Mixed Base of Hydrophilic Ointment and Purified Lanolin to Improve the Drug Release Rate and Absorption of Water of Minocycline Hydrochloride Ointment for Treatment of Bedsores

Masato SHIGEYAMA,*[,a] Toyoaki OHGAYA,[a] Yoshiaki KAWASHIMA,[b] Hirofumi TAKEUCHI,[b] and Tomoaki HINO[b,1)]

Department of Pharmacy, Takayama Red Cross Hospital,[a] 3–11 Tenman-cho, Takayama, Gifu 506–8550, Japan, and Department of Pharmaceutical Engineering, Gifu Pharmaceutical University,[b] 5–6–1 Mitahora-higashi, Gifu 502–0003, Japan. Received December 10, 1998; accepted March 17, 1999

A desired ointment bases for better treatment of bedsores was developed to improve the release rate of minocycline hydrochloride (MH) and the water absorption capacity using various types of hydrophobic to hydrophilic ointment base. The influence of purified lanolin (PL) on the release behavior of MH from hydrophilic ointment (HO) base was primarily focused on. It was found that the release rate of drug increased with increase in the hydrophilicity of the base. A linear correlation between the apparent release rate constant of drug from the HO and PL mixed ointment base at various combination ratios and the elution of ointment base was noted. The HO ointment base containing 30% PL had the highest apparent release rate constant of MH. The mixed ointment base with the lowest viscosity showed the highest absorption of water and elution of ointment base. In conclusion, it was found that HO (70%) and PL (30%) mixed ointment base was a promising candidate for better treatment of bedsores.

Key words minocycline hydrochloride; ointment base; release rate constant; drug release; Franz diffusion cell; absorbed water

The treatment of bedsores and refractory skin ulcers has become an important issue in modern medical care due to the increase of bedridden people in the aging society. We have been concerned about local treatments for these problems, and have conducted clinical trials of antibiotic ointments, *e.g.* minocycline hydrochloride (MH) ointment, applied against bacterium for the treatment and prevention of infection.[2—6)] The characteristics of the ointment base are critically important in improving the therapeutic effects: requirements are that they be only slightly stimulative and easily washed away with water. Good capacity for water absorption is desired especially at the inflammatory stage which is the highest level of exudation. Kiyohara and his colleagues[7,8)] reported that oily bases improved the efficacy of treatment of open lesions and bedsores, more than did water-soluble or emulsified bases.

A series of specialized analyses of the local environmental factors has recently revealed that humidity is the most important key for healing of lesions.[9—11)] The exudation from the wounded surface or part of the corium layer forms scabs when exposed to air, and this causes the epithelium to be regenerated beneath the scabs. However, when the surface is covered and kept moist, scabs are not formed and the epithelium is regenerated smoothly along the surface of the wound beneath the exudation. Furthermore, it is also assumed that the exudation contains various growth factors generated from macrophage, which hold the key to recovery and that dry conditions delay the healing process due to the disappearance of these factors.[12,13)] Thus, an ointment base must be capable of regulating the humidity of the exudation. Wound dressings are usually airtight films containing no antibacterial substances; thus, the degree of infection frequently becomes worse. In our hospital, bedsore treatment has been conducted using only a lipophilic ointment base admixed with antibiotics, irrespective of disease stage including the infectious period, necrosis and agglutination period, proliferation period of granulation and the formative period of epidermis. The treatment, therefore, has not always been ideal. We wanted to develop a desirable ointment base having a high water absorbing capacity and improved drug releasing property for the incipient stages of bedsores: the infectious period and necrosis and agglutination periods. In the infectious period, an antibiotic substance is applied to lower the degree of infection, where it is necessary to enhance the drug release rate in order to shorten the period of treatment and avoid creating tolerant bacteria. Identification of bedsore bacteria made during our treatment showed that MH had the highest efficacy, so MH was chosen for the present study.

An improved drug MH release rate and water absorption capacity was sought using various types of hydrophobic and hydrophilic ointment bases to provide better treatment of bedsores and refractory skin ulcers. From preliminary drug release tests, a hydrophilic ointment (HO) with the highest rate of drug release was chosen as a basic ointment to use in coformulation with purified lanolin (PL) in an attempt to obtain even greater drug release rate and water absorption capacity. The drug release behavior, the water absorption capacity, the viscosity and the elution of ointment base during the drug release test of the mixture of HO and PL were investigated to determine the optimal formulation parameter, *i.e.* mixing ratio.

Materials and Methods

Materials Powdered MH, (Japan Lederle) for injection, which had been passed through a JIS sifter of 180 μm was used as a model drug. Ointment bases used were hydrophilic petrolatum (HP), absorptive ointment (AO) and HO purchased from Maruishi Pharmaceutical Co., macrogol ointment (MO) (Dainippon Pharmaceutical Co.), white petrolatum (WP) (Nikko Pharmaceutical Co.), PL (Yoshida Pharmaceutical Co.), and petrolatum polyethylene ointment (PO) (Taisho Pharmaceutical Co.). All ointment bases were used as received.

Lactated Ringer's injection (Lactec®) as a medium for the drug release tests (Otsuka Pharmaceutical Co.) was used as received.

Preparation of Ointment Containing MH The ointment bases and

* To whom correspondence should be addressed.

MH were kneaded and homogenized with ointment spatulas on a ceramic slab. The kneaded mixture was homogeneously mixed and dispersed in a warmed water bath at 80 °C and the system was then cooled to room temperature. The drugs were dissolved in the formulations with HO, HP, AO and MO bases and were dispersed uniformly in those of WP and PO. The concentration of MH was 1% in all formulations prepared in the studies.

Evaluation of the Release Rate of MH from Ointment A Franz diffusion cell with membrane installed horizontally was used to evaluate the drug release from the ointment.[14] Seamless cellulose tubing (Visking Co., size 30/32) was used as the membrane after 2 h of washing in distilled water at 80 °C. Five grams of ointment was placed on the cellulose membrane in the receiver cell (the area of the membrane in contact with the ointment: 8.03 cm^2; volume of the cell: 45 ml). Then, 50 ml of distilled water or lactated Ringer's injection solution was introduced into the receiver cell and stirred with a magnetic stirrer. The assembled cell was placed in the water bath thermally controlled at 37 °C. Every 30 min for 3 h 1 ml of the solution was withdrawn and was replaced by 1 ml of the dissolution medium. The MH released in the medium was measured spectrophotometrically at 349 nm (105-40 type, Ultraviolet spectrophotometer, Hitachi, Japan). The data of the drug release test were represented by the mean value of triplicate runs. The diffusion coefficients of MH through the cellulose membrane (seamless cellulose tubing) in distilled water and lactated Ringer's injection solution were measured using the same diffusion cell at 37 °C, and were found to be 6.27×10^{-7} cm^2/s and 1.24×10^{-7} cm^2/s, respectively.

Water Absorption and Elution of Ointment Five grams of the ointment sample was applied to the cellulose membrane mounted on the Franz diffusion cell and 50 ml of the medium was introduced to the receiver cell. The system was placed in the water bath thermally controlled at 37 °C. After 3 h, the ointment and the water it had absorbed was removed from the membrane and weighed (W_1). The ointment completely desiccated with silica gel was weighed (W_2). The percentages of the eluted ointment base (E) and of absorbed water (A) were calculated by the following equations:

$$E=(W_2+W_c+A-W_1)/5\times100 \quad (1)$$
$$A=(W_1-W_2-W_c+E)/5\times100 \quad (2)$$

where W_c is the amount of the water content in the ointment base.

Measurement of Apparent Viscosity The apparent viscosities of ointment were measured by a rheometer (NRM 100-0 model type, Japan Rheology). The radius and edge angle of corn were 6.4 cm and 20°, respectively. The maximum shear rate applied during the measurement was 1800 s, and the acceleration and deceleration of corn speed was programmed to be constant within 60 s. The shear rate *vs*. stress curve of all tested ointment bases exhibited almost a straight line as found by Õishi *et al*.[15] The apparent viscosity of the ointment base at room temperature was determined by calculating the ratio of its slope of straight line to that of standard calibration liquid.

Measurement of Diffusion Coefficient of Drug The diffusion coefficients of MH in distilled water and in lactated Ringer's injection at 37 °C were measured through cellulose membrane inserted in the Franz diffusion cell. The drug solution of test medium with a concentration of 900 μg/ml and drug free medium were introduced into the donor and receptor cells, respectively. The diffusion rate of drug from the donor to receptor medium through the membrane is described by Eq. 1, where V is the volume of medium in the receptor cell, S is the area of cellulose membrane and h is thickness of the membrane.

$$V\cdot dc/dt=DS(C_0-C)/h \quad (1)$$

An integrated form of Eq. 1 is expressed as Eq. 2, where C_0 is the initial concentration of drug solution in the donor cell.

$$\ln\{C_0/(C_0-C)\}=D/h\cdot S/V\cdot t \quad (2)$$

A linear relationship between $\{C_0/(C_0-C)\}$ and t ln was found, consequently the apparent diffusion coefficient (D) was calculated by inserting the slope of straight line obtained (tan α), h (0.0305 cm), V (45 ml) and S (5.3066 cm^2) into Eq. 3

$$D=V\cdot h/S\cdot\tan\alpha \quad (3)$$

Results and Discussion

Release Behavior of MH from Ointment Base To obtain maximum efficacy of the drug contained in the ointment base, the drug released from the base should permeate deeply underneath the skin. Accordingly, the degree of drug release

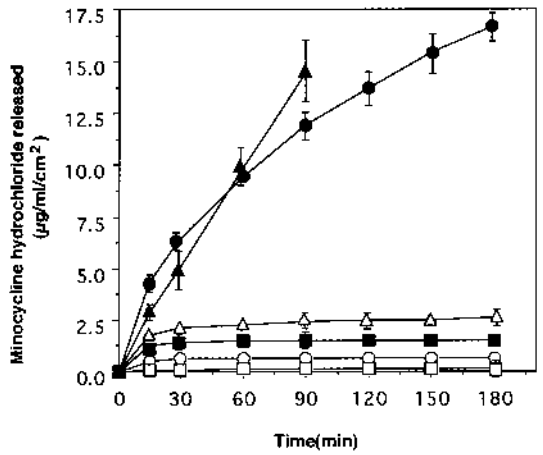

Fig. 1. Release of Minocycline Hydrochloride from Various Ointment Base in Distilled Water

○, White petrolatum (WP); ●, hydrophilic ointment (HO); □, petrolatum polyethylene ointment (PO); ■, hydrophilic petrolatum (HP); △, absorptive ointment (AO); ▲, macrogol ointment (MO). Measurement temperature: 37 °C. Data represent the mean± S.E. of three experiments.

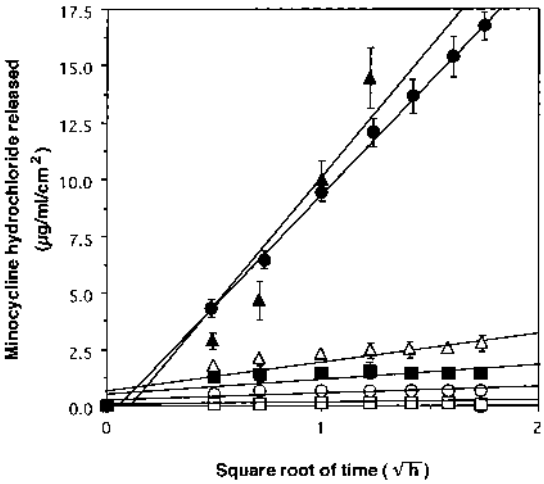

Fig. 2. Drug Release Plots According to Higuchi's Equation for Various Ointment Bases in Distilled Water

○, White petrolatum (WP); ●, hydrophilic ointment (HO); □, petrolatum polyethylene ointment (PO); ■, hydrophilic petrolatum (HP); △, absorptive ointment(AO); ▲, macrogol ointment (MO). Measurement temperature: 37 °C. Data represent the mean± S.E. of three experiments.

is one of the most important factors to determine the absorption of drug by the skin. The drug release behaviors of ointment in distilled water are shown in Fig. 1, where the amount of released MH from six different bases *vs*. time are plotted. In Fig. 2 the relationship between amount of the released MH and the square root of time are displayed according to Higuchi's equation[16,17] assuming that the diffusion of drug through the matrix structure of ointment is the rate determining step. Good linear correlations for almost all bases were found except MO as shown in Fig. 2. Since the MO is water soluble, the matrix structure of the base was easily destroyed, resulting in immediate release of the drug. When the drug is completely dissolved in the base, the release amount can be described by Eq. 4,

$$Q=2C_0\sqrt{Dt/\pi} \quad (4)$$

where Q is the amount of released drug at time t, C_0 is the initial concentration contained in the base and D is the diffu-

Table 1. Apparent Release Rate Constant of Minocycline Hydrochloride from Various Ointments (Distilled Water and Lactated Ringer's Injection)

Ointment	*k*w	*k*l
WP	0.29±0.057	0.22±0.071
HO	9.86±0.459	6.10±0.502
PO	0.09±0.021	0.04±0.018
HP	0.65±0.106	0.50±0.072
AO	1.28±0.268	1.02±0.212

*k*w: apparent release rate constant (μg/ml/cm^2/$\sqrt{h}$) in water. *k*l: apparent release rate constant (μg/ml/cm^2/$\sqrt{h}$) in lactated Ringer's injection. Data represent the mean±S.E. of three experiments.

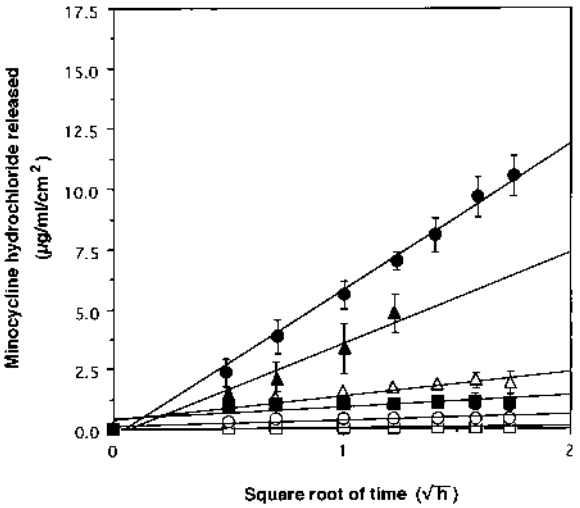

Fig. 3. Release of Minocycline Hydrochloride from Various Ointment Bases in Lactated Ringer's Injection

○, White petrolatum (WP); ●, hydrophilic ointment (HO); □, petrolatum polyethylene ointment (PO); ■, hydrophilic petrolatum(HP); △, absorptive ointment (AO); ▲, macrogol ointment (MO). Measurement temperature: 37 °C. Data represent the mean±S.E. of three experiments.

sion constant of drug in the base. When the drug is suspended in the base, the release amount is calculated by Eq. 5.[18]

$$Q=\sqrt{2ADCst} \quad (5)$$

where A is the concentration of drug, and *C*s is solubility of the drug in the matrix base. Since this latter factor was unmeasurable, the dissolution rates under both dissolved and dispersed drug conditions were evaluated as the apparent velocity by employng Eq. 6:

$$Q=k\sqrt{t} \quad (6)$$

where *k* is the apparent release rate constant.

The apparent release rate constants calculated from the slope of straight line determined by a least squares method are tabulated in Table 1. The rank order of the release rate of MH from ointment base found in Figs. 1 and 2 was: MO> HO≫AO, HP, WP, PO. This order was followed to the hydrophilicity of the base. The HO, an emulsified base having an aqueous outer layer, exhibited the second highest release rate. The AO, w/o type emulsified base, showed a moderate rate ranked between HO and HP. Hydrophobic WP and PO bases reduced significantly the drug release rate. The drug release test conducted with lactated Ringer's injection to simulate a body fluid displayed similar rank order of the release rate to that found in water, as shown in Fig. 3 and Table 1, although the release rates were considerably reduced. This finding might be due to the higher diffusion coefficient of drug in water ($0.627\times10^{-6}\pm0.11$ cm^2/s) than in lactated Ringer's injection ($0.124\times10^{-6}\pm0.04$ cm^2/s) filling the channeled pores of the ointment matrix. Based on the above results, HO was chosen as a basic ointment to investigate improvements in the drug release rate and the water absorption capacity of the ointment base by coformulary PL, because of its excellent water absorbable property.

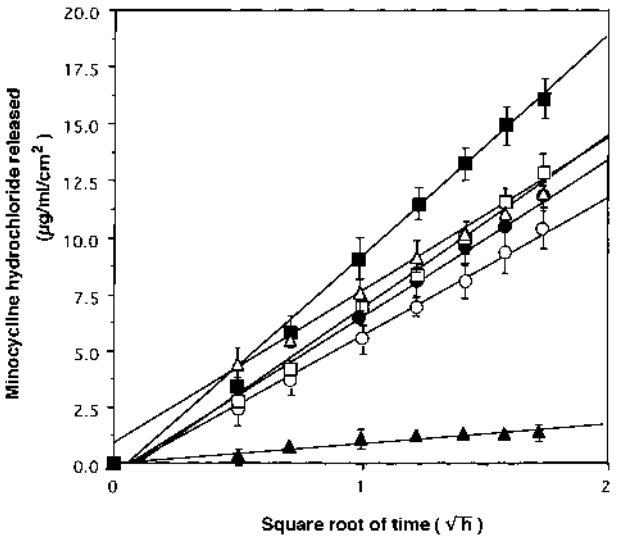

Fig. 4. Effects of Purified Lanolin Concentration on the Release Behavior of Minocycline Hydrochloride from Hydrophilic Ointment in Distilled Water

○, Hydrophilic ointment 100% (HO100); ●, hydrophilic ointment 90%: purified lanolin 10% (HO90 : PL10); □, hydrophilic ointment 80%: purified lanolin 20% (HO80 : PL20); ■, hydrophilic ointment 70%: purified lanolin 30% (HO70 : PL30); △, hydrophilic ointment 60%: purified lanolin 40% (HO60 : PL40); ▲, purified lanolin 100% (PL100). Measurement temperature: 37 °C. Data represent the mean±S.E. of three experiments.

Table 2. Effect of Composition of Ointment Base on Its Apparent Viscosity and the Release Rate of Minocycline Hydrochloride from the Ointment

HO : PL[a]	*k*[b]	*V*[c]
100 : 0	6.10±0.502	1.121±0.161
90 : 10	6.95±0.372	1.085±0.079
80 : 20	7.59±0.488	0.995±0.163
70 : 30	9.72±0.528	0.926±0.106
60 : 40	6.73±0.543	1.110±0.183
0 : 100	0.83±0.121	1.651±0.353

a) HO: hydrophilic ointment, PL: purified lanolin. *b*) *k*: apparent release rate constant (μg/ml/cm^2/$\sqrt{h}$) in lactated Ringer's injection. *c*) *V*: apparent viscosity (Pa·s). Data represent the mean±S.E. of three experiments.

Influence of PL on Release Behavior of Drug of HO Base The influence of HO intermixed with PL on the release of MH from the ointment base is shown in Fig. 4. The amount of release of MH from each base was correlated linearly to the square root of time, in which the apparent release rate, *i.e.* slope of straight line, differed depending on the PL concentration. The drug release rate increased with increasing PL ratio up to 30%, having the highest drug release rate constant (Table 2). The amount of water absorption and elution of ointment base during the drug release test were measured. As shown in Fig. 5, it was found that HO 70% added with PL 30% maximally eluted the base, whereas the absorption of water of ointment increased steadily with increasing

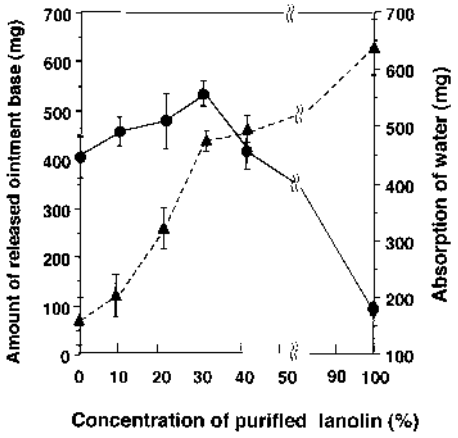

Fig. 5. Effects of Concentration of Purified Lanolin on Absorption of Water and Amount of Released Ointment Base

▲, Absorption of water *vs.* concentration; ●, amount of released ointment base *vs.* concentration. Data represent the mean±S.E. of three experiments.

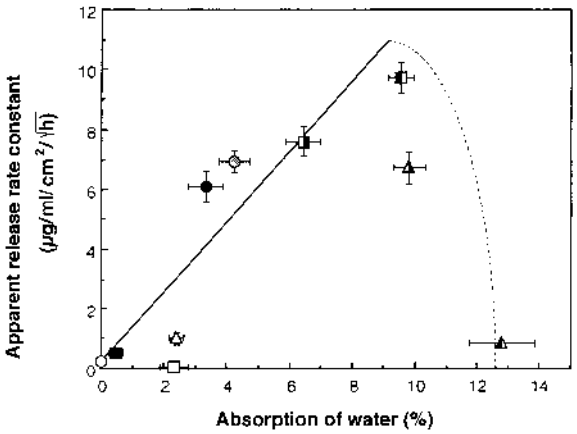

Fig. 6. Relationship between Release Rate and Absorption of Water

○, White petrolatum (WP); ●, hydrophilic ointment (HO); □, petrolatum polyethylene ointment (PO); ■, hydrophilic petrolatum (HP); △, absorptive ointment (AO); ◍, hydrophilic ointment 90%: purified lanolin 10% (HO90 : PL10); ◨, hydrophilic ointment 80%: purified lanolin 20% (HO80 : PL20); ◧, hydrophilic ointment 70%: purified lanolin 30% (HO70 : PL30); ◭, hydrophilic ointment 60%: purified lanolin 40% (HO60 : PL40); ◭, purified lanolin 100% (PL100). Data represent the mean±S.E. of three experiments.

concentration of PL in the ointment base. Water absorbed by PL in the ointment base swelled the matrix structure of the base, promoting the elution of water soluble material from the base as well as the diffusion of drug. Therefore, correlations of drug release rate with water absorption and elution of the ointment base were investigated as in the next paragraph.

Relationships between Drug Release Rate Constant and Water Absorption, and Elution of Ointment Bases A linear correlation of the apparent release rate constant with the absorption of water was found with the exception of the ointment base with 40% and 100% PL (Fig. 6). With increasing PL at a higher ratio than 30% the structure of ointment transformed to a gel-like form, in which HO was entrapped. Therefore, the apparent viscosity was increased as shown in Table 2 and the diffusion rate of drug decreased as shown in Fig. 6. In contrast, a linear correlation between the apparent release rate constant and the amount of released ointment base was found without exception in Fig. 7. The elution of water soluble materials from the ointment base left pores

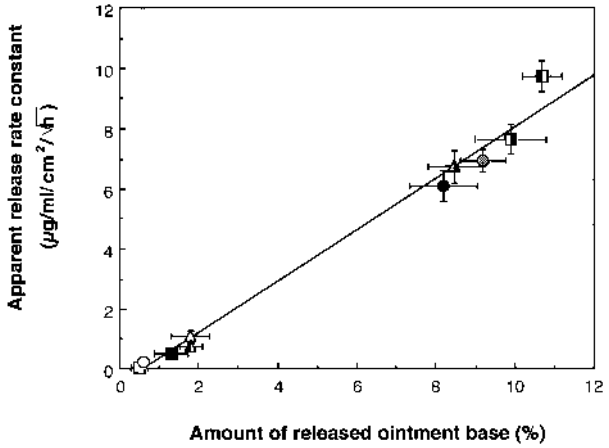

Fig. 7. Relationship between Release Rate and Amount of Released Ointment Base

○, White petrolatum (WP); ●, hydrophilic ointment (HO); □, petrolatum polyethylene ointment (PO); ■, hydrophilic petrolatum (HP); △, absorptive ointment (AO); ◍, hydrophilic ointment 90%: purified lanolin 10% (HO90 : PL10); ◨, hydrophilic ointment 80%: purified lanolin 20% (HO80 : PL20); ◧, hydrophilic ointment 70%: purified lanolin 30% (HO70 : PL30); ◭, hydrophilic ointment 60%: purified lanolin 40% (HO60 : PL40); ◭, purified lanolin 100% (PL100). Data represent the mean±S.E. of three experiments.

filled with water, which reduced the apparent viscosity of the base as shown in Table 2 and enhanced the diffusion rate of drug in the base.

Conclusion

In conclusion, it was found that the ointment base with HO 70% and PL 30% was the most useful ointment base for clinical use, because the increased drug release rate and sufficient absorption of water lowered its viscosity for application to the skin. The drug released amount of the mixed base of HO 70% and PL 30% was approximately 10% in 3 h in the distilled water. This amount was much higher than that of WP applied effectively to 20 human subjects in our hospital. Therefore, an improved drug release from the ointment base might increase the amount of penetration of drug to the epidermal deficiency. Useful basic data were provided in this study for designing an application to treat epidermal deficiency. Bedsore treatment with the mixed base of HO 70% and PL 30% containing MH was significantly effective for 11 human subjects during their infection period in our hospital. The detailed results will be reported in our next paper.

References and Notes

1) Present address: *Faculty of Pharmaceutical Sciences*, *University of Tokushima*, *1–78–1 Shoumati*, *Tokushima 770–0044*, *Japan*.
2) Shigeyama M., Ohtani R., Yamazaki F., Sakaguchi Y., Murakawa T., *Kiso To Rinshou* (*Clin. Rep.*), **21**, 3793—3801 (1987) (in Japanese).
3) Shigeyama M., Ohtani R., Yamazaki F., Murakawa T., *Kiso To Rinshou* (*Clin. Rep.*), **22**, 3767—3775 (1988) (in Japanese).
4) Shigeyama M., Ohtani R., Yamazaki F., Murakawa T., *Kiso To Rinshou* (*Clin. Rep.*), **22**, 5876—5884 (1988) (in Japanese).
5) Shigeyama M., Ohtani R., Yamazaki F., Hashimoto N., Murakawa T., Miyata Y., Hirose Y., *Kiso To Rinshou* (*Clin. Rep.*), **24**, 2951—2960 (1990) (in Japanese).
6) Ushimaru K., Imai T., Shigeyama M., Ohtani R., Ohgaya T., Date K., Miyata Y., Idani C., *Med. J. Takayama R.C.H.*, **17**, 13—18 (1993).
7) Takami M., Nishiguchi K., Komada F., Iwakawa S., Okumura K., *Yakuzaigaku*, **53**, 185—190 (1993).
8) Kiyohara Y., Komada F., Iwakawa S., Okumura K., *Yakuzaigaku*, **54**, 117—121 (1994).
9) Ichikawa H., Nakamura K., Yamaura Y., Koike H., *Prog. Med.*, **12**,

2781—2789 (1992).
10) Ishibashi Y., Eto T., Anzai T., Jitsukawa K., Harada S., Nakanishi H., Yoon S. H., Fujisawa R., Iijima M., Sueki H., Tomisawa T., Yamada K., Kawashima M., Nishiwaki M., Urushibata O., Kase K., Ohara K., Ohta T., Kawabata Y., Watanabe R., Nanko H., Uemura T., Atsumi R., Shishiba T., Harada S., Tei I., Hino H., *Rinshouiyaku* (*J. Clin. Ther. Med.*), **6**, 1227—1240 (1990) (in Japanese).
11) Koyama H., Akamatsu J., Kawai K., Matsushita Y., Issiki N., Sawada M., Kimura T., Yoshida Y., Kurimoto K., Nose K., Moriguchi T., Tanaka H., Hiramoto M., Aoki Y., Nishihata K., Ohi K., *Kiso To Rinshou* (*Clin. Rep.*), **26**, 667—673 (1992) (in Japanese).
12) Dvonch V. M., Murphey R. J., Matsuoka J., Grotendorst G. R., *Surgery*, **112**, 18—23 (1992).
13) Matsuoka J., Grotendorst G. R., *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 4416—4420 (1989).
14) Vlachou M. D., Rekkas D. M., Dallas P. P., Choulis N. H., *Int. J. Pharm.*, **82**, 47—52 (1992).
15) Ōishi H., Takehara M., Koike M., *Yakuzaigaku*, **32**, 110—118 (1972).
16) Higuchi W. I., Higuchi T., *J. Am. Pharm. Assoc.*, **49**, 598—606 (1960).
17) Higuchi W. I., *J. Pharm. Sci.*, **51**, 802—806 (1962).
18) Higuchi T., *J. Soc. Cosmetic Chemists*, **11**, 85—97 (1960).