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Studies on Improvement of Pharmaceutical Preparations Prescribed in Hospitals. III.[1] Prevention of Fading by Use of Solid Dispersion System of Ointment Containing Methylrosaniline Chloride[2]

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Conventionally, an aqueous solution of methylrosaniline chloride kneaded with 10% zinc oxide ointment has been used for disinfection and drying of operative wounds in our hospital. However, this ointment undergoes remarkable fading with time, and this is assumed to indicate a loss of antibacterial activity. Therefore, an attempt was made to improve the pharmaceutical characteristics of the ointment. Since methylrosaniline chloride in solid form is known to show little or no fading, an aqueous solution of methylrosaniline chloride was adsorbed on microcrystalline cellulose, which was then dried. The powder obtained by grinding the dried substance was kneaded with a zinc oxide ointment. The resulting ointment was found to be an effective preparation, showing less fading and an extremely small loss of antibacterial activity.

Keywords——methylrosaniline chloride; 10% zinc oxide ointment; fading; antibacterial activity; microcrystalline cellulose

Methylrosaniline chloride (abbreviated as MRC hereinafter) is an effective bactericidal disinfectant against gram-positive bacteria, in particular *Staphylococcus* and *Pseudomonas aeruginosa*, as well as fungi.[3] Aqueous solutions of MRC are widely used in clinical practice. At our hospital, MRC in aqueous solution is used for the purpose of disinfection and drying of affected areas after kneading with a 10% zinc oxide ointment incorporating rape seed oil and white beeswax. However, the MRC contained in the ointment fades remarkably from bluish purple to colorless, and this is assumed to indicate a loss of antibacterial activity. Since MRC in solid form shows little or no color changes, we adsorbed an aqueous solution of MRC on microcrystalline cellulose, dried it, and kneaded the resultant powder into a zinc oxide ointment. This preparation was then examined for fading and loss of bactericidal activity in comparison with the conventional aqueous solution of MRC kneaded into the zinc oxide ointment.

Materials and Methods

Materials——MRC (Kanto Chemical Co., Inc.), microcrystalline cellulose (abbreviated as MCC hereinafter; Asahi Chemical Industry Co., Ltd.; Avicel PH-M06), zinc oxide, white beeswax and rape seed oil were all products meeting the standards of JPX. Other reagents were commercial products of special grade.

Preparation of Ointment——Table I shows the formulations of the ointment prepared by kneading an aqueous solution of MRC with a zinc oxide ointment (abbreviated as P-ZS) and the ointment prepared by kneading MCC powder containing MRC with the zinc oxide ointment (abbreviated as PM-ZS).

Figure 1 shows the methods used for preparation of P-ZS and PM-ZS.

TABLE I. Formulations of P-ZS and PM-ZS

	P-ZS (g)	PM-ZS (g)
Zinc oxide	10	10
White beeswax	20	20
Rape seed oil	70	70
Methylrosaniline chloride	0.06	0.06
Microcrystalline cellulose (Avicel PH-M06)	—	6

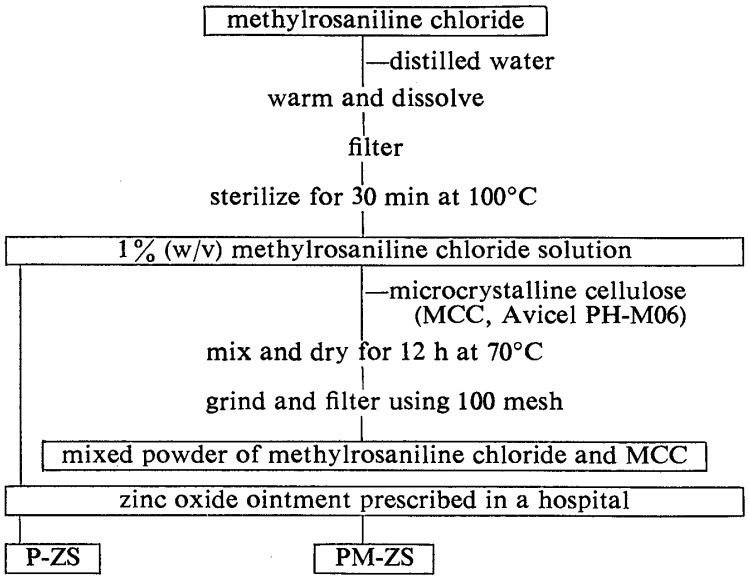

Fig. 1. Method of Preparation for P-ZS and PM-ZS

Observation of Fading of Ointment——P-ZS and PM-ZS were stored at a temperature of 40 °C and the color changes of the ointments were examined by taking photographs on days 0, 7, 14, and 28.

Measurement of Degree of Fading of Ointments——P-ZS and PM-ZS were each packed in a cylindrical glass cell 25 mm in diameter and 10 mm in height and sealed to prevent contact with air. These glass cells were stored at 5, 25 and 40 °C for 35 d. Then the glass cell was applied to an integrating sphere accessories of 60ϕ and the absorbances at 400 to 800 nm were determined by using a model 228A spectrophotometer (Hitachi Ltd.). A graph was drawn by plotting the wavelength on the abscissa and the absorbance on the ordinate. The area under the curve was defined as the color concentration. Taking the initial color concentration at the time of preparation of each ointment as 100%, the degree of change in the color of the ointment was calculated by comparing the color concentrations.

Determination of Antibacterial Activity of Ointment——P-ZS and PM-ZS were stored at 5, 25 and 40 °C for 35 d and the antibacterial activity of the ointment was determined at various time points. One gram of the ointment was accurately weighed into a separatory funnel and 10 ml of ether, 5 ml of isopropyl myristate and 15 ml of sterile water were added. In this way, the MRC contained in the ointment was extracted into the aqueous phase. Phosphate buffer (pH 6.0) was added to 2 ml of the extract to give 20 ml, and this solution was used as the test solution. The antibacterial activity was determined by the cup-plate method in accordance with the methods established in the Minimum Requirements for Antibiotic Products, issued by the Japanese Ministry of Health and Welfare.[4] *Micrococcus luteus* ATCC 9341 was used as the test strain. The inhibition zone diameter formed after 18 to 20 h of incubation at 32 °C was determined by using a ZA-FX zone analyzer system (Toyo Sokki Co., Ltd.).

Determination of Biological Activity of Ointment——To determine the biological activity of P-ZS or PM-ZS, the following experiments were performed. Stainless steel cylinders of 7.9 to 8.1 mm in outer diameter, 5.9 to 6.1 mm in inner diameter and 9.9 to 10.1 mm in height were filled with P-ZS or PM-ZS and placed on an agar medium covered with the test strain, *Micrococcus luteus* ATCC 9431; this system was designed to simulate the skin surface of affected sites. The composition of the agar medium used for this experiment was the same as that used for the antibacterial activity determinations. Similarly, a stainless steel cylinder was filled with a standard solution of MRC, placed on the same agar medium and incubated for 18 to 20 h at 32 °C. The biological activity of P-ZS or PM-ZS in this skin model

was determined by directly comparing the inhibition zone diameters formed by the standard solution and by the ointment, on the assumption that all the MRC in the standard solution penetrated into the medium.

Results and Discussion

Fading of MRC-Containing Ointment

Figure 2 shows the changes in color concentration of P-ZS and PM-ZS as a function of time during storage at 40 °C for 28 d. For P-ZS, a significant change was seen in the color concentration, and the color of the ointment had faded completely at 14 d. PM-ZS, however, did not show fading or color change even at 28 d after preparation.

Figure 3 shows the changes in the color concentrations of P-ZS and PM-ZS *versus* time at 5, 25 and 40 °C. P-ZS showed greater fading than PM-ZS after storage at each of the temperatures. In particular, rapid fading was seen within 7 d after preparation, and its extent

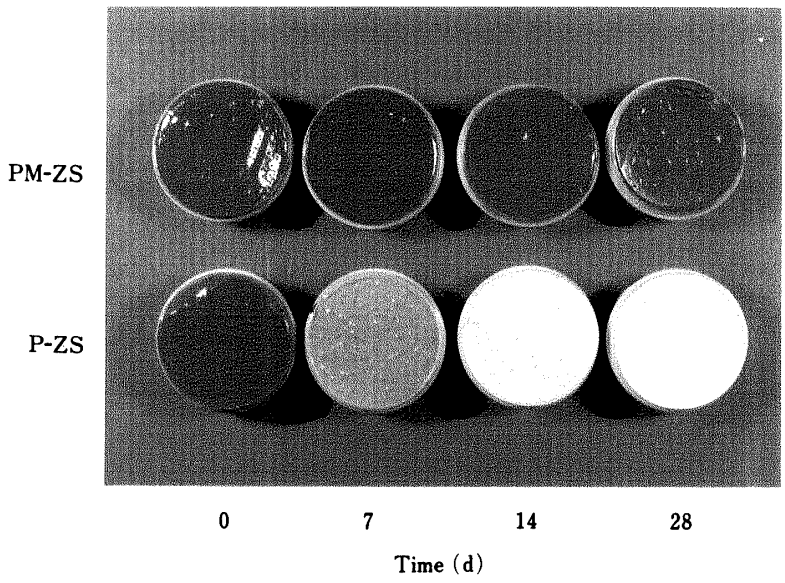

Fig. 2. Change in Color Concentration of PM-ZS and P-ZS Kept at 40 °C for 28 d

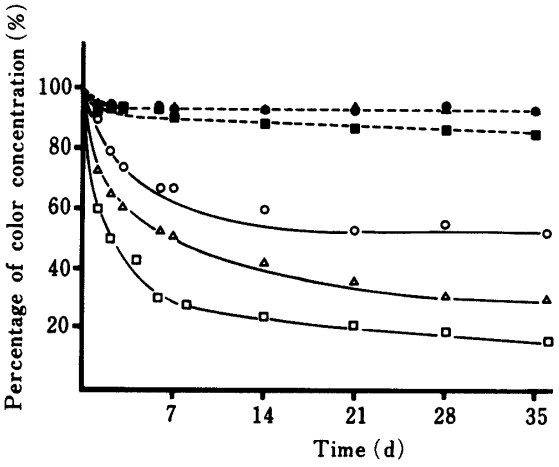

Fig. 3. Change in Percentage of Color Concentration of P-ZS and PM-ZS during Storage at Three Temperatures

P-ZA: ○, 5 °C; △, 25 °C; □, 40 °C. PM-ZS: ●, 5 °C; ▲, 25 °C; ■, 40 °C. Initial color concentration: P-ZS, 111.9 abs*nm; PM-ZS, 111.8 abs*nm.

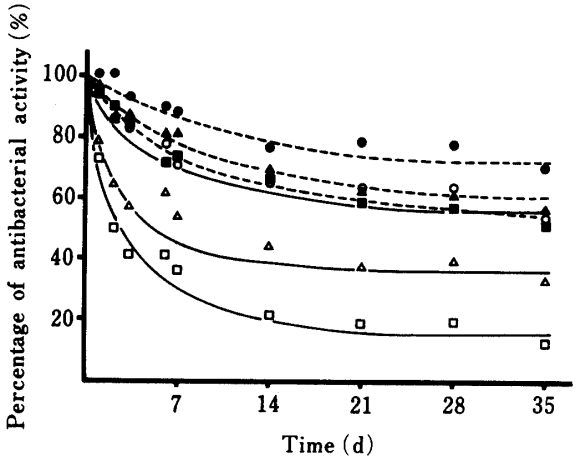

Fig. 4. Change in Percentage of Antibacterial Activity of P-ZS and PM-ZS during Storage at Three Temperatures

P-ZS: ○, 5 °C; △, 25 °C; □, 40 °C. PM-ZS: ●, 5 °C; ▲, 25 °C; ■, 40 °C. Initial antibacterial activity: P-ZS, 4.6 mg/g; PM-ZS, 5.0 mg/g.

was proportional to the temperature. In contrast, PM-ZS retained more than 90% of its initial color concentration even after 35 d of storage at 40 °C, indicating that it undergoes little fading during storage.

It has been reported that fading of MRC is caused by reduction of purple-colored MRC to the colorless leuco methyl-*p*-rosaniline form.[5] The fading of the ointment containing MRC is also assumed to be related to reduction of the MRC in the ointment due to unknown causes into the leuco form. Less change in the color concentration was observed with PM-ZS than with P-ZS. This may be explained by the fact that the MRC contained in MCC is present in a solid form with a lower rate of reduction (conversion to the leuco form) compared to P-ZS, which was kneaded with an aqueous solution of MRC.

Antibacterial Activity of Ointment

P-ZS and PM-ZS were stored at 5, 25 and 40 °C for 35 d and the antibacterial activities of the ointment were determined at various periods. Figure 4 shows the changes in antibacterial activity expressed as percentage, taking the initial potency as 100%. Compared with PM-ZS, P-ZS showed greater loss of antibacterial activity during storage, with the difference becoming greater as the temperature of storage increased. From these results, it was considered that the loss of antibacterial activity can be prevented by using a solid dispersion of MCC powder containing MRC.

Relationship between Change in Color Concentration of MRC-Containing Ointment and Its Antibacterial Activity

Figure 5 shows the relationship between the color concentration and the antibacterial activity; the initial color concentration is taken as 100% (plotted on the abscissa), while the initial antibacterial activity is taken as 100% for P-ZS, which showed greater color changes and loss of potency. As can be seen in Fig. 5, a linear relationship was found between these parameters at each storage temperature. The slope of the line was almost 1. From this result, it can be assumed that colorless leuco methyl-*p*-rosaniline has little or no antibacterial activity.

Biological Activity of the Ointment

Figure 6 shows the amount of methylrosaniline chloride that penetrated from P-ZS or PM-ZS into the medium simulating the skin. Compared to P-ZS, PM-ZS showed greater

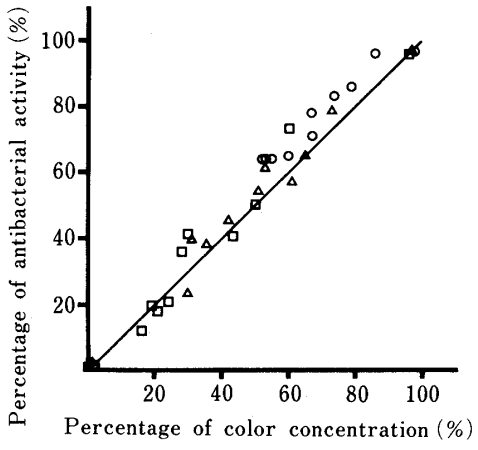

Fig. 5. Relationship between Percentage of Color Concentration and Antibacterial Activity of P-ZS Stored at Three Temperatures

○, 5 °C; △, 25 °C; □, 40 °C.

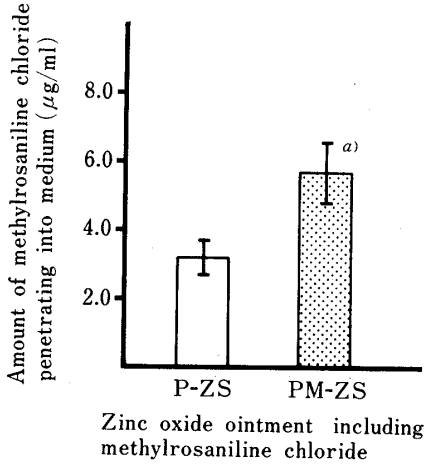

Fig. 6. Amount of Methylrosaniline Chloride Penetrating into the Medium from P-ZS and PM-ZS

Each bar represents the mean ± S.E. ($n=6$). *a*) $p<0.05$ *vs.* P-ZS.

penetration of MRC into the agar medium, and the difference between them was significant.

In this study, the amount of MRC which penetrated into the medium was measured by a method based on the antibacterial activity of unchanged MRC. Therefore, strictly speaking, Fig. 6 shows the difference in the amount of unchanged MRC. Taking into account the fact that antibacterial activity is expected to be obtained from unchanged MRC, the results of the present study can be interpreted as indicating that the stability and the availability of the modified MRC-containing ointment PM-ZS, were considerably improved. Thus, PM-ZS should be useful in clinical practice.

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References and Notes

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