so that the treatment of oral lesions with nicotinamide oral gels may become possible, resulting in increasing the benefits to patients and reducing the traditional therapy with topical corticosteroids which is likely to cause undesirable effects.

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