Stability of Mixed Preparations Consisting of Commercial Moisturizing Creams with an Ointment Base Investigated by Magnetic Resonance Imaging

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A moisturizing cream mixed with a steroid ointment is frequently prescribed to patients suffering from atopic dermatitis. However, there is a concern that the mixing operation causes destabilization. The present study was performed to investigate the stability of such preparations closely using magnetic resonance imaging (MRI). As sample preparations, five commercial moisturizing creams that are popular in Japan were mixed with an ointment base, a white petrolatum, at a volume ratio of 1:1. The mixed preparations were stored at 60°C to accelerate the destabilization processes. Subsequently, the phase separations induced by the storage test were monitored using MRI. Using advanced MR technologies including spin–spin relaxation time (T2) mapping and MR spectroscopy, we successfully characterized the phase-separation behavior of the test samples. For most samples, phase separations developed by the bleeding of liquid oil components. From a sample consisting of an oil-in-water-type cream, Urepearl Cream 10%, a distinct phase-separation mode was observed, which was initiated by the aqueous component separating from the bottom part of the sample. The resultant phase separation was the most distinct among the test samples. To investigate the phase separation quantitatively and objectively, we conducted a histogram analysis on the acquired T2 maps. The water-in-oil type creams were found to be much more stable after mixing with ointment base than those of oil-in-water type creams. This finding strongly supported the validity of the mixing operation traditionally conducted in pharmacies.

Key words mixed external preparation; magnetic resonance imaging; quantitative T2 map; emulsion stability

Atopic dermatitis is one of the most common skin disorders in Japan. Dermatologists frequently prescribe moisturizing creams mixed with steroid ointments.1–4 This treatment approach is based on the concept that moisture retention of the skin is important for the treatment of atopic dermatitis, as is steroid therapy. To facilitate application of the cream and ointment, they are mixed in an operation that is widely performed at pharmacy level.

Moisturizing creams are classified into two types: oil-in-water (o/w) emulsions, which are composed of small oil droplets dispersed in a continuous aqueous phase, while water-in-oil (w/o) creams are composed of small water droplets dispersed in a continuous oily phase.5 The basic concept concerning the compatibility of the different types of emulsions with steroid ointments is described in the dispensing guidelines in Japan, Chozai Shishin.6 The guidelines show a greater preference for w/o-type moisturizing creams in mixing creams with ointments than for o/w-type creams. This description is often used as a rationale to argue that the mixing operation performed in the pharmacy is appropriate. However, as a whole, pharmaceutical emulsions are not designed under the assumption that they would be mixed with other preparations. Therefore, it remains a concern that the mixing operation could be attributed to a substantial decrease in the emulsion stability. To date, there is insufficient scientific evidence concerning this issue.

From this perspective, we investigated the relative stability of various mixed preparations. We employed magnetic resonance imaging (MRI)7 as a key technology to evaluate the stability of emulsions. As well as a standard nondestructive method, it enables the visualization of the molecular mobility of compounds incorporated in the sample by using magnetic resonance (MR) parameters. We have recently verified that MRI is a promising tool by which to investigate the stability of emulsions.8–10 In this study, we selected five different commercial products, which are popularly used in Japan as moisturizing creams. They include both o/w and w/o-type creams. To prepare samples, these creams were mixed with white petrolatum (WP) at a volume ratio of 1:1. Subsequently, the samples were stored at 60°C for designated intervals to accelerate destabilization. After the 60°C storage test, the samples were examined using MRI. We successfully characterized the phase-separation behavior of the samples.

Experimental

Materials Hirudoid soft ointment 0.3% (HS) and Hirudoid cream (HC) were purchased from Maruho Co. (Osaka, Japan). Urepearl Cream 10% (UC) was purchased from Osuka Pharmaceutical Co. (Tokyo, Japan). Pastaron soft ointments 10% (P10) and 20% (P20) were purchased from Sato

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Sample Preparation The moisturizing cream was mixed with WP at a weight ratio of 1:1 for 3 min using a revolution/rotation-type hybrid mixer (Model HM-500; Keyence, Osaka, Japan). The revolution and rotation speeds were 2000 and 800 rpm, respectively. After preparing 13 g of mixed preparation, 1 mL of the sample was packed in a tube having a volume of 1.5 mL and stored at 4°C until it was used in the experiments. To accelerate the destabilization processes in the samples, these samples in the tubes were stored at 60°C for a designated period. Afterwards, the samples were monitored using MRI.

MRI Study The MRI experiments were performed at room temperature using a 9.4 T vertical MRI scanner (Varian, Palo Alto, CA, U.S.A.). MR images were acquired using a spin-echo pulse sequence with a repetition time (TR) = 2000 ms, echo times (TEs) = 25, 40, and 60 ms, filed of view (FOV) = 35 × 35 mm², matrix size = 128 × 128, number of excitations = 2, and slice thickness = 1 mm. A quantitative spin-spin relaxation time or transverse relaxation time (T²) map was constructed from the three MR images with different TE values. MR spectroscopy (MRS) was performed using a stimulated-echo acquisition mode sequence (acquisition bandwidth = 5 kHz); a spectrum of regions of interest (ROIs) were acquired. The acquisition parameters were TR = 2000 ms and TE = 10.20 ms from an average of 10 scans. The voxel size of the ROIs was 2 × 2 × 2 mm³. The ROIs were positioned in the center of the upper and lower layers.

Data Analysis Origin Pro 2015 (Origin Lab, Northampton, MA, U.S.A.) was used for histogram analysis of T² maps. After acquisition of T² maps, the T² values were extracted from the maps, and then their histograms were created. The peak fitting was performed using a Gauss function. The peak areas of the fitting curves were calculated and then ratio of the area of the upper layer’s peak to the total was observed to evaluate phase-separation behavior.

Results and Discussion

In the initial phase of this study, we continuously monitored the phase separation produced with the storage test using MRI. We tested five commercial creams. HS, P10, and P20 are classified as w/o-type moisturizing creams, while HC and UC are o/w-type moisturizing creams. P10, P20, and UC are urea-containing creams. Figure 1 shows MR images of the w/o-type cream-containing samples in the course of the 60°C storage test. The MRI image contrast is generated by the differences in the NMR signal intensity, and the NMR signal intensity largely depends on the density and the relaxation properties of the protons therein. Phase separations appeared to occur at the top surface of the sample. After an upper separated layer was detected in the samples, the separation gradually spread to the deeper part of the tube with prolonged storage. From a visual observation, the upper separated layer appeared to be liquid, suggesting the bleeding of liquid oil components from the mixed preparation. These images also clarified that there is no remarkable difference in the phase-separation behavior between P10-WP and P20-WP, suggesting that the urea in these creams barely affected the stability of the mixed preparation. The stability of HS-WP seemed to be a little better than P10-WP and P20-WP. The stability was considered better because the phase separation was barely observed in the 4 h-storage sample, and the volume of the upper layer at the end of the experiment (120 h) was obviously less than that of the other samples.

We have previously investigated the compatibility of w/o-type moisturizing creams with steroid ointments using a centrifugation protocol. After preparing, the mixed samples were centrifuged at 2000 × g for 3 min and then evaluated by using MRI and near-infrared (NIR) spectroscopy. From the centrifuged HS-WP, three distinct layers (upper, middle, and lower) were observed. Subsequently, we analyzed the components of each layer and found that the upper and middle layers were oily, while the lower layer was a water and oil emulsion. The oil components of the upper layer were more fluid than those of the middle layer. In the present study, we found the liquid component as an upper separated layer. We consider that the mode of phase separation induced by the storage test was similar to that found using the centrifugation protocol.

The phase separation of o/w-type cream-containing samples was much more substantial than that shown in Fig. 1, in particular, the UC-WP separated into three distinct phases after 120 h storage (Fig. 2). This result is quite reasonable because o/w-type creams have more hydrophilic nature than w/o-type creams. The phase separation of HC-WP began at the top surface of the sample; thus, the phase-separation mode was considered the same as that shown in Fig. 1. By contrast, different phase-separation behavior was observed from UC-WP. Considerable phase separation suddenly appeared starting from the bottom of the tube after 1 h; subsequently, the upper layer gradually separated into two distinct phases. MR images indicated that the emulsion of the o/w-type moisturizing creams was significantly changed by mixing. Samples stored for 0 and 0.5 h appeared to be irregular even before the phase separation occurred. The images of the original creams appeared to be smooth like those of the w/o-type cream-containing samples (data not shown). This change in the emulsion associated with mixing of the ointment base probably contributed to the substantial phase separation.

To investigate the phase separation of the samples in more detail, we conducted a component analysis of the phase-sep-
Separated layers after 12 h storage by MRS. MRS is a technique to acquire the NMR spectrum of selected ROIs. We observed similar 1H-NMR spectra for the upper and lower layers from the w/o-type cream-containing samples (Fig. 3). The spectra of the lower layers showed noteworthy peaks assigned to water (peak at around 4.7 ppm) and hydrocarbon groups of oil (peaks at around 1.3 ppm), indicating that this layer was an emulsion consisting of oil and water. By contrast, the spectra of the upper layers showed only oil peaks at around 1.3 ppm; thus, this layer was mainly composed of oils. Therefore, the phase separation of these samples was produced by bleeding of liquid oils from the mixtures. The spectral patterns of HC-WP (Fig. 4) were overall the same as those shown in Fig. 3; the upper layer consisted of oil, while the lower layer was an emulsion. An exception was that the oil peaks ranging from 0 to 1 ppm observed from the lower layer were much smaller than those shown in Fig. 3, which is quite reasonable because HC was an o/w-type cream and contained less oil than the w/o-type creams. The upper and lower layers of UC-WP showed only oil and water peaks, respectively, while the middle layer showed both peaks. Thus, not only oil components, but also water separated from the preparation in the course of its destabilization, which then formed distinct oily and aqueous layers as the upper and lower layers, respectively.

We next investigated the phase-separation behavior objectively and quantitatively. We employed HS-WP and HC-WP as representative preparations consisting of o/w- and w/o-type creams, respectively. We acquired quantitative \( T_2 \) maps and then analyzed their histograms. \( T_2 \) is a time constant that corresponds to the decay of transverse magnetization arising from natural interactions at the atomic or molecular level. The \( T_2 \) value of a sample changes according to its composition and the molecular mobility of its components. We have already found that \( T_2 \) mapping is a powerful tool with which to evaluate the stability of emulsions.8-9,12) The acquired \( T_2 \) maps clearly display the phase separation with distinct \( T_2 \) values (Figs. 5a, c). Figures 5b and d show the histograms of \( T_2 \) values created from their \( T_2 \) maps. The peak corresponding to the upper layer of the HS-WP sample, was small and barely detectable after 24 h (Fig. 5b). The peak area slightly increased with prolonged storage. The peak from the upper layer of the HC-WP sample was clear after storage for 0.25 h (Fig. 5d). The peak area steadily increased until the end of the experiment (120 h), indicating the development of phase separation. We also note that the upper layers showed almost the same \( T_2 \) values regardless of the type of emulsion. After storage for 120 h, a peak position of 24.2 ms was found for samples of HC-WP and 21.5 ms for HS-WP, indicating that the oil components of the upper layers were quite similar to each other in terms of molecular mobility. For further information, these \( T_2 \) values were comparable to that of fluid oils observed in the previous study (27.3 ms).9) Therefore, although the components have not yet been identified, fluid oils were separated from both preparations as the upper layer. By contrast, the \( T_2 \) values of the lower layers were quite different. The peak position

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Fig. 2. Continuous Monitoring of the Phase Separation of the Mixed Preparations Consisting of O/W-Type Moistening Creams

The preparations were stored at 60°C, and then their MR images were acquired at designated intervals. HC-WP separated into two distinct phases (the interface is marked with an arrow). UC-WP separated into three distinct phases; the interfaces between the upper and middle layer (arrow) and between the middle and lower layers (arrowhead) are shown.

Fig. 3. 1H-NMR Spectra of Each Phase-Separated Layer Acquired from the Preparations Consisting of W/O-Type Moistening Creams

The test samples were stored for 120 h at 60°C.

Fig. 4. 1H-NMR Spectra of Each Phase-Separated Layer Acquired from the Mixed Preparations Consisting of O/W-Type Moistening Creams

The test samples were stored for 120 h at 60°C.
was 62.1 ms for HC-WP and 18.0 ms for HS-WP after storage of the preparations for 120 h. It is likely that the $T_2$ values of the lower layers depend mostly on the molecular mobility of water. That is because water peak at 4.7 ppm was obviously observed in the $^1$H-NMR spectra (Figs. 3, 4), and its proton density is higher than that of oil hydrocarbons. Therefore, this result represents the difference in the state of water in the lower layers caused by their different compositions.

Figure 6 shows a time-dependent increase in the volume of the upper layer. The volume of the upper layer of the HC-WP sample was enlarged from 5.0 ± 0.7 to 18.8 ± 1.5% when the storage time was prolonged from 0.25 to 2 h. The volume eventually reached 28.6 ± 1.8% by 120 h. In contrast, the increase in volume of the upper layer of HS-WP was small; it increased from 4.7 ± 2.2 to 6.6 ± 1.8% during 24 to 120 h. We concluded that the w/o-type cream (HS) was more compatible with the ointment base than the o/w-type cream (HC).

Dermatologists often prescribe mixing of moisturizing...
creams with ointments.1–4) This mixing is aimed at improving the patient compliance by reducing the frequency of application and modifying the texture of the preparations. Cream preparations are thermodynamically unstable emulsions; therefore, to prepare a stable mixed preparation, appropriate selection of the moisturizing cream is crucial. We previously investigated the compatibility of different steroid preparations with moisturizing creams using visual and microscopic observations.5) We found betamethasone valerate-containing cream (Rinderon V cream; Shionogi & Co., Ltd., Osaka, Japan) was compatible with moisturizing creams (HS and HC). However, a preparation consisting of its ointment (Rinderon V ointment) resulted in substantial phase separation after centrifugation (16500×g, 7 min). We found that the emulsion of moisturizing creams was changed substantially by mixing with the Rinderon V ointment. A number of other studies found significant destabilization of the mixed preparations (e.g., phase separation into distinct layers and bleeding of oil components).1,3,13,14) In the present study, we focused on the compatibility of moisturizing creams with an ointment base. We tested various commercial creams that are popularly used in Japan. We included both o/w- and w/o-type moisturizing creams. We also considered it important to select a sensitive method to assess the stability of the emulsions. To date, various methods have been employed. These include droplet size analysis,15,16 tight scattering,15,17,18 and turbidity measurements.19) We believe that our MRI method is better than conventional methods. T2 mapping can depict slight changes in the emulsions with high sensitivity, and MRS is an effective tool for nondestructive component analysis. We successfully characterized the phase-separation behavior of the test samples, and then confirmed that the compatibility of the w/o-type creams with ointment base was better than that of o/w-type creams. These findings strongly support the validity of the mixing operation described in the dispensing guidelines in Japan. For mixing with ointments w/o-type moisturizing creams are preferred to o/w-type creams. The present study may provide valuable information for mixing operations used daily at pharmacies.

Conclusion

We conducted a comparative study of the stability of preparations consisting of various commercial moisturizing creams mixed with steroid ointment base. We gained a comprehensive understanding of the phase-separation behavior of the test samples with the MR techniques used (T2 mapping and MRS). The findings supported the conventional mixing operation of using a w/o-type emulsion to mix with steroid ointments. The present study offers insight into the stability of the preparations.

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Conflict of Interest

The authors declare that they do not have any financial or noncompeting interests concerning this manuscript. The Department of Pharmaceutical Technology, University of Toyama, is an endowed department, supported by an unrestricted grant from Nichi-Iko Pharmaceutical Co. (Toyama, Japan).

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