MRI Monitoring of the Mixed State of Admixtures Consisting of Moisturizing Cream and Steroid Ointment during the Mixing Process by a Revolution/Rotation-Type Hybrid Mixer

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The admixture of a steroid ointment and a moisturizing cream is frequently prescribed to patients suffering from atopic dermatitis. For the mixing operation, a revolution/rotation-type hybrid mixer is widely used in pharmacy. The purpose of this study was to monitor the mixed state of the admixtures during the mixing process of the hybrid mixer. The key technology used in this study was magnetic resonance imaging (MRI). Two different commercial mometasone furoate-containing ointments were used as a test steroid ointment. After layering the moisturizing cream and the steroid ointment in an ointment bottle, the sample was mixed for a predetermined period using the hybrid mixer. According to MRI transverse relaxation time (T2) mapping for nondestructive monitoring, it was confirmed that the Flumeta® ointment-containing admixture became homogeneous by mixing for 60 s or more. As for the mometasone furoate ointment 0.1%-containing admixture, the mixed state, after becoming homogeneous, was separated into two layers again by the prolonged mixing process. From the 1H-NMR spectra of the phase-separated layers, re-separation was caused by removing aqueous components from the bottom of the samples. MRI is a powerful tool for monitoring the mixed state of the admixture during the mixing process. We believe that our findings offer profound insights into the clinical practice of the mixing operation using a hybrid mixer.

Key words mixed state; hybrid mixer; magnetic resonance imaging; nondestructive monitoring; mometasone furoate-containing ointment

Steroid ointments are often prescribed together with moisturizing creams for the treatment of atopic dermatitis and psoriasis, and then they are mixed in an operation that is widely performed at the pharmacy level. The mixing operation is aimed at improving patient compliance; the admixture allows the patients to reduce the frequency of application and modify the sticky and uncomfortable texture of the ointment base.1–5) For the mixing operation, a centrifuge-type apparatus called “a revolution/rotation” hybrid mixer is widely used in pharmacy.6) This apparatus comprises a main rotary head mounted on a vertical axis for the rotation motion and a secondary rotor mounted in bearings on the main head for the revolution motion. Samples are supposed to be centrifuged by the pseudo-planetary motion. This uninterrupted planetary motion creates a considerable centrifugal force that removes small air bubbles from the materials and mixes them at the same time.

The mixing efficiency of the hybrid mixer is much superior to that of hand mixing, but there is still a concern about insufficient mixing of admixtures. According to on-the-job experience of pharmacists, an insufficient mixed state is occasionally pointed out by patients who were prescribed the admixtures. From this perspective, this study was dedicated to nondestructive (ND) monitoring of the mixed state of the admixtures. The key technology of this study was magnetic resonance imaging (MRI).3,7) MRI is a popular molecular imaging method based on the principle of NMR. Beside ND monitoring, it can also visualize the molecular mobility of a compound in a sample using magnetic resonance parameters including the T1 and T2 relaxation times.8) In our previous MRI studies, we investigated the physical stability of mixed preparations consisting of moisturizing creams with a steroid ointment. After preparing homogeneous mixed preparations, an accelerated destabilization testing was conducted using centrifugation or storage at high temperature. During the accelerated testing, their emulsion states were monitored by T2 mapping.9,10) From these studies, T2 mapping proved to be effective for detecting the change in emulsion state (e.g., creaming and phase separation).

The purpose of this study was to monitor the mixed state of admixtures consisting of a steroid ointment and a moisturizing cream during the process of mixing by the hybrid mixer. As result of this study, we confirmed that the MRI technique was a powerful tool for this purpose.

Experimental

Materials Heparinoid cream 0.3% [YD], a moisturizing cream containing heparinoid, was purchased from Nihon Generic Co., Ltd. (Tokyo, Japan). Flumeta® cream (FLU) (the original brand of the ointment containing mometasone furoate), was purchased from Shionogi & Co., Ltd. (Osaka, Japan), whereas mometasone furoate ointment 0.1% (FUR), the generic equivalent, was purchased from Iwaki Seiyaku Co., Ltd. (Tokyo, Japan). All other chemicals used were of analytical grade.

Sample Preparation The moisturizing cream (5 g) was placed at the bottom of a 12-mL plastic ointment jar, and then

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an equal weight of steroid ointment was piled on the cream. The revolution/rotation-type hybrid mixer (Model HM-500; Keyence, Osaka, Japan) operates in two standard modes, one for mixing and the other for degassing. The revolution and rotation speeds of the mixer were 2000 and 800rpm, respectively. The treatment modes are different in terms of the ratio of motions of rotation and revolution. First, the degassing mode treatment of the mixer was applied to the admixture for 30s to remove air bubbles; this treatment is similar to normal centrifugation. In this experiment, the sample was defined as “initial period” (0 h). Afterwards, the mixing was performed using the mixing mode of the hybrid mixer for predetermined periods up to 180s.

MRI Study The MRI experiments were performed at room temperature using a 9.4T 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) of 2000ms, echo times (TEs) of 25, 40, and 60ms, with a field of view of 35×35mm² and a matrix size of 128×128; the number of excitations was 2, and the slice thickness was 1mm. A quantitative spin–spin relaxation time or transverse relaxation time ($T_2$) map was constructed from the three MR images with different TE values. The acquisition of the $T_2$ map was triplicated for each sample. Afterwards, histograms of $T_2$ data were created by random sampling of 1000 data from the maps, and then the peak fitting of the histograms was performed using a Gauss function. Origin Pro 2015 (Origin Lab, Northampton, MA, U.S.A.) software was used for curve fitting. Then, the mean peak positions were calculated from three individual maps. The acquisition of the $^1$H-NMR spectrum of the sample was performed by an SPLUS sequence with a TR of 500ms and a number of excitations of 128.

Results and Discussion We first monitored single steroid ointments and the moisturizing cream by $T_2$ mapping. Uniform features were observed from the maps of these products (Fig. 1A) and then their histograms were created (Fig. 1B). Histogram analysis allows us to discuss the emulsion state quantitatively. The mean values were 20.13±0.51, 23.47±0.44, and 18.60±0.26 ms, respectively for FLU, FUR, and the moisturizing cream. The values for FLU and FUR were distinct from those of the moisturizing cream, indicating that $T_2$ mapping makes it possible to distinguish each pharmaceutical in the admixture as long as they are unmixed.

In the next phase of this study, we evaluated the mixed state of the admixtures following mixing by the hybrid mixer. According to the $T_2$ mapping, the change in the mixed state
induced by the mixing process was clearly characterized (Fig. 2). Two distinct layers could be observed in the admixtures before the mixing process (0s). Once the admixtures were treated with the hybrid mixer, the distribution was substantially changed. The mixed state developed clearly from the center of the sample bottle outwards. For the FLU admixture, after only 15 s, an orange region especially located in the center part had clearly disappeared; the steroid ointment was significantly mixed with the moisturizing cream. After mixing for 30 s, the mixing had steadily proceeded, and a small amount of unmixed ointment remained in the left outer part. After 60 s mixing, the admixture appeared to be homogeneous. The homogeneous mixed state did not change even though the mixing period was extended up to 180 s. As for the FUR admixture, the mixing behavior was similar to that of the FLU admixture until the mixed state became homogeneous. After mixing for 15 s, the thickness of the upper steroid ointment layer become significantly thinner by mixing with the moisturizing cream, and after mixing for 30 s, a small amount of steroid ointment still remained in the outer parts of the samples. After mixing for 60 s, the admixture appeared to be almost homogeneous. The FUR admixture was re-separated into two distinct phases from the homogeneous mixed state by excessive mixing; the bottom right corner of the mixing admixture after 60 s showed slightly longer $T_2$, and then two distinct phases were clearly observed after 180 s mixing.

The histograms of the $T_2$ maps of the admixtures were also created (Fig. 3A), and then the peak positions in the histograms were calculated by curve fitting (Fig. 3B). Before the mixing process, the histograms showed two distinct peaks (here, “Peak 1” is the peak with the shorter $T_2$ and “Peak 2” is the peak with the longer $T_2$). The means of Peaks 1 and 2 for the FLU admixture were 17.95±0.08 and 20.83±0.17, and for the FUR admixture the means were 17.33±0.24 and
23.40 ± 0.28; these values were consistent with those of single pharmaceuticals observed in Fig. 1. After 15 s mixing, the initial two distinct peaks turned into a single one. Afterwards, the single peak became sharpened with a prolonged mixing period. In particular, a sharp and symmetric peak was observed for FLU admixtures with 30 s mixing or more (Fig. 3A); the mean peak position was 18.41 ± 0.17 s for 30 s mixing admixture (Fig. 3B); this value was comparable to the average of the two pharmaceuticals. Thus, the FLU admixture reached a homogeneous mixed state by mixing for 30 s, and then the state did not change even though the mixing period was prolonged to 180 s. By contrast, the histograms of the FUR admixture were substantially changed with prolonged mixing period. In the initial phase of the mixing process (until 60 s), the two distinct peaks disappeared and turned into a single peak; this behavior was similar to that of the FLU admixture. As for the admixture after 180 s mixing, two distinct peaks were clearly detectable in the histogram; these mean values eventually led to peak positions of 22.20 ± 0.38 and 30.53 ± 1.98 s, respectively. It represents the re-phase separation. We stressed that these $T_2$ values were quite different from the original FUR ointment or the moisturizing cream. Thus, the phase re-separation was caused by removing some specific components from the admixture.

Component analysis based on $^1$H-NMR spectra was also performed in the present study to identify the mechanism responsible for the phase re-separation observed for the FUR admixture. First, we examined single pharmaceuticals and homogeneous admixtures. Concerning the FLU and FUR ointments, a single peak occurring around 1.6 ppm was observed (Figs. 4A, B). It denotes the hydrocarbon groups of the oily base of the ointment, such as white petrolatum. The moisturizing cream showed two main peaks occurring at 1.6 and 4.7 ppm (Fig. 4C). They were assigned to the hydrocarbon groups of the oils and to water, respectively. As a homogeneous admixture, the FLU admixture mixed for 180 s and the FUR admixture mixed for 30 s were tested (Figs. 4D, E). A similar peak intensity ratio between the hydrocarbon peak (1.6 ppm) and the water peak (4.7 ppm) was observed for the homogeneous admixtures (Figs. 4D, E). Subsequently, phase-separated layers were examined; fractions of each layer were sampled and then their $^1$H-NMR spectra were acquired. Figures 4F and G presents the $^1$H-NMR spectrum of the upper and lower phase-separated layers of the FUR admixture after 180 s mixing. From these spectra, two main peaks at around 1.5 and 4.7 ppm were observed. The lower and upper layers showed differences in their peak intensity ratio. Namely, the oily base peak (1.5 ppm) of the upper layer was obviously
higher than that of the lower layer (Fig. 4F), and a higher water peak (4.7 ppm) was observed for the lower layer (Fig. 4G); thus, the upper and lower layers were oil- and water-enriched emulsion layers, respectively. From these results, the mechanism of the phase re-separation of the FUR admixture was fully understood: namely, water was removed from the admixture because of excessive mixing. Although the exact reason is still unclear, several possible reasons are cited to explain the difference in the stability of the admixtures. For one thing, their formulations are different. FUR contains bleached beeswax and propylene glycol stearate, while FLU contains N-methylpyrrolidone, propylene carbonate, and liquid paraffin. In addition, we observed that the viscosity of FUR admixture was lower than that of FLU admixture (see Supplementary material, Fig. S1). There are several reports suggesting that the rheological properties of steroid ointments significantly affect the emulsion stability and then viscous mixed preparations were generally more stable.12,13)

In clinical practice, the hybrid mixer is frequently used for the preparation of admixtures of a steroid ointment and moisturizing cream. We stress that this study could offer a sensible solution to monitor changes in the mixed state occurring during the mixing process. Furthermore, this study clarified the risk of serious destabilization of admixtures by excessive mixing. It is necessary to keep in mind that this problem might arise whenever a mixing operation is clinically performed.

Conclusion

This is the first technical report on ND monitoring of the mixing process by the revolution/rotation-type hybrid mixer. According to the $T_2$ mapping, we successfully characterized the difference in mixing behaviors between the FLU and FUR admixtures. The FLU admixture became homogeneous after mixing for 60–180 s. As for the FUR admixture, after achieving a homogeneous mixed state, it was separated again into two distinct phases by excessive mixing. From the component analysis using NMR spectra, the phase re-separation was a result of removing aqueous components from the bottom of the sample. Our study presents valuable information for the clinical practice of the mixing operation using a hybrid mixer.

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Conflict of Interest

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Supplementary Materials

The online version of this article contains supplementary materials.

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