Release Profiles of Dexamethasone Dipropionate from Admixtures of Steroid and Heparinoid Ointments Prepared by Different Mixing Methods

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Characterization and release profiles of commercial dexamethasone dipropionate (DDP) from an innovator and 2 generic ointments (Methaderm (IM), Promethasone (GP), and Mainvate (GM)) and their admixtures with heparinoid ointment (Hirudoid Soft) were investigated. The admixtures were prepared using 2 mixing methods (slab or rotation/revolution mixer). Microscopic and FT-Raman spectrometric analyses revealed that the ointments, except for IM, contained DDP crystals. A silicone membrane was used for the evaluation of the DDP permeation. The permeated DDP amounts from GP and GM were lower than that from IM, indicating that DDP solubility in the ointment vehicle affected the release of DDP from the ointment. No significant differences were observed in DDP release between IM alone and its admixture prepared using a slab; however, DDP release from the admixture prepared using a rotation/revolution mixer was significantly lower than those from IM alone and its admixture by slab. In the GP system, DDP release from the admixtures by the 2 mixing methods was higher than that from GP alone, whereas no significant difference in DDP release between the 2 mixing methods was observed. No significant differences were observed between the GM and admixtures. The apparent solubility of DDP in the admixtures as determined by the ultracentrifugal separation method indicated that the DDP amount in the liquid phase of admixtures with GP was 6 times higher than that of admixtures with IM or GM. Therefore, the apparent solubility of DDP in the liquid phase in the GP system might influence the DDP release in admixtures.

**Key words** steroid; heparinoid ointment; release profile; mixing method; FT-Raman spectroscopy; apparent solubility

Given that generic substitution can greatly reduce total drug costs, the Japanese government strongly supports the use of generic drugs. However, the promotion of generic drug use has proved to be a major challenge. Bioequivalence assessment is carried out for orally administered drugs intended for systemic circulation by measuring plasma drug concentrations following administration of a test and reference dosage form in humans. However, bioequivalence assessment is not done for topical formulations, as these are not distributed in the systemic circulation.

In semisolid formulations, the additives in generic drugs are different from those in innovators. Previous studies reported that the vasoconstrictive effect could differ between generic and innovator topical steroids.1–4 Because the thermodynamic activity and vehicle solubility of the drug affect its release characteristics from topical formulations,5,6 drug permeation across the skin might be different between innovator and generic formulations. Therefore, it is necessary to compare the drug release profiles of innovator and generic formulations.

Topical corticosteroids are frequently prescribed for dermatological conditions, particularly for the treatment of psoriasis7 and atopic dermatitis.8 They have various strengths and formulations, giving clinicians substantial flexibility in their approach to treatment. Although topical steroid formulations are applied alone, admixtures of commercial topical steroid formulations are prepared upon the physician’s request.9 In addition, the use of an admixture of a topical steroid formulation with another semisolid formulation is a common approach to improve patient compliance.9

Admixtures of a steroid and another component show varying anti-inflammatory10 and vasoconstrictive effects.11 Gibson et al. showed no difference in the vasoconstrictive effect between 0.05% clobetasol propionate ointment and its 1 : 10 dilution (0.005% clobetasol propionate ointment).12 On the other hand, hydrocortisone was reported to show accelerated skin permeation with an admixture of urea, with the permeation rate being dependent on the urea concentration in the formulation.13 Several studies demonstrated that admixture of steroidal ointment with another component leads to changes in the physicochemical properties of the steroid and their changes significantly affects its permeation characteristics and stability.10,14

The mixing method is a contributing factor to the physical property variations of topical formulations, such as demulsification and change of rheologic properties.14 Ohtani et al. reported that mixing steroidal ointment with water-in-oil (w/o) type cream resulted in demulsification and decreased preservative activity.15

The aim of the present study was twofold. First, it aimed to compare the physicochemical and permeation properties of innovator and generic topical steroid formulations. Three different commercially available 0.1% dexamethasone dipropionate (DDP) ointments, Methaderm ointment (IM), Promethasone ointment (GP), and Mainvate ointment (GM), were selected as model ointments. As shown in Table 1, these commercial ointments contain different additives. Although Ohtani et al. reported on the permeation properties of admixtures of innovative steroidal formulations with another semisolid formulation,16 no study has been carried out to compare in detail innovator and generic ointments in admixtures of steroid with another ointment. The second aim of this study was to elucidate the effect of mixing method on DDP release. Heparinoid
0.3% ointment, Hirudoid Soft ointment (HS), was selected as the admixed ointment, since the heparinoid ointment was recommended for slight skin eruptions in the atopic dermatitis guidelines.\textsuperscript{17} And it is well known that the combination of steroid ointment and heparinoid ointment is widely used in dermatological practice.\textsuperscript{18} We also investigated the effects of mixing method on the release of DDP from the admixtures of different DDP ointments with HS.

In all experiments, the cumulative amount of DDP that permeated across a silicone membrane was measured using a Franz-type diffusion cell. Because silicone membrane was applied to investigate the release profile,\textsuperscript{19} it was also used for the comparison of DDP release from the ointments. Microscopic analysis and Raman spectroscopy were carried out for the characterization of DDP ointment and admixtures. Raman spectroscopy is a nondestructive and rapid measurement method and requires no sample preparation, and it has been utilized for the characterization of various dosage forms such as tablets, injection, inhalation, and semisolid formulations.\textsuperscript{20—24} To investigate the distribution of DDP in the admixture of DDP ointment and HS, the admixtures were subjected to ultracentrifugal separation and DDP concentration was measured in the separated phases. Only the soluble part of DDP is able to diffuse across the silicone membrane. Therefore, it was important to determine the apparent solubility of DDP in the different ointments in order to reveal the influence of excipients on DDP release.\textsuperscript{6}

**Experimental**

**Materials** Three DDP 0.1% ointments (innovator: IM (Taicho Pharmaceutical Co., Ltd., Tokyo, Japan); generic: GP (Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan)) and GM (Sato Pharmaceutical Co., Ltd., Tokyo, Japan) and HS (Maruho Co., Ltd., Osaka, Japan) were used. DDP (molecular weight (MW), 504.59; mp 200—206°C; LogP, 3.66; solubility in water at 20°C, 0.016 mg/mL), fluocinonide (internal standard), and butylparaben (internal standard) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals and solvents were of reagent grade or HPLC grade and used without further purification. The silicone membrane (TLC-SI-75, thickness of 75 μm) was a kind gift from Lintec Co., Ltd. (Tokyo, Japan).

**Preparation of Admixtures of DDP Ointments with HS** Admixtures of DDP ointment with HS were prepared using an ointment slab and an ointment spatula (method S) or a rotation/revolution mixer (NR-500; Thinky Co., Ltd., Tokyo, Japan) (method M). In the former method, DDP ointments and HS were weighed out 5 g on the spatula and equally mixed by the slab over 5 min. In the latter method, samples were mixed by rotating and simultaneously revolving the vessel in the mixer at a fixed rotation and a revolution speed of 1000 rpm for 30 s. The mixing ratio of DDP ointment and HS was fixed as the equal proportions between the DDP and the heparinoid ointment. Admixtures of HS with IM, GP, and GM by method S were designated as IMHm, GPHm, and GMHm, respectively. Similarly, IMHm, GPHm, and GMHm denote the admixtures of DDP ointment and HS prepared by method M.

**Microscopy and Raman Spectrometry** Microscopic images were obtained using an optical microscope (BH-2; Olympus Corporation, Tokyo, Japan). The Raman spectra data were collected using a dispersive Raman spectrometer (Nicolet Almega XR with a 532 nm laser; Thermo Fisher Scientific K.K., Kanagawa, Japan). Spectra were obtained for 10 scans at 3-s exposure time.

**Silicone Membrane Permeation Experiments** The permeation analysis of DDP from samples across the silicone membrane was performed according to the method of Ishii et al., with slight modifications.\textsuperscript{5} Briefly, the silicone membrane was set in a Franz-type diffusion cell with an effective permeation area of 0.38 cm\textsuperscript{2}. The test ointments were applied to the donor cell side, and phosphate buffered saline (pH 7.4) (5.0 mL) was applied to the receiver cell side. The diffusion cell was kept at 37°C with a water jacket connected to a water bath. The receiver cell was agitated using a magnetic stirrer bar. At the indicated times, an aliquot (200 μL) was withdrawn from the receiver solution and the same volume of fresh phosphate buffered saline was added to keep the volume constant. Samples were taken at 0, 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 h. An aliquot (50 μL) was injected into the HPLC system. The HPLC conditions for the determination of permeated DDP are described in the “HPLC analysis” section.

**Ultracentrifugal Separation of Admixtures and Extraction of DDP** The admixtures were phase-separated by a modified centrifugal separation method, and DDP was extracted following a modified extraction procedure as described by Lombardi Borgia et al.\textsuperscript{25} In brief, 7 g of each admixture was placed in a centrifuge tube and centrifuged at 60000 rpm at 20°C for 2 h. A liquid phase and 2 solid phases (upper is solid phase 1 and lower is solid phase 2) were obtained after ultracentrifugation. To extract DDP from each separated phase, 700 μL of liquid phase and 10 mg of each solid phase were dispersed in 7 and 10 mL of chloroform, respectively. Following the addition of fluocinonide as internal standard, 1 mL of the sample was withdrawn and exsiccated by vacuum rotation. In the IM system, butylparaben was used as internal standard instead of fluocinonide. Residues were extracted 3 times with 500 μL of methanol. 10 μL of the sample was injected into the HPLC system.

**HPLC Analysis** The HPLC system consisted of a pump (LC-10AT; Shimadzu, Kyoto, Japan), a column (COSMOSIL SC18-MS-II Packed Column, 4.6×150 mm; Nacalai Tesque, Kyoto, Japan), an auto-injector (SIL-10AXL), a UV detector

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Excipients</th>
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<tbody>
<tr>
<td>Methaderm® ointment</td>
<td>White petrolatum, liquid paraffin, petrolatum, propylene glycol, propylene carbonate, glycercyl mono-stearate, lanolin alcohol</td>
</tr>
<tr>
<td>Promethasone® ointment</td>
<td>Petrolatum, crotamiton, oil of olive, white beeswax, methylparaben, butylparaben</td>
</tr>
<tr>
<td>Mainvate® ointment</td>
<td>Petrolatum, propylene glycol, polyoxyethylene hydrogenated castor oil 40</td>
</tr>
<tr>
<td>Hirudoid® Soft ointment</td>
<td>White petrolatum, white beeswax, methylparaben, light liquid paraffin, propylparaben, glycercin, squalene, cerasin, glycercin fatty acid ester, dibutylhydroxytoluene, edetate sodium hydrate</td>
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Table 1. Qualitative Composition of the 3 Different DDP Ointments and HS
The mobile phase was used by changing the appropriate ratio of distilled water/acetonitrile from 35:65 to 60:40 to avoid the additional peaks due to the additives in the formulations. The flow rate was 1.0 mL/min. Detection was performed at UV 254 nm. The limit of detection of DDP was 300 pg.

Statistical Analysis
Permeation data at 4 h (given as mean±S.D. (n=3—6)) were analyzed by Tukey’s multiple comparison test with one-way analysis of variance (ANOVA). Statistical analysis was carried out using SPSS software version 17.0 (SPSS Inc., Chicago, IL, U.S.A.). A p value of <0.05 was considered to be significant.

Results and Discussion
In semisolid formulations, the physicochemical properties, particularly the solubility, of the drug are important factors for the drug permeation. In this study, 3 topical DDP ointments were selected as model topical ointments and characterized.

Figure 1 shows the microscopic images of GP and GM. No crystals were formed in IM (data not shown). In GP and GM, centrosymmetric rod-like and needle crystals were observed, suggesting that DDP was crystallized in GP and GM.

To identify the crystals, a comparative study using Raman spectroscopy was performed for the DDP standard and observed crystals. The results are shown in Fig. 2. The Raman spectrum of the DDP standard exhibited sharp characteristic peaks between 1600 and 1700 cm⁻¹. Fini et al. reported that the Raman spectra of several steroidal molecules gave characteristic peaks between 1600 and 1700 cm⁻¹, which could be attributed to the carbon–carbon double bond and carbonyl stretching vibration of the steroidal backbone. These peaks coincided with those of the observed crystals in GP and GM; therefore, the observed crystals in GP and GM were identified as DDP. Refai and Muller-Goymann reported that changes in steroid permeation are dependent on the concentration of dissolved corticosteroid in the vehicle. Since thermodynamic activity and drug solubility in the ointment vehicle affect drug permeation, we analyzed whether DDP permeation differed dependently on DDP solubility in the ointment vehicle.

The permeation profiles of DDP from the 3 different ointments across the silicone membrane were investigated (Fig. 3). Significant differences in DDP permeation were observed among the 3 ointments. The cumulative amounts of DDP permeated from IM, GP, and GM in the reservoir solution after 4h were 3.14, 1.53, and 0.77 μg/cm², respectively, showing that the cumulative amounts of DDP permeated from GP and GM
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were 2- and 4-times lower, respectively, compared with the IM system. These results indicated that the DDP solubility in the ointment vehicle of IM was higher than those in the ointment vehicles of GP and GM.

Ohtani et al. reported that the permeability and vasoconstrictor activity of steroid changed with the admixture of another ointment.11) Since the 3 ointments showed different DDP permeation characteristics, their admixtures with HS were predicted to also show varying permeation and release profiles.

Fig. 4. Microscopic Images of GPHs (a), GMHs (b), GPHm (c), and GMHm (d)

In the GM and GP systems, some crystals in GM or GP alone were observed in the admixtures. On the other hand, DDP was still solubilized after mixing with heparinoid ointments in IM system (data not shown).

Fig. 5. Microscopic Images of IMHs (a), GPHs (b), GMHs (c), IMHm (d), GPHm (e), and GMHm (f)

The gray and white parts in the admixtures of DDP ointment with HS are indicated by white and black arrows, respectively. The 2 types of mixing method had no effect on the GP and GM systems. Different microscopic images were obtained in IM system (a,d).
To investigate the physicochemical properties and permeation profiles of DDP from the admixtures, DDP ointment was admixed with HS using different mixing methods. On visual analysis, homogeneous admixtures of DDP ointment and HS were obtained, and no effect of mixing method on the homogeneity was observed. Figures 4 and 5 show microscopic images of the admixtures of DDP ointments and HS. As shown in Fig. 4, no changes in the DDP crystal shape in GPH and GMH were observed as compared with the DDP ointment, thus indicating that the mixing method did not affect the crystal shape. In the IM system, no crystallization occurred with the admixture of HS (data not shown). In all the admixture systems, 2 phases of gray (white arrow) and white regions (black arrow) were observed (Fig. 5). Microscopic analysis showed that mixing method had no effects on appearance of the admixtures, except for the IMHm system. In the IMHm system, microscopic images exhibited numerous white regions with a clear border between the white and gray regions. Raman spectrometry was performed to investigate the composition of the white and gray regions in the admixture. Figure 6 shows the Raman spectra of the IMH systems with HS or IM alone. HS and the white region of the IMH displayed a broad band from 3100 to 3700 cm$^{-1}$ for the stretching bands of hydroxyl groups in the additive molecules of HS. On the other hand, no absorption bands due to the stretching vibration of hydroxyl groups were observed in the IM and gray region of the IMH. The spectrometry results suggested that 2 types of phases, white and gray regions with hydrophilic and hydrophobic compositions, existed in the admixture.

On the basis of the microscopic observations described above, apparent DDP solubility was expected to differ among the admixtures. To evaluate the apparent solubility of admixtures of DDP and HS, the admixtures were ultracentrifuged for 2 h at 60000 rpm at 20°C. Three separated phases were obtained and the DDP amount in each phase was determined. Table 2 summarizes the DDP amount in each phase in the admixtures. The weight in the liquid phase ranged from 25.9 to 35.8%. DDP showed varying amounts and distribution among the 3 phases in all the admixtures. The DDP amounts in the liquid phase of GPHs and GPHm were more than 6 times higher as compared with the DDP amounts in the liquid phase of the IM and GM systems. The mixtures of the liquid phase of DDP ointments and the lipophilic compounds of HS might be shown DDP solubility different from the liquid phase of ointment alone. Therefore, admixture of HS might have caused changes in the DDP permeation profile.

Figure 7 shows the cumulative curve of DDP permeated across the silicone membrane from DDP ointment and their admixtures with HS. Various cumulative curves of DDP from each ointment were obtained. In the IM system, no significant difference in DDP permeation between IM and IMHs was observed. On the one hand, DDP from the IMHm showed decreased permeation. These results suggested that a change in the dispersion state by method M influenced the DDP permeation. For the GP system, although the cumulative amount of DDP from admixtures by the 2 mixing methods increased compared with the GP alone, no differences in the cumulative amount of DDP between GPHs and GPHm were observed. The highest DDP solubility was observed in the liquid phase in all systems, with no significant difference found among the systems (Table 2), indicating that...
DDP crystals were not observed in solid phase 1 of IMHs and GPHs (data not shown), and the sum of the amount of DDP dissolved in liquid phase and solid phase 1 of IMHs and GPHs were similar value. Therefore, DDP dissolved in solid phase 1 might be related to permeation.

In conclusion, we found that the dispersion state of DDP varied in the different formulations, and the release of DDP from suspended ointments was less than that from nonsuspended ointments. Moreover, the release of DDP from admixtures with HS was dependent on the system. Except for the IMHM system, no significant changes in DDP release were observed with the admixture of HS in the IM and GM systems. In the GP system, the DDP release from GPHs and GPHm was increased compared with that from GP alone. Taken together, the results of this study suggest that the steroid ointment used for generic substitution and the mixing method for the preparation of admixtures of generic steroid ointment and HS should be carefully selected, as these influence drug potency.

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Conflict of interest The authors declare no conflict of interest.

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