Formulation Design of Ointment Base Suitable for Healing of Lesions in Treatment of Bedsores

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We intended to develop a desired ointment base suitable for treatment of bedsores including the proliferation of granulation and epidermis. The main bedsore bacteria detected in our hospital were *S. aureus* in gram-positive coccus and *P. aeruginosa* in gram-negative *bacillus*. As the macrogol ointment (MO) was found to have bactericidal effects on these bacteria, MO was adopted as the base for the objective ointment. To improve the properties of the ointment base such as regulating the humidity of the exudation and controlling the release of antibiotics formulated in the ointment, co-formulating effects of various additives to MO were evaluated. The sustained release function of the ointment base was obtained by adding hydrophilic petrolatum (HP) to MO. However, the resultant ointment was found to have a poor humidity regulating property. On the other hand, MO containing 5% of hydroxypropyl cellulose (HPC) showed both the humidity regulating and the controlled drug releasing properties. It was considered that HPC particles dispersed in the ointment could be swelled by absorbing water to form a gel network. The curd tension meter tests for the ointments prepared with the various polymers showed that the MO–HPC base, which showed the highest sustained drug releasing property, was found to have the highest hardness. This result means that HPC formulated into the base forms the most rigid gel structure to resist the erosion of the ointment and to control the drug release.

Key words minocycline hydrochloride; sustained release ; hardness; rotation disk method; Franz diffusion cell; hydrophilic polymer

In clinical treatment of bedsores and refractory skin ulcers, the most suitable types of ointment should be applied considering the various disease stages such as the infectious period, necrosis and agglutination period and proliferation period of granulation and epidermis.

Although some commercial ointments with different clinical properties are available, it is necessary to develop multifunctional ointments suitable to the disease stages of bedsores and refractory skin ulcers.

In our hospital, a simple lipophilic ointment base, in which antibiotics are formulated, has been used with expectation of antibacterial action in the infectious period of bedsores.[2—5)] In the previous paper, we reported a special formulation of the bedsore ointment to improve the drug release rate and water absorption property.[6)] These properties of ointment are preferable in treating bedsores at the incipient stages: the infectious period and the necrosis and agglutination periods.

For healing of lesions in the treatment of bedsores, a function of regulating the humidity of the exudation is required for the ointment base. A series of specialized analyses of the local environmental factors controlling the lesions has recently revealed that humidity is one of the most effective factors in the healing of lesions.[7—9)] It is also assumed that the exudation contains various growth factors generated from macrophages, which hold the key to recovery, and that dry conditions delay the healing process due to the disappearance of the growth factors.[10,11)] The airtight films used for wound dressings are effective in regulating the humidity of the exudation, however, they sometimes cause infections due to the humid conditions. Considering these facts, the requirement for the ointments applicable to the healing of lesions are regulating both the humidity of the exudation and the antibacterial action.

In this paper we intended to design an ointment having suitable properties for the treatment of the last stage of bedsores. Macrogol ointment (MO) was used as the base of the objective ointment because MO was reported to have some characteristics of bactericidal activities.[12,13)] The antibacterial activity of MO was evaluated based on the clinical data collected in our hospital. An antibacterial substance was formulated to the ointment to prevent the infection during the clinical treatment. Co-formulation of additives such as hydrophilic polymers to MO was examined to improve the properties of the resultant ointment bases with respect to drug releasing and humidity regulation.

Experimental

Materials Powdered minocycline hydrochloride (MH), (Japan Lederle) for injection, passed through a JIS sifter of 180 μm opening was used. Hydrophilic polymers used were sodium alginate (Kibun Food Chemifa Co.), gum arabic (Kishida Chemical Co.), carmellose sodium (Cellogen® BS-H) (Daiichi Industry Pharm. Co.), macrogol 1,500 (Maruishi Pharm. Co.), hydroxypropyl cellulose-H (HPC-H) (Nihon-Soda Co.), hydroxypropyl methyl cellulose (HPMC) 4000 (60SH), 10,000 (60SH), and 15,000 (90SH) (Shinetsu Chemical Co.), and Carbopol 934P (CP) (BF Goodrich Co.).

Ointment bases used were hydrophilic petrolatum (HP), absorptive ointment (AO) and hydrophilic ointment (HO), (Maruishi Pharm. Co.), and MO (Dainippon Pharm. Co.).

Lactated Ringer,s injection(Lactec®) (Otsuka Pharm. Co.) as the medium for the release test was used as received.

Preparation of Ointment Containing MH The ointment bases were prepared with the fusing method. In the procedure, the ointment bases and MH were kneaded and homogenized with an ointment spatula on a ceramic slab. When the hydrophilic polymer was added to the ointment base, it was also mixed in the same manner. The kneaded mixture was further mixed on a warmed water bath at 80 °C and then cooled down to room temperature. The concentrations of MH and hydrophilic polymer in the ointment were 1% and 5% , respectively (fusing method).

Separation and Identification of Bedsore Bacterium The subjects

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(n=378) were the inpatients at our hospital from the 10th of Jan. in 1994 to the 26th of Dec. in 1997, having complications of bedsores. The infusion solution (1 ml), which was a normal saline solution combined with material from bedsore gauze, was cultured in heart infusion agar (HIA, Eiken Chemical Co.) or GAM semi solid (Nissui Pharm. Co.). After that it was determined that the bacterium to use was Sceptor® Panel, made by BBL Co.

Antibacterial Action of Ointment Base The antibacterial action against the typical two types of bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, collected from the bedsores of the inpatients was investigated. The ointment base of 5 g each was dissolved in 10 ml of normal saline in the test tube at a warm temperature. After adding 1 ml of the bacterium solution 10^6 CFU/ml to the test solution of 9 ml, it was cultured at 37 °C. After cultivation, the number of bacteria was measured until the 7th day.

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 washing for 2 h in distilled water at 80 °C. Five grams of ointment was mounted on the cellulose membrane placed on 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 ringers 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 release 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.

In the rotation disk method, the surface area of the disk (the area of contact with ointment) was 8.03 cm^2, the rotation speed was 100 rpm, the elution solution was Lactated Ringer's injection (Lactec®), and the test temperature was 37 °C. The amount of MH released was determined spectrophotometrically at 349 nm.

Water Absorption and Elution of Ointment In the Franz diffusion cell method, 5 g of the ointment sample was applied to the cellulose membrane mounted on the apparatus, and 50 ml of the medium was introduced into the receiver cell. The system was placed in a water bath thermally controlled at 37 °C. After 1 h, the ointment absorbing water was removed from the membrane and weighed (W_1). The ointment completely desiccated with silica gel was weighed (W_2). The eluted ointment base (E) and absorbed water (A) were calculated from the following equations.

$$E=5.0-W_1 \quad (1)$$

$$A=W_1—W_2 \quad (2)$$

The amounts of water absorbed and ointment eluted were measured with the rotation disk method in the same manner used for the release test.

The Measurement of Hardness The ointment samples were prepared by adding 5% of hydrophilic polymers to the MO. The ointment samples were placed carefully without making bubbles in the plastic container, having sizes of 40 mm in internal diameter and 20 mm in depth. The solidity of the ointment was measured by a curd tension meter (Techno Corporation) with 100, 200 or 400 g of the weight and the spring suitable for each weight. The contacting disks of 8 or 11.3 mm in diameter were used, and the speed of rising board was 0.36 cm/s.

Results and Discussion

Antibacterial Action of Ointment Base The antibacterial action is important in designing the bedsore ointments. To clarify the optimum antibacterial property, we identify the types of bacterium collected from the inpatients in our hospital having complications of bedsores. The data collected for 4 years revealed that the major types of bacterium were *S. aureus*, *S. epidermidis*, *E. faecalis*, and *P. aeruginosa* (Fig. 1).

According to the report stating that MO showed a bactericidal action, we checked the antibacterial property of several hydrophilic ointment bases including MO.[12,13] The bacteria used were the two major bacteria shown in Fig. 1: *S. aureus* of gram-positive *coccus* and *P. aeruginosa* of gram-negative *bacillus*. In both cases, MO showed a considerably higher bactericidal action compared to other ointment bases tested. Based on these experimental results, MO was selected as a basic material for designing the ointment base suitable for treatment in the recovery stage of bedsores. It was found that MO had a bactericidal action for *S. aureus* and *P. aeruginosa*, while the other ointment bases tested showed little effect on the bacterial growth (Fig. 2).

MO–HP Base Although the antibacterial function of MO was confirmed, we formulated an antibiotic to the ointment base to ensure the complete antibacterial action for the resultant ointment. MH was chosen as the drug because it had less appearance of tolerant bacterium.[15] One of the problems for the MH containing MO ointment was the drug releasing property. In the previous paper, we examined the

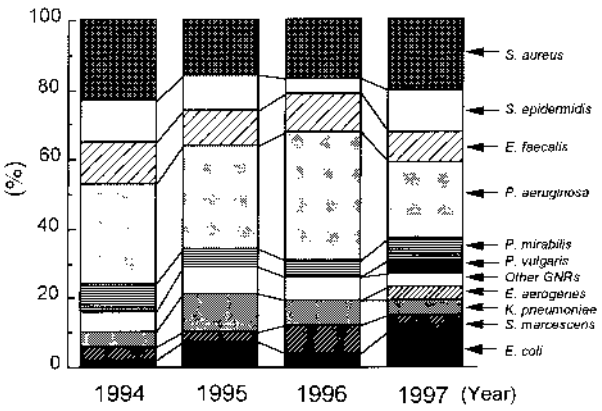

Fig. 1. Transition of Causative Organism of Bedsores

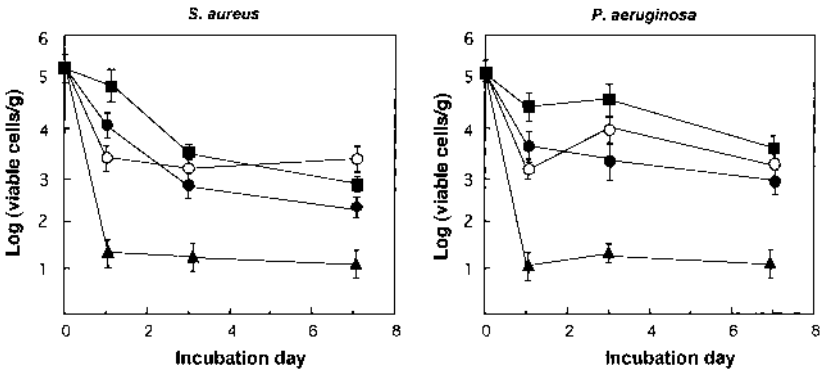

Fig. 2. Effect of Ointment Base on Viability of Bacteria

○, Control; ▲, MO; ■, AO; ●, HO. Data represent the mean±S.E. of three experiments.

drug releasing property of various hydrophilic ointment bases to confirm the fastest drug releasing rate for MO and the latest one for the HP among them.[6)]

In using MO as the bedsore ointment base, the drug release property should be improved. We tested the co-formulation of HP with MO to sustain the drug release from MO ointment. As shown in Fig. 3, the drug release rate decreased with the increase of HP content in the base formulation. When the HP content was low, the ointment set in the Franz diffusion cell was dissolved before the drug release was completed. The critical points were indicated by the arrows for each drug dissolution plot (Fig. 3). This release test revealed that low content of HP in the ointment formulation caused the burst of the drug within a short time in the actual clinical application. The MO base containing 50% of HP was able to keep the shape to continue the drug release up to 360 min in the dissolution test. In measuring the amount of the ointment bases released at 1 h in the dissolution test, we confirmed the decrease in the amount of ointment released with the increase of the HP content in the ointment formulation (Fig. 4). Admixing HP to MO in the base formulation may cause hardening in its structure to reduce the erosion. This structural change well explained the change in the drug release rate.

However, a further experiment found that the addition of HP into the MO base also caused the decrease in the water uptake as shown in Fig. 4. The lack of water absorbing property of HP is responsible for this phenomenon. Considering the desired properties for the bedsore ointment, such as the modulation of humidity, it was concluded that MO–HP mixture was not an optimum formulation for the ointment base.

MO–Hydrophilic Polymer Base The improvement in the properties of MO base was tested by co-formulating hydrophilic polymer instead of HP to the ointment base. Table 1 lists the polymers used, all of which are expected to have a gel-forming property in the presence of water. To evaluate the properties of the bases, the rotation disk method (Fig. 5) was devised instead of the Franz cell method, because the latter method could not evaluate the drug release caused by erosion of the ointments. In the rotation disk method, the ointment set on the disk was gradually eroded and simultane-

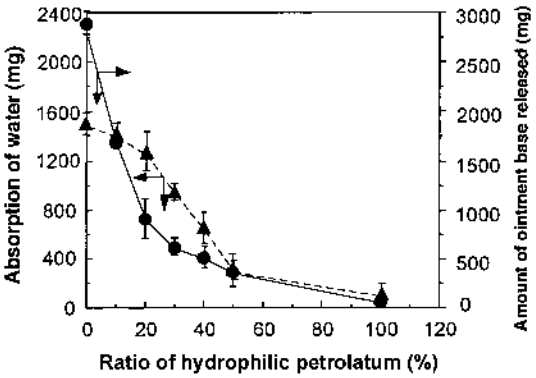

Fig. 4. Effect of Concentration of HP on Absorption of Water and Amount of Ointment Base Released

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

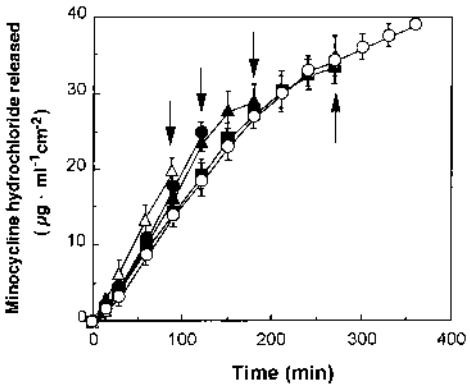

Fig. 3. Effect of HP Concentration on the Release of MH from MO

△, MO90: HP10; ●, MO80: HP20; ▲, MO70: HP30; ■, MO60: HP40; ○, MO50: HP50. Data represent the mean±S.E. of three experiments.

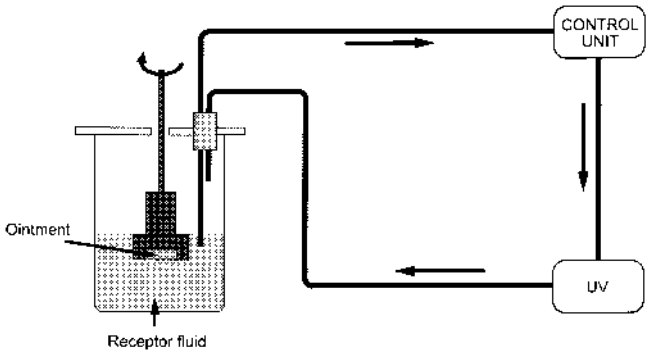

Fig. 5. Rotation Disk Method for the Release Test of MH from Ointment

Table 1. Effect of Addition of Hydrophilic Polymer in MO Base on Dissolution Time of the Ointment Base

Hydrophilic polymer[a)]	Dissolution time (min)	
	Without adding water to the formulation[b)]	Water-coformulated[c)]
No additives	70±7	65±8
Gum arabic	80±5	105±4*
Sodium alginate	80±2	105±7*
HPC	120±4**	190±9***
HPMC (4000)60SH	90±8	110±6*
HPMC (10000)60SH	110±8	140±7**
HPMC (15000)90SH	110±5	145±7**
Carmellose sodium	95±8*	140±5**
Carbopol 934P	95±9	110±6*

a) Concentration of additives in ointment base was 5%. *b*) ∗: $p<0.05$ *vs.* water-coformulated. ∗∗: $p<0.01$ *vs.* water-coformulated. Data represent the mean±S.E. of three experiments. *c*) Water content of ointment base was 0.5%. ∗: $p<0.05$ *vs*. none. ∗∗: $p<0.01$ *vs*. none. ∗∗∗: $p<0.005$ *vs.* none.

ously the drug, MH, was released in the ointment. Thus, the dissolution time shown in Table 1 means the time when the ointment as well as the drug completely released. The dissolution test revealed that formulation of 5% of HPC most improved the drug release property of the ointment compared with other polymers. When the small amount of water was co-formulated into the base, the drug release was more prolonged in any base formulation (Table 1). This may be attributed to the facilitation of gel formation of the polymers in the ointment by the added water.

MO–HP ointment base showed a defect in that the water absorption property was decreased with an increase in the amount of HP formulated, although the improved drug release property was conferred. On the other hand, the water absorption property of MO–HPC was found to be retained up to 5% of HPC content when the HPC concentration in the ointment base was increased (Fig. 6). At 8% of HPC content, the water absorption was a little bit disturbed, but a considerable amount of water was absorbed into the base. HPC, as well as the other hydrophilic polymers tested, can hold the water absorbed by forming a gel phase with water in the ointment, resulting in the preferable water absorption property.

Figure 7 schematically shows the structure of the ointment containing HPC and MH applied to the dissolution test. The gel layer is formed by water penetration to the ointment. The erosion occurs on the surface of the ointment as it absorbs the excess water. When the amount of polymer formulated is higher, the gel layer may tolerate more erosion caused by the mechanical stirring. The gel formation of the hydrophilic polymers in the ointment bases may be reflected by the hardness of the ointments. We measured the hardness of the ointment bases containing the polymers by using the curd tension meter. The hardness measured was plotted against the elution time for various ointment bases (Fig. 8). There was a good correlation between them; the MO–HPC base, which showed the highest drug sustained property, was found to have the highest hardness. This result means that HPC formulated into the base forms the most rigid gel structure to resist the erosion of the ointment and a drug formulated.

It is concluded that MO–HPC is a suitable ointment base for the recovery stage of bedsores. The base itself possesses an antibacterial activity and can sustain the release of the antibiotic formulated. It can also absorb the exudation solution of bedsores to keep fibroblast cells or the various growth factors in treating. As HPC is one of the most popular pharmaceutical polymers, a clinical trial can be easily carried out. Bedsore treatment with this HPC mixed MO containing MH was significantly effective for 11 human subjects during their proliferation period of granulation and epidermis in our hospital. The detailed results will be reported in our following paper.

Reference and Notes

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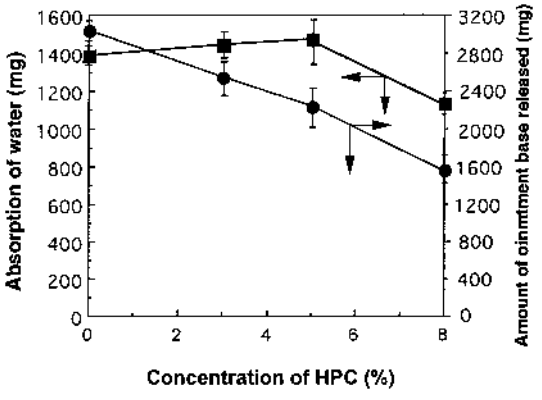

Fig. 6. Effect of Content of HPC in MO Base on Absorption of Water and Amount of Ointment Base Released

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

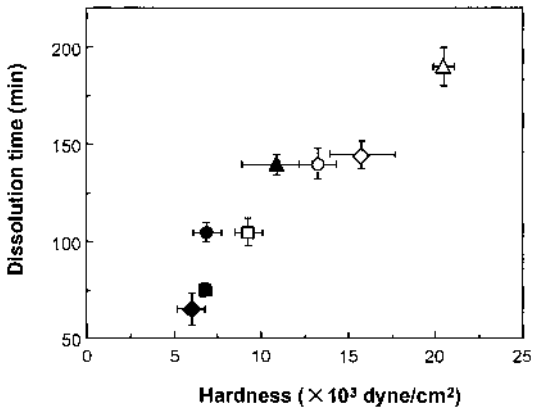

Fig. 8. Relationship between Dissolution Time and Hardness of MO

●, Gum arabic; □, Sodium alginate; □, Macrogol 1500; △, HPC; ○, HPMC (10000); ◇, HPMC (15000); ▲, Carmellose sodium; ◆, None. Data represent the mean±S.E. of three experiments.

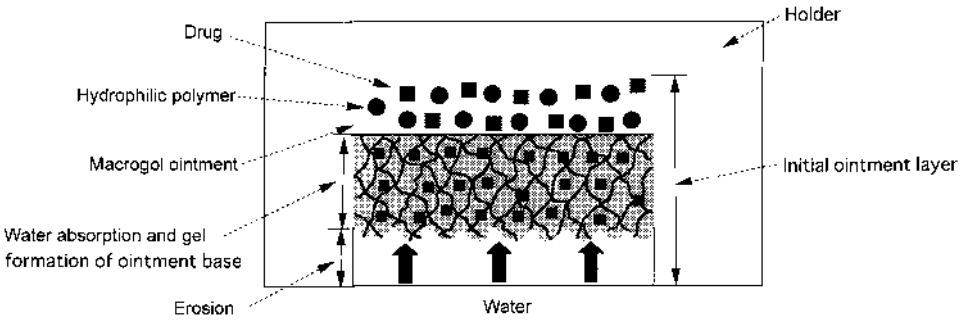

Fig. 7. Schematic Representation of Gel Formation of MO

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