

Effects of mixing heparin analog formulations on moisturizing effects and formulation changes

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Abstract

Moisturizers are important in treating atopic dermatitis, and in mild cases, moisturizers alone can improve symptoms. In moderate or severe disease, moisturizers are generally used in combination with topical steroids. However, as topical agents were originally designed for use alone, formulation changes during mixing can be problematic. In this study, Hirudoid® soft ointment and Hirudoid® cream, which are the most widely prescribed heparin analogs, were used as test drugs by mixing them with white petrolatum, which is also a base for topical steroids, to investigate changes in formulation and effects on moisturizing properties.

No changes in formulation were observed after 8 weeks of application of the Hirudoid® soft ointment and white petrolatum mixture. However, the Hirudoid® cream and white petrolatum mixture demonstrated emulsification breakdown after 4 weeks. The Hirudoid® soft ointment and white petrolatum mixture significantly decreased moisturizing efficacy compared with application of the mixture alone. Similarly, the Hirudoid® cream and white petrolatum mixture significantly decreased moisturizing efficacy.

To prevent a decrease in moisturizing efficacy owing to the mixture of moisturizers and topical steroids, careful attention should be paid to the type of base agent, and mixing the two should be carefully decided. This study provides important insights into the use of topical agents to maximize the moisturizing effects of atopic treatments.

Key words: atopic dermatitis, cream, ointment, moisturizer

1. Introduction

Skin care by moisturization plays an important role in the treatment and prevention of the recurrence of atopic dermatitis^{1,2)}. Currently, urea- and heparin-containing preparations with moisturizing properties are used as topical products because they are highly effective for dry skin³⁾.

Although moisturizers alone can improve mild cases of atopic dermatitis, they are often used in combination with topical steroids and other topical skin preparations in cases of moderate or severe disease. Mixed preparations are used in combination with topical steroids and other topical skin preparations to improve patient adherence and

reduce steroid dose⁴⁾. In a survey of dermatologists and paediatricians reported that 88.5% prescribed mixed preparations⁵⁾. In this report, heparin analogues were reported to be used most frequently as a mixture with topical steroids (34.8%). However, topical skin preparations are originally designed to be used alone, as the base agent is selected considering drug properties, disease, and indicated site. Hence, in medical practice, mixing and dispensing are performed with reference to a book summarizing the changes in formulations.

Regarding formulation changes due to mixed preparations, according to the Handbook of Changes in Ointment and Cream Formulations, Second

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Edition (Jiho Co., Ltd.), a 1:1 mixture of Hirudoid® soft ointment 0.3%, a water-in-oil base, and white petrolatum will separate after 4 weeks. In addition, a mixture of oil-in-water base Hirudoid® cream 0.3% and white petrolatum will be stable after 8 weeks⁶⁾. Nevertheless, oil-in-water base agents are typically incompatible with fat-based agents because of the instability of the emulsion upon mixing with fat-based agents, which allows air to enter the emulsion⁷⁾.

A study investigating the moisturizing effects of topical skin preparations has reported that a 1:1 mixture of steroid ointment and moisturizer increased steroid permeation through the skin⁸⁾. However, the effect of mixed preparations on moisturizing properties was significantly reduced in a 1:1 mixture of heparin analog ointment (Hirudoid® soft ointment) and white petrolatum, which is assumed to be a steroid ointment, compared with the mixture alone⁹⁾.

In this study, Hirudoid® soft ointment and Hirudoid® cream, the most widely prescribed heparin analog preparation, were used as test drugs by mixing them with white petrolatum to investigate changes in formulation and effects on moisturizing properties.

2. Methods

2.1. Formulation changes

2.1.1. Samples

The following samples were used: Hirudoid® cream 0.3% in an oil-in-water emulsion base, Hirudoid® soft ointment 0.3% in an oil-in-water emulsion base, which are preparations containing heparin-like substances, and white petrolatum, which is a base for topical steroids.

2.1.2. Preparation

Mixing and adjustment were performed at a 1:1 ratio of 0.3% of Hirudoid® soft ointment and white petrolatum (ointment MIX) and 0.3% of Hirudoid® cream and white petrolatum (cream MIX). The mixing was adjusted manually using an ointment plate and spatula.

2.1.3. Observation

The prepared ointment MIX and cream MIX were stored at room temperature (25 °C) and in a cool

place (4 °C), and any changes in the formulation were monitored weekly by visually examining the ointment jars and performing a microscopic examination. The observation period was 8 weeks, as described in the Handbook on Ointment and Cream Formulation Changes.

2.2. Moisturizing effect

2.2.1. Participants

Subjects were recruited between October and December 2023: nine healthy male and female volunteers aged 10-60 years (five males and four females, mean age 27.8 ± 18.1 years), without skin diseases or allergies, with the forearm as the target site.

2.2.2. Equipment used

A portable skin moisture meter HP10-N (Integral Corporation, Tokyo, Japan) was used.

2.2.3. Clinical study

The moisturizing effect was determined by measuring the water content of the stratum corneum at four points on the forearm and target site.

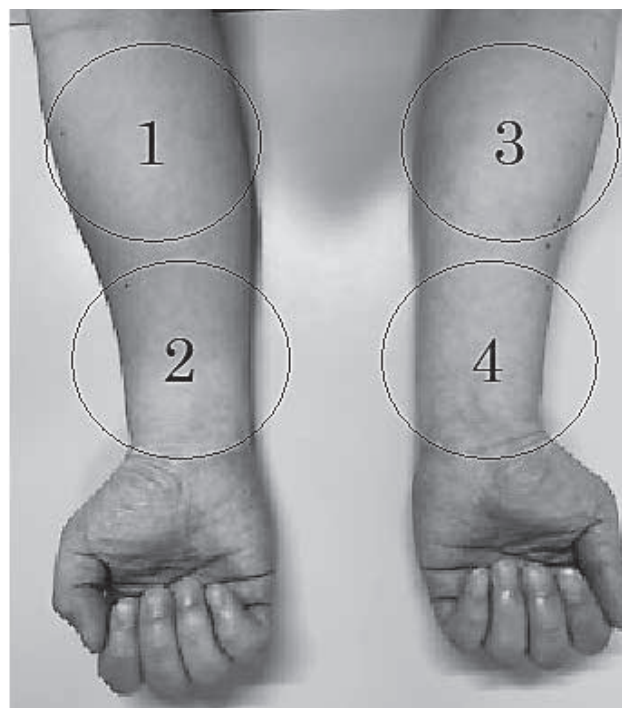


Fig. 1. Four measurement sites in clinical trials.

Four points on the forearms of the subjects were used to measure the water content of the stratum corneum. Measurements were taken five times consecutively on one occasion, and the average value was used as the measured value for analysis.

area (Fig. 1). The water content of the corneum was measured before, immediately after, 1 h after, and 4 h after application of the test drug. Measurements were performed five times consecutively on one occasion, and the average value was used for the analysis. The moisture meter uses the capacitance method, which indicates the water content of the stratum corneum as a relative value from 0 to 99 a.u.; the higher the water content of the skin, the higher the value^{10,11)}.

2.2.4. Statistical analysis

Results are expressed as mean \pm standard deviation. The water content of the stratum corneum after application of the test drug was compared at different time points using the Student's *t*-test. Statistical significance was set at $P < 0.05$.

2.2.5. Ethical considerations

The study protocol conformed to the Ethical Guidelines for Life Sciences and Medical Research involving Human Subjects and was approved by the Ethics Committee of Iryo Sosei University (approval number: 22-03). Written informed consent was obtained from all the participants.

3. Results

3.1. Formulation changes

Changes in the formulation of the ointment MIX and cream MIX were observed by visually inspecting the ointment jars immediately after mixing, and after 1, 2, 4, and 8 weeks. No changes in the formulation were observed at room temperature or during cold storage.

Microscopic observations ($\times 120$) showed uniform results of the ointment MIX both at room

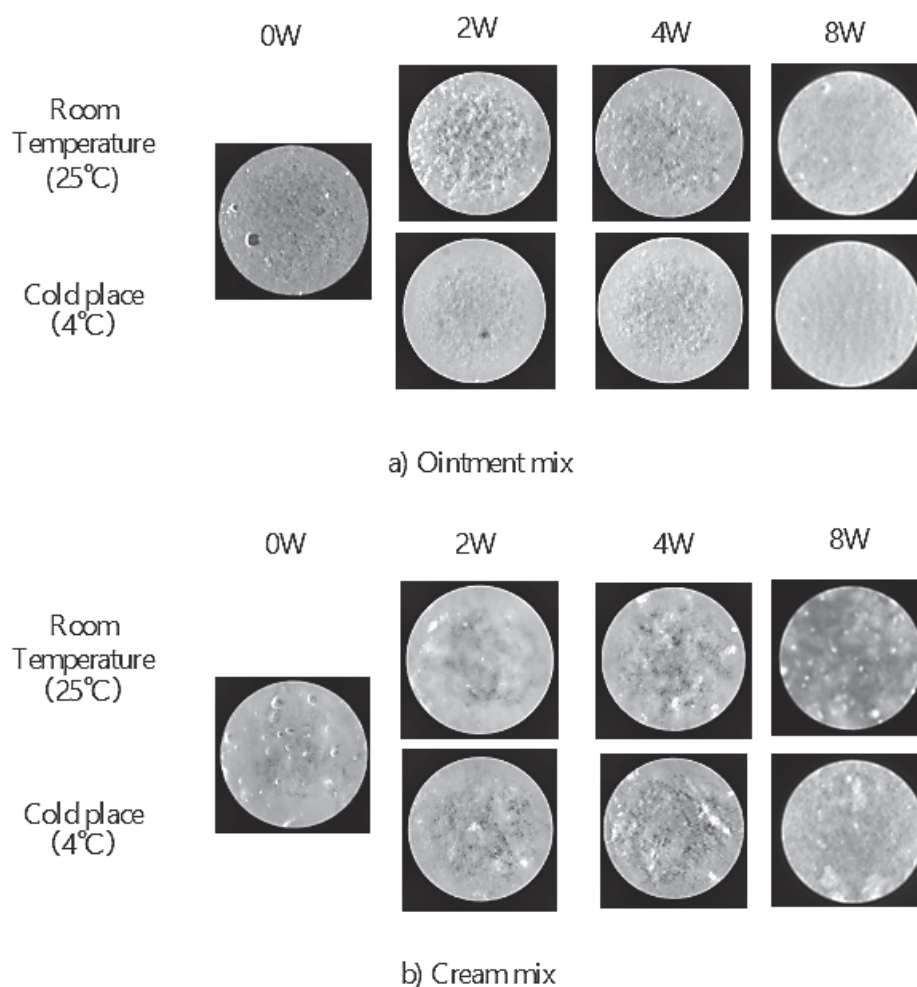


Fig. 2. Microscopic images.

Changes in the formulation of ointment MIX and cream MIX stored at room temperature (25°C) and cold place (4°C) were observed using a microscope ($\times 120$) just after mixing (0W), after 1 week (1W), after 2 weeks (2W), after 4 weeks (4W) and after 8 weeks (8W).

temperature and under cold conditions, from immediately after mixing to after 8 weeks. However, in the cream MIX, small lumps were observed after 4 weeks at room temperature, and after 8 weeks, the cream became lighter overall. Similarly, white lumps were observed after 8 weeks under cold conditions (Fig. 2).

3.2. Moisturizing effects

The water content of the stratum corneum up to 4 h after application showed no significant differences between the ointment MIX and cream MIX (Fig. 3). Immediately after application of both agents, the water content of the stratum corneum decreased, with values of -1.42 ± 9.34 and -1.68 ± 7.59 , respectively. At 1 h after application, the water content of the stratum corneum applied with the ointment MIX remained unchanged at -1.86 ± 16.99 , while that for the stratum corneum applied with the cream MIX increased to 2.95 ± 14.91 . At 4 h after application of the ointment MIX and cream MIX, the water content increased to 9.60 ± 11.30 and 12.1 ± 14.16 , respectively.

The water content of the stratum corneum after the application of Hirudoid® soft ointment alone was 23.95 ± 18.20 just after application, $16.86 \pm$

11.64 at 1 h post-application, and 14.00 ± 9.36 at 4 h post-application. A significant difference in water content between the ointment MIX and Hirudoid® soft ointment alone was observed after administration, 1 h post-application, and 4 h post-application ($P < 0.01$, Fig. 4a). Additionally, the water content of the stratum corneum after the application of Hirudoid® cream alone was 46.82 ± 18.55 just after application, 15.15 ± 9.56 at 1 h post-application, and 11.68 ± 5.33 at 4 h post-application. A significant difference in water content between the cream MIX and Hirudoid® cream alone was observed after administration, at 1 h post-application ($P < 0.01$), and at 4 h post-application ($P < 0.05$, Fig. 4b). The water content of the stratum corneum after the application of white petrolatum alone was -29.31 ± 21.97 just after application, -7.97 ± 26.80 at 1 h post-application, and 1.42 ± 12.93 at 4 h post-application. The water content of the stratum corneum increased over time and returned to pre-application levels 4 h post-application (Fig. 5).

4. Discussion

According to a survey on the mixing of topical skin products, >80% of dermatologists mix topical

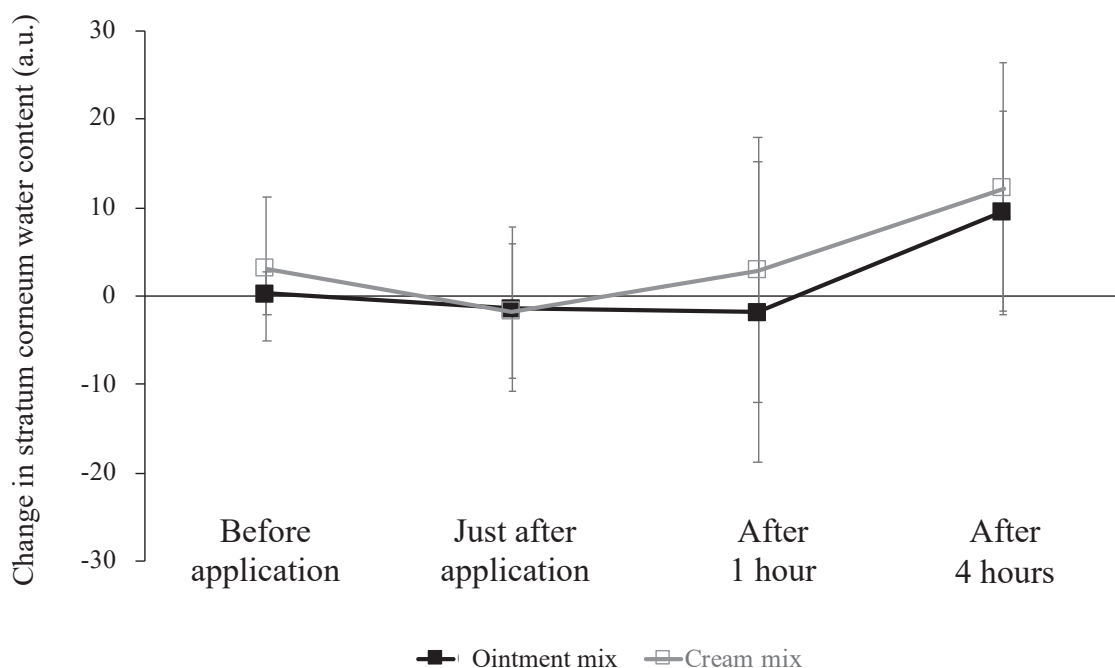
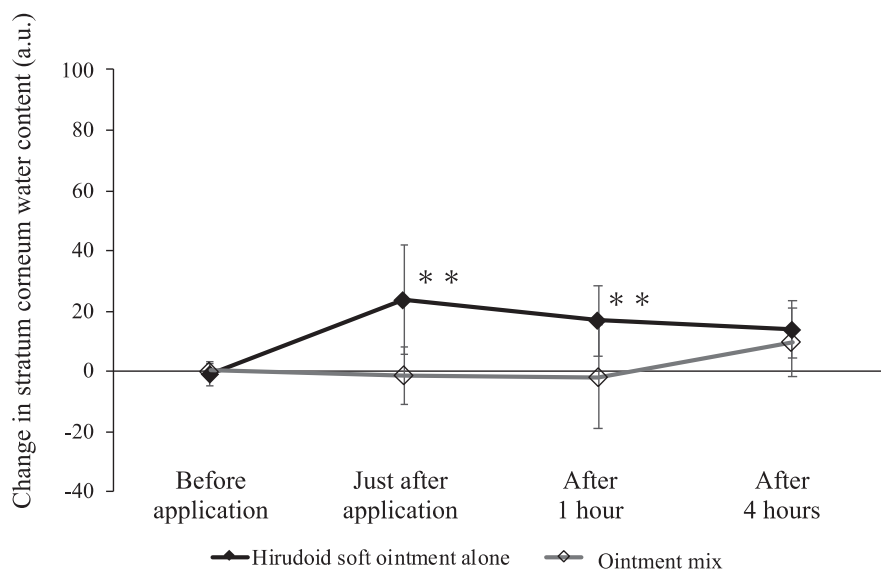
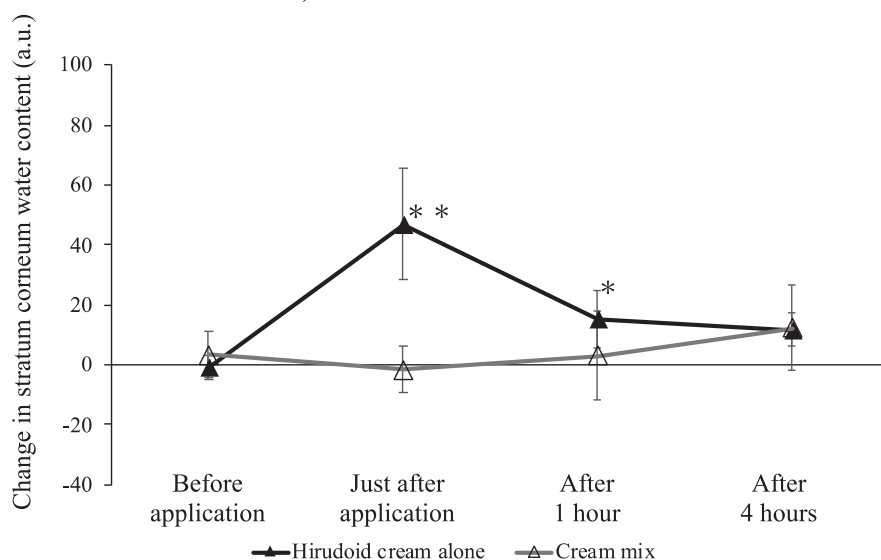


Fig. 3. Comparison of the water content of the stratum corneum of ointment mixes and cream mixes.

The water content of the stratum corneum of the target areas to which the ointment mix and cream mix were applied was measured over time before, just after, 1 h after and 4 h after application of the test drugs. Data are expressed as the mean \pm SD (n = 9).



a) Hirudoid soft ointment



b) Hirudoid cream

Fig. 4. Comparison of the water content of the stratum corneum of each mix and alone.

The water content of the stratum corneum of the target areas to which each mix and alone were applied was measured over time before, just after, 1 h after and 4 h after application of the test drugs. Data are expressed as the mean \pm SD (n = 9). **: $P < 0.01$, *: $P < 0.05$

steroids with topical skin products⁴⁾. This is because skin care is important in the treatment of atopic dermatitis in addition to drug therapy with topical skin products. Moreover, the use of topical steroids and topical skin products with moisturizing effects increases the frequency of application and reduces adherence. Therefore, mixing is often used to avoid the hassle of application and to improve adherence. In clinical practice, the most common

ratio of topical steroids to topical skin products is 1:1¹²⁾. Therefore, a 1:1 ratio was used in the present study.

In this study, formulation changes were first examined. The Handbook of Changes in Ointment and Cream Formulations, Second Edition indicates that a 1:1 mixture of Hirudoid® soft ointment 0.3% and white petrolatum separates after 4 weeks, whereas a mixture of Hirudoid® cream 0.3% and

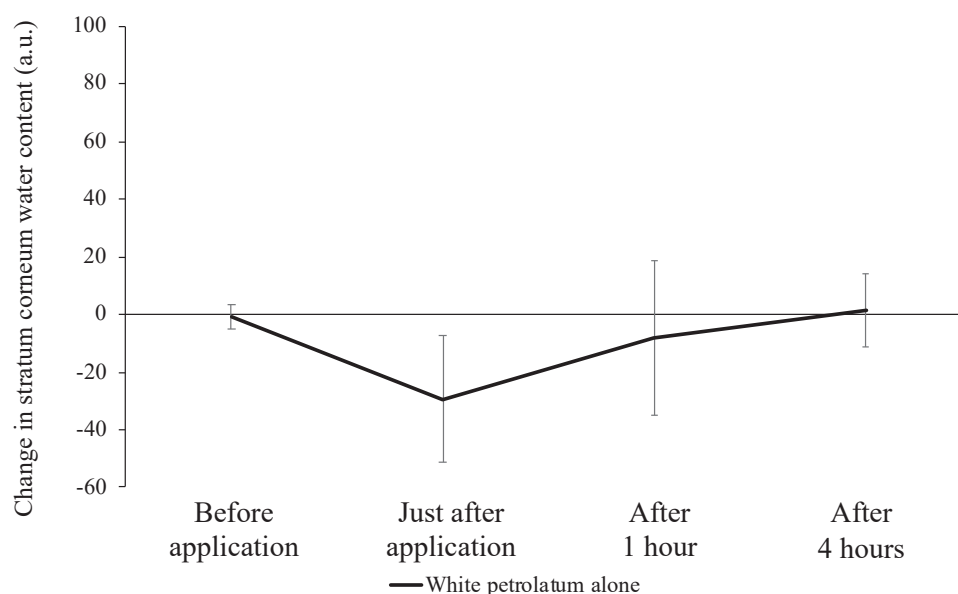


Fig. 5. Changes in the water content of the stratum corneum with white petrolatum.

The water content of the stratum corneum of the target areas to which white petrolatum was applied was measured over time before, just after, 1 h after and 4 h after application of the test drugs. Data are expressed as the mean \pm SD ($n = 9$).

white petrolatum remains stable after 8 weeks. By contrast, in the present study, although changes in appearance were visible in plain sight, no changes were observed in the mixture of Hirudoid® soft ointment 0.3% and white petrolatum after 8 weeks. Furthermore, microscopic observations showed that the Hirudoid® soft ointment 0.3% and white petrolatum mixture remained uniform and stable after 8 weeks. Thus, the mixture of Hirudoid® cream 0.3% and white petrolatum demonstrated emulsification breakdown over time.

In addition, the difference between the moisturizing effects of the ointment MIX and cream MIX was examined. The moisturizing effect was evaluated by comparing the water content of the stratum corneum measured using a portable skin moisture meter. No significant differences were found in measurements at four points on the forearm. As in previous reports, compared with ointment alone, both ointment MIX and cream MIX significantly decreased the water content of the stratum corneum⁹⁾. No significant differences in the measurements up to 4 h after application were observed between the ointment MIX and cream MIX. Both results showed an increase in the water content of the stratum corneum after application over time. This was similar to the course observed

with the water content of the stratum corneum after the application of white petrolatum alone. The water content of the stratum corneum showed negative values from immediately after application (-29.31 ± 21.97) to 1 h post-application (-7.97 ± 26.80). This suggests that white petrolatum may have covered the surface of the skin, preventing accurate capacitance measurements. Based on these results, it is speculated that the ointment MIX and cream MIX did not enhance the moisturizing effect of the skin after application but rather provided a protective function.

A study on the effect of the frequency of topical application on the moisturizing effect of heparin analog formulations has reported that twice-daily application increased the moisturizing effect by 2.5 to 4 times compared with once-daily application¹³⁾. Mixed formulations of topical steroids and heparin analogs often provide instructions for once-daily application as the main purpose of topical steroids. However, the present findings indicate that the moisturizing effect is significantly reduced when white petrolatum is mixed with Hirudoid® cream as well as with Hirudoid® soft ointment 0.3%. In this study, the combination of Hirudoid® soft ointment, Hirudoid® cream and white petrolatum was investigated until only. Future studies should also

pay attention to the possible loss of moisturising effect due to less frequent application of topical steroids.

Therefore, when mixing topical skin medicines, carefully considering the type of base agent and possibility of reduction in moisturizing effects is necessary.

5. Acknowledgements

Not applicable.

6. Competing Interests.

There are no conflicts of interest to declare.

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