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Novel chitosan wound dressing loaded with minocycline for the treatment of severe burn wounds

Shuichi Aoyagi, Hiraku Onishi *, Yoshiharu Machida

Department of Drug Delivery Research, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

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Abstract

Novel wound dressings composed of chitosan (CH) film and minocycline hydrochloride (MH) were prepared using commercial polyurethane film (Tegaderm) as a backing. CHs with deacetylation degrees of 67%, 83% and 96% (mol/mol), named CH67, CH83 and CH96, respectively, were used. Wound dressing with a large piece of Tegaderm film (4 cm × 4 cm), named CH-MH-N, and wound dressing prepared by cutting CH-MH-N to the wound size, named CH-MH-A, were developed. As CH67-MH-N and CH83-MH-N showed the sustained release of minocycline in vitro, CH67 and CH83 were used as chitosan in the in vivo studies. Various formulations were applied to severe burn wounds in rats in the early stage, and the wound status and change in the wound surface area were examined. The use of 10 mg of MH and complete sealing with Tegaderm had a negative effect. MH ointment was not effective, but Geben cream was fairly effective. However, CH83-MH-A containing 2 mg of MH (CH83-MH2-A) and CH83 film showed an excellent effect. Considering the elimination of pus, CH83-MH2-A tended to be better than CH83 film. CH83-MH2-A is suggested as a useful formulation for the treatment of severe burn wounds.

Keywords: Novel wound dressing; Chitosan film; Minocycline; Polyurethane film; Severe burn wound

1. Introduction

Various formulations such as ointments and wound dressings have been developed for the treatment of severe skin wounds or ulcers including bedsores and burn wounds (Susuki et al., 1983; Sone et al., 1984; Imamura et al., 1989; Yamamoto et al., 1990; Niimura et al., 1990; Machida et al., 1997). Wounds recover through several disease stages. In bedsores and burn wounds, the wound stages are often divided into an infectious period, necrosis and agglutination period, proliferation period and epidermis formation period (Shigeyama et al., 2001a; Shigeyama, 2004). Generally, formulations are selected based on the disease stage; namely, the application period is a critical factor in the choice of formulation. For example, sulfadiazine silver cream (Geben cream) and purified sucrose–povidone iodine paste (U-PASTA) are chosen in the infectious period, and tretinoin tocoferil ointment (Olcenon ointment) is used in the proliferation period (Susuki et al., 1983; Imamura et al., 1989; Yamamoto et al., 1990). Therefore, it has also been studied to identify the stage at which the developed formulation works effectively (Shigeyama et al., 2001a).

In the treatment of bedsores or serious burn wounds, wound dressings are often used to protect the wound and/or to enhance healing. Previously, chitosan (CH) and chitin films were prepared as wound dressings, and their effects were examined using a severe burn wound model in rats (Tachihara et al., 1997a,b). CH films were more effective than chitin film or gauze alone. CH films were considered to be effective due to good protection of the wound, absorption of exudate and antibacterial action. In the infectious period, bacterial infection delays healing and may cause serious problems such as sepsis. Recently, minocycline hydrochloride (MH) was shown to be effective for the suppression of bacteria in skin ulcers (Shigeyama et al., 1999, 2000, 2001b), and MH ointments have therefore been examined clinically for the treatment of skin ulcers (Katsuura et al., 2002). However, when ointments or creams are used for the treatment of skin wounds, their frequent reapplication and washing of the wound region are required, often leading to pain or burden on the patient. On the other hand, CH films can be dealt with more simply, and are more durable during application. Thus, we decided to develop a novel wound dressing by combining CH film and MH.

* Corresponding author. Tel.: +81 3 5498 5724; fax: +81 3 3787 0036.
E-mail address: onishi@hoshi.ac.jp (H. Onishi).

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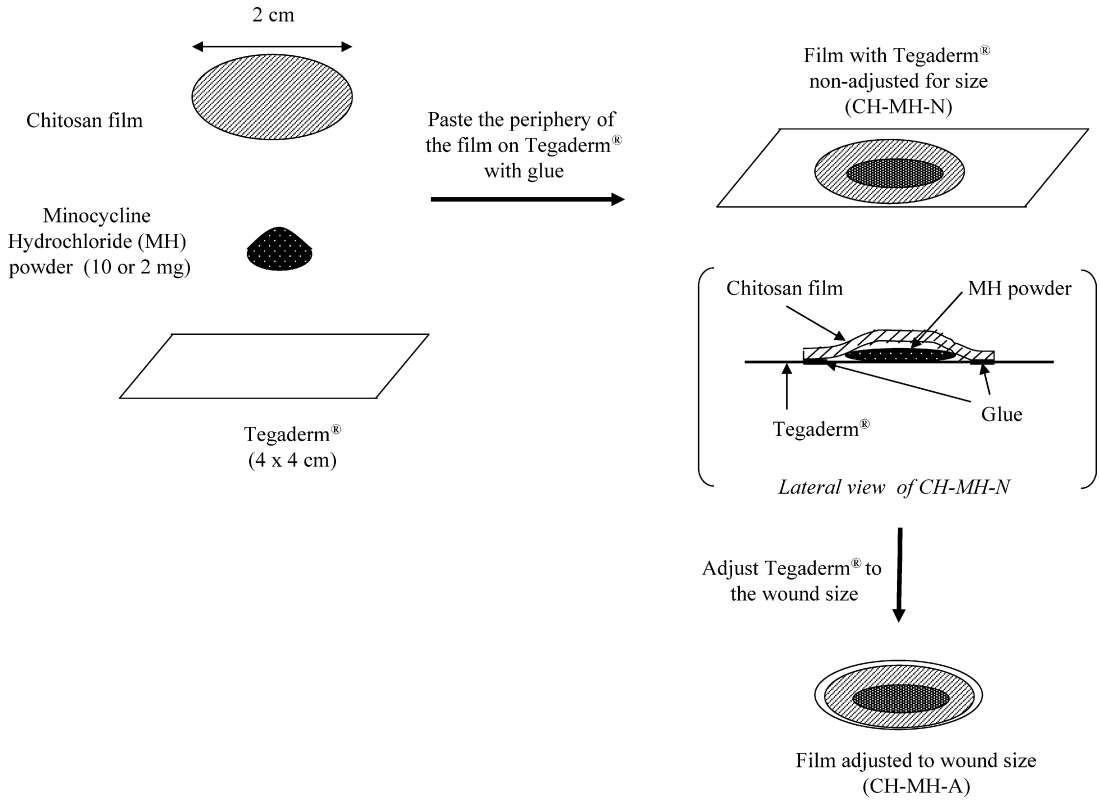

Fig. 1. Preparation of novel wound dressings made of chitosan film, minocycline hydrochloride and Tegaderm backing, named CH-MH-N and CH-MH-A.

The novel wound dressing consists of a backing of commercial polyurethane film (Tegaderm) (Holland et al., 1984), CH film and MH, with MH sandwiched between the films (Fig. 1). Water vapor and oxygen can permeate the Tegaderm film but water cannot. Namely, as Tegaderm can protect the wound from not only solid substances but also liquid and remains attached to the skin for a long period, it was chosen as the backing film. CH film is expected to protect the wound and to achieve the controlled release of minocycline. Thus, this wound dressing was first examined in vitro for drug release and swelling characteristics, and then its efficacy was evaluated in vivo by comparing with other formulations including those clinically available.

2. Materials and methods

2.1. Materials

Chitosans (CHs) with a deacetylation degree of 96% (mol/mol) and viscosity grade of 57 cP (0.5% (w/v) at 20 °C) and with a deacetylation degree of 83% (mol/mol) and viscosity grade of 758 cP (0.5% (w/v) at 20 °C), named CH96 and CH83, respectively, were kindly supplied by Katakura Chikkarin Co. Ltd. (Japan). Chitosan (CH) with a deacetylation degree of 67% (mol/mol) and viscosity grade of 225 cP (0.5% (w/v) at 20 °C) (CH67), and minocycline hydrochloride (MH) were obtained from Wako Pure Chemical Industries Ltd. (Japan). Polyurethane film (Tegaderm) was purchased from 3M Health Care (Germany). Beschitin W was obtained from Unichika Ltd. (Japan) (Oshima et al., 1987). White petrolatum was purchased from Miyazawa Yakuhin Co. Ltd. (Japan), and was used to prepare the MH ointment (Katsuura et al., 2002). Geben cream was obtained from Tokyo Tanabe Pharmaceutical Co. Ltd. (Japan) (Susuki et al., 1983). All other chemicals were of reagent grade.

MH ointment was prepared as follows (Katsuura et al., 2002): MH (20 mg) was mixed with liquid paraffin (0.2 ml), and the mixture was levigated into a liquid sludge using a pestle and mortar. White petrolatum (20 g) was then added gradually to the sludge. The ointment produced was used as MH ointment.

2.2. Animals

Male Wistar rats weighing 220 g (7-week old) were purchased from Tokyo Laboratory Animals Science Co. Ltd. (Japan), and soon used for animal experiments. They were kept on the breeding diet MF (Oriental Yeast, Japan) with water ad libitum, at room temperature maintained at 23 ± 1 °C and relative humidity of 60 ± 5%. The experimental protocol was approved by the Committee on Animal Research of Hoshi University, Tokyo, Japan, and the animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University, Japan.

2.3. Preparation of CH films

CH (1.2 g) was put into 40 ml of 2% (v/v) acetic acid aqueous solution, and dissolved by stirring for 2 h. The solution was poured into a Teflon mold (6 cm length × 6 cm width × 0.2 cm depth) until the mold was filled completely. The mold loaded

with the solution was immersed in an excess amount of a 1 M sodium hydroxide aqueous solution for 10 min. The mold with swollen CH gel was taken out and put into water, and the medium pH was neutralized to pH 7 with 0.1 M hydrochloric acid aqueous solution. The gel was further washed with water, and dried in air for 7 days while the central part (4 cm length × 4 cm width) was exposed to air and the peripheral part was fixed with a Teflon frame. The film produced in the central part (4 cm length × 4 cm width) was used as CH film.

2.4. Preparation of wound dressing

The novel wound dressing was prepared using CH film, MH and Tegaderm as shown in Fig. 1. MH (2 mg or 10 mg) was applied in a circle (1 cm in diameter) to the adhesive face of Tegaderm. The drug was covered with round CH film (2 cm in diameter), and the Tegaderm and CH film were attached with surgical glue Aron Alpha A (Toagosei Co. Ltd., Japan). The obtained wound dressing was named CH-MH-N (Fig. 1). Next, the central part of CH-MH-N, including the CH film covering MH, was placed on the wound surface area, and its Tegaderm edge was cut to the size and shape of the wound surface, yielding CH-MH-A (Fig. 1). Each preparation was named by additionally showing the deacetylation degree of CH and amount of MH. For example, when CH83 was used, containing 2 mg of MH, the non-adjusted and adjusted wound dressings were named CH83-MH2-N and CH83-MH2-A, respectively.

2.5. In vitro release of minocycline from wound dressing

CH-MH-N was fixed to a Teflon frame (inside open space: 2.5 cm × 2.5 cm) with the CH film face as shown in Fig. 2. Namely, the adhesive face of the Tegaderm edge was attached to the under surface of the Teflon frame. A release test was performed using the paddle method at 60 rpm and 37 °C for 48 h using 500 ml of phosphate-buffered saline, pH 7.4 (PBS) as the release medium. At appropriate time points, aliquot samples (1 ml) were taken, and immediately after each sampling, the same volume of fresh buffer was added to the incubation medium. The concentration of minocycline in the sample was measured by HPLC.

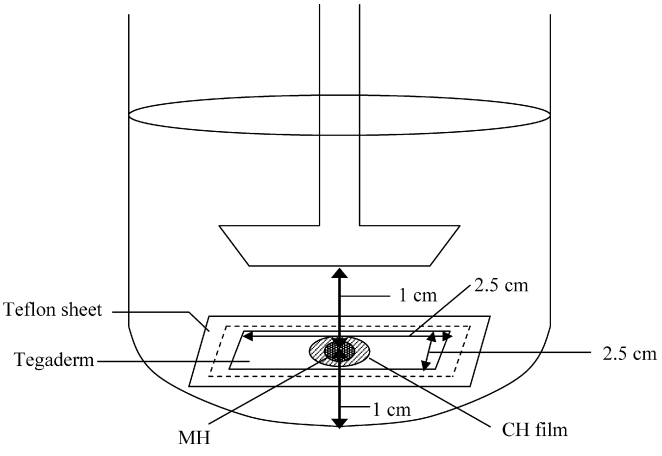

Fig. 2. Apparatus and conditions for in vitro release test.

2.6. Buffer absorption by wound dressing

Immediately after the in vitro release test described above, the region of the wet CH film and remaining MH was removed by cutting along the edge of the CH film. This region was weighed as wet weight after simply wiping with filter paper, and then dried completely in a desiccator and weighed as dry weight. The extent of buffer (PBS) absorption by the wound dressing was determined from the ratio of the wet to dry weight.

2.7. Stability of minocycline in PBS at 37 °C

MH (10 mg) was dissolved in PBS (500 ml) pre-warmed to 37 °C, and stirred at 60 rpm and 37 °C with a paddle in a manner similar to that described in the in vitro release. The test was performed for 72 h ($n = 1$). At appropriate time points, aliquot samples (1 ml) were taken, and immediately after each sampling, fresh PBS (1 ml) was added to the incubation medium. The concentration of minocycline in each sample was measured by HPLC.

2.8. Creation of a burn wound model in rats

The rats were anesthetized with ethyl ether, the hair on their upper back was shaved, and they were made to lie on their front. Then, the circular flat face (1 cm diameter) of the top of an electric soldering iron cylindrical bar, heated to 200 °C, was applied for 20 s with the weight of the apparatus on the shaved dorsal skin at the point 1.5 cm right-laterally from the back midline toward the axillary region. After 48 h, the resultant dead area was removed surgically under anesthesia with ethyl ether to obtain a severe burn wound model. The wound was almost round and slightly larger than 2 cm in diameter, and reached the surface of the back muscle in depth. This wound was used as a burn wound model for the in vivo experiments.

2.9. In vivo release of minocycline from wound dressing

CH83-MH2-N was applied to the wound surface, and was removed 6, 12, 24 and 48 h after covering the wound. The Tegaderm was removed, the remaining MH and CH film were weighed, and the CH film was cut into small pieces. The remaining MH and all the pieces of CH film were mixed with a five-fold volume of PBS, homogenized with a glass homogenizer using a Teflon pestle, and mixed with 1% (w/v) hydrochloric acid aqueous solution (five-fold volume) to dissolve the film. Water (44-fold volume) and 0.5 M phosphate buffer, pH 7.0, (55-fold volume) were then added. After the addition of methanol (55-fold volume), the mixture was centrifuged, and the supernatant was analyzed by HPLC for minocycline. The determined amount of minocycline was corrected with the recovery ratio, which was examined as follows: namely, a Tegaderm–CH83 combined film with no MH, that is, CH83-MH2-N without MH, was applied to the wound surface. Six,

12, 24 and 48 h after application, the Tegaderm–CH83 film was removed, the Tegaderm was separated, and the remaining CH film was weighed and cut into small pieces. One hundred fifty microliters of MH solution (1, 1.67 and 5 mg/ml) was added to the CH film pieces, and left to stand for 10 min. The CH film pieces containing MH were treated and analyzed in the same manner as the tested samples. The recovery ratio was obtained as the ratio of the observed MH amount to the calculated amount.

2.10. In vivo examination of efficacy

CH-MH-N, CH-MH-A, gauze alone, Beschitin W, CH films, MH ointment and Geben cream were applied to the wound surface. In the control, the wound surface was covered directly with four sheets of gauze laid one on top of another. Each formulation was applied to the wound surface by covering with 4 sheets of gauze laid one on top of another except as otherwise specially described, and fixed to the wound surface by adhesive surgical tape. After application, each rat was fed separately with one animal per cage. Every time the wound was observed, the gauze, formulation and surgical tape were replaced with new ones. The details of the application were as follows.

First, CH-MH10-N was changed 3 days after the start of application, new film was applied for another 3 days, and then gauze was applied. CH-MH2-N was applied for 2 days, new film was applied for another 2 days, and then gauze was applied. Beschitin W covered with four sheets of gauze laid one on top of another was applied for 14 days.

Secondly, when CH-MH2-A, CH film, MH ointment (10 g) and Geben cream (10 g) were applied, each was applied for 2 days, reapplied as new for another 2 days, and then Beschitin W was applied without gauze. Beschitin W was used after application of these formulations because there was less bleeding with Beschitin W than with gauze when changing the formulations.

The formulations were checked visually for wound sticking, adsorption of exudate and elimination of pus 2 and 4 days after the start of application except for CH-MH-N (Fukawa et al., 1982), which was checked 3 and 6 days after the start of application. Results were defined as ++, +, ± and − for very good, good, neutral and negative responses, respectively, as compared with the control (gauze alone). Furthermore, the ratio of the wound surface area to the initial area, named the wound area ratio, was calculated as the healing index as follows (Fukawa et al., 1982; Yamashita et al., 1989):

$$\text{Ratio of the wound surface area to the initial one (wound area ratio) (\%)} = 100 \times \frac{\text{wound length} \times \text{wound width}}{\text{initial wound length} \times \text{initial wound width}}$$

2.11. HPLC assay

High performance liquid chromatography (HPLC) was used for the assay of minocycline at room temperature using a pump, Shimadzu LC-6AD, equipped with a Unishil Q C8 column (4.6 mm Ø × 15 cm length) and a UV–VIS absorption detector, Shimadzu SPD-10AV, set at 280 nm. A mixture of 0.2 M ammonium oxalate, *N*,*N*-dimethylformamide and 0.1 M ethylenediaminetetraacetic acid disodium (11:5:4, v/v/v), the pH of which was adjusted to 6.2 by the addition of tetrabutylammonium hydroxide, was used as the mobile phase and eluted at 1.2 ml/min. The sample (20 μl) was injected onto the column. The absolute calibration curve method was used for the calculation.

2.12. Statistical analysis

Statistical analysis was performed using the unpaired *t*-test, and significant difference was set as $P < 0.05$.

3. Results and discussion

3.1. In vitro release and buffer absorption

CH films were prepared by drying in air at 60 °C for 24 h or in air at room temperature for 7 days. The films produced by drying at 60 °C were too hard to apply to the wound surface, whereas the films obtained by drying at room temperature could be applied to the wound surface as they were somewhat flexible. CH films with a thickness of approximately 0.13 mm were chosen and used for all experiments.

The in vitro release profiles of minocycline from CH-MH-N are shown in Fig. 3. In the early phase for CH-MH10-N, the release rate was faster in the order CH96-MH10-N > CH83-MH10-N > CH67-MH10-N, and the latter two formulations showed gradual drug release. For CH-MH2-N, CH96-MH2-N exhibited the fastest drug release. Accordingly, CH83-MH-N and CH67-MH-N were considered adequate for gradual drug release. The buffer absorption of CH films after the release test was evaluated based on the ratio of the weight of the region containing the wet CH film and remaining MH (wet weight) to the dry weight of this region (Fig. 4). In both CH-MH10-N and CH-MH2-N, buffer absorption was larger in the order of CH67-MH-N > CH83-MH-N > CH96-MH-N. Buffer absorption was influenced markedly by the type of CH, but the amount of loaded MH did not appear to cause so much difference ($P < 0.05$ for CH83-MH10-N versus CH83-MH2-N, but $P > 0.05$ for CH96-MH10-N versus CH96-MH2-N and CH67-MH10-N versus CH67-MH2-N). In CH-MH10-N, since the CH67 film swelled the most, causing the narrowest free space around the MH powder region, the flow of water to the powder was prevented, resulting in slower dissolution and release of minocycline. On the other hand, in CH-MH2-N, as the drug amount was small, swelling of the CH film did not inhibit the flow of water into the powder. Namely, the release from CH-MH2-N was considered to be governed by the diffusion of minocycline through the membrane rather than the dissolution rate of the drug.

In the later phase, the concentration of minocycline in the medium decreased gradually. As this was considered a result of the stability of MH (Pawelczyk and Matlak, 1982; Orti et al., 2000; Honnorat-Benabbou et al., 2001), the stability test was performed at 37 °C. The results are shown in Fig. 5. After 48 h, the remaining percentage of minocycline was about 70%. Therefore, the decrease in minocycline concentration in the latter

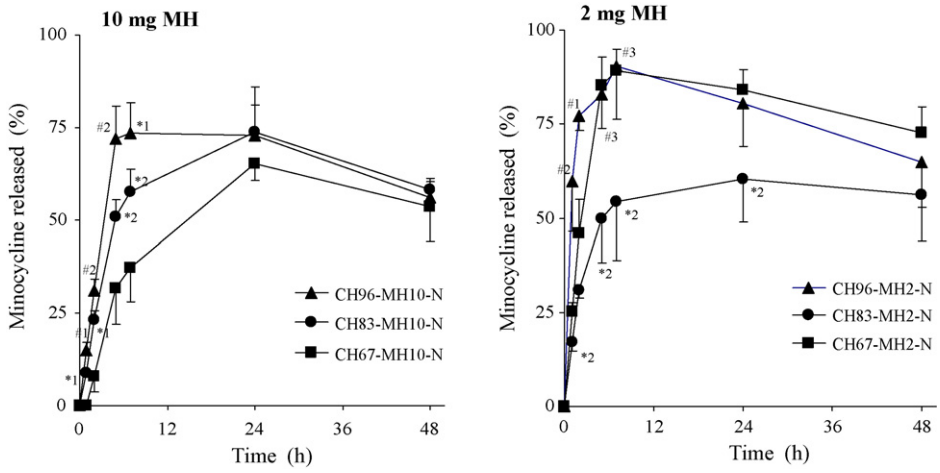

Fig. 3. Release of minocycline from CH-MH loaded with different amounts of minocycline hydrochloride. The results are expressed as the mean ± S.D. ($n = 3$). For comparison between two groups of the same MH amount: $^{\#}1$, $P < 0.01$ vs. CH83-MH-N and $P < 0.01$ vs. CH67-MH-N; $^{\#}2$, $P < 0.05$ vs. CH83-MH-N and $P < 0.01$ vs. CH67-MH-N; $^{\#}3$, $P < 0.05$ vs. CH83-MH-N; $^{*}1$, $P < 0.01$ vs. CH67-MH-N; $^{*}2$, $P < 0.05$ vs. CH67-MH-N.

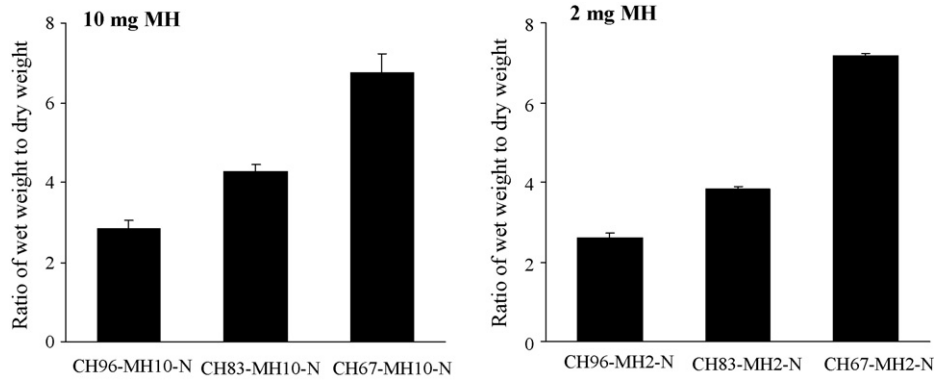

Fig. 4. Buffer absorption by CH-MH after incubation in phosphate-buffered saline, pH 7.4, at 37 °C for 48 h. The results are expressed as the mean ± S.D. ($n = 3$). For comparison between two groups: $P > 0.05$ for CH96-MH10-N vs. CH96-MH2-N and CH67-MH10-N vs. CH67-MH2-N, $P < 0.05$ for CH83-MH10-N vs. CH83-MH2-N, and $P < 0.01$ for other combinations.

stage of the in vitro release test was considered to be mainly due to the decomposition of minocycline. On the whole, CH67 and CH83 films were considered appropriate for gradual release; thus, formulations prepared with CH67 and CH83 were studied in the in vivo experiments.

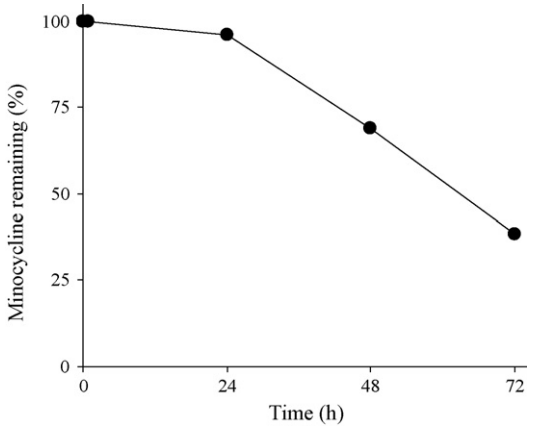

Fig. 5. Stability of minocycline in phosphate-buffered saline, pH 7.4, at 37 °C.

3.2. In vivo release of minocycline

In this study, CH83-MH2-N was applied to the wound surface, and the minocycline remaining in the formulation was investigated at appropriate time points after the start of application. More than 80% of the drug was released within 6 h after the start of application, and then little drug was released (Fig. 6). This was different from the in vitro release. Lysozyme in the exudate might accelerate the degradation of the CH film, leading to the faster release of minocycline. The maintenance of minocycline in the formulation from 6 h after the start of application was considered to be due to the highly viscous state of the exudate-swollen CH film and due to suppressed permeation caused by accumulation of the drug at the wound. This indicated that minocycline was released well in vivo and that the wound was exposed to the drug for a long period over 48 h.

3.3. Evaluation of wound status in the early stage

The wound status was evaluated for formulation sticking to the wound, absorption of exudate and elimination of pus 2 and

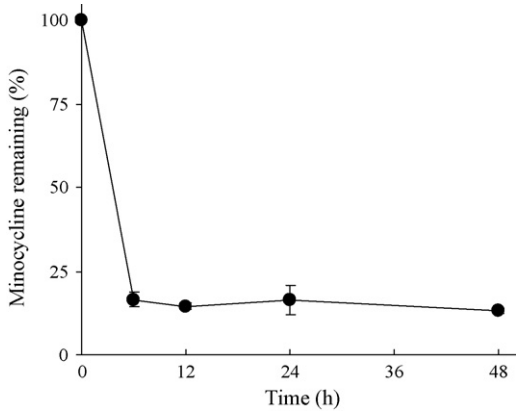

Fig. 6. Change of minocycline remaining in CH83-MH2-N after application to the burn wound. The results are expressed as the mean ± S.E. ($n = 3$).

Table 1
Effect of each preparation on wound sticking, absorption of exudate and elimination of pus in burn wound

Formulation	Animal number	Sticking to wound surface	Absorption of exudate	Elimination of pus
Gauze alone (control)	1	±	±	±
	2	±	±	±
	3	±	±	±
CH83-MH10-N	4	+	−	+
CH67-MH10-N	5	+	−	±
CH83-MH2-N	6	+	−	++
	7	++	−	+
	8	+	−	+
CH67-MH2-N	9	−	−	±
	10	+	−	+
	11	+	−	±
CH83-MH2-A	12	++	+	++
	13	++	++	++
	14	++	++	++
CH67-MH2-A	15	+	++	±
	16	+	++	+
	17	−	±	±
CH83 film	18	++	++	++
	19	++	++	+
	20	++	+	+
CH67 film	21	+	++	±
	22	+	++	+
	23	+	++	+
Beschitin W	24	+	±	±
	25	±	±	±
	26	±	±	±
MH ointment	27	++	±	++
	28	++	−	+
	29	++	±	+
Geben cream	30	±	++	++
	31	±	++	±
	32	−	±	±

The results are expressed as ++, +, ± and − for very good, good, neutral and negative responses, respectively.

4 days after the start of application except for CH-MH10-N, which was evaluated 3 and 6 days after the start of application. The results are shown for every rat tested in vivo (Table 1). In CH-MH-N, the absorption of exudate was inferior because the Tegaderm film covered the wound completely and prevented the absorption of exudate. Furthermore, the retained exudate was not good for sticking to the wound. In particular, in CH-MH10-N, as the wound status was inferior and the severe deposition of MH on the wound obviously prevented wound healing, the experiment was not repeated ($n = 1$). The wound status of CH-MH2-N was not as bad as CH-MH10-N, but the retention of exudate and MH deposition on the wound were still observed. On the other hand, CH-MH2-A showed good wound sticking, good absorption of exudate and fairly good elimination of pus. As the size and shape of CH-MH2-A were adjusted to the wound surface, the exudate could pass through the small gap between the wound and the formulation, which allowed the removal of exudate. In particular, CH83-MH2-A gave a very good wound status.

CH films and clinically used formulations were also examined for their effect on the wound status. Overall, CH83 film displayed a good effect. CH67 film exhibited good absorption of exudates, but its wound sticking and elimination of pus were not as good as CH83 film, because CH67 film was more swollen and more biodegradable. MH ointment was not good for the absorption of exudate, that is, the exudate remained at the wound. Geben cream absorbed the exudate well, but became hard, resulting in less wound sticking and insufficient elimination of pus. Thus, in the early stage, the wound status was excellent in CH83-MH2-A and CH83 film.

3.4. Effect of each formulation on wound healing

The change in the wound surface area for each formulation is shown in Figs. 7 and 8. In Fig. 7, gauze was applied to the

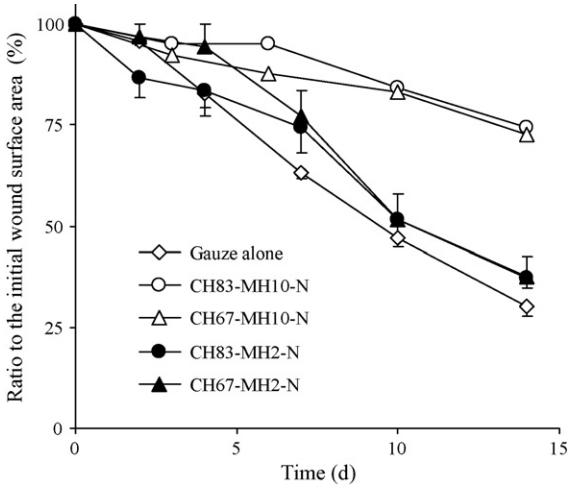

Fig. 7. Change in the wound surface area after the application of CH-MH-N to the burn wound. The results are expressed as the mean ± S.E. ($n = 3$) except CH-MH10-N ($n = 1$). There was no significant difference ($P > 0.05$) for gauze alone vs. CH83-MH2-N, gauze alone vs. CH67-MH2-N and CH83-MH2-N vs. CH67-MH2-N.

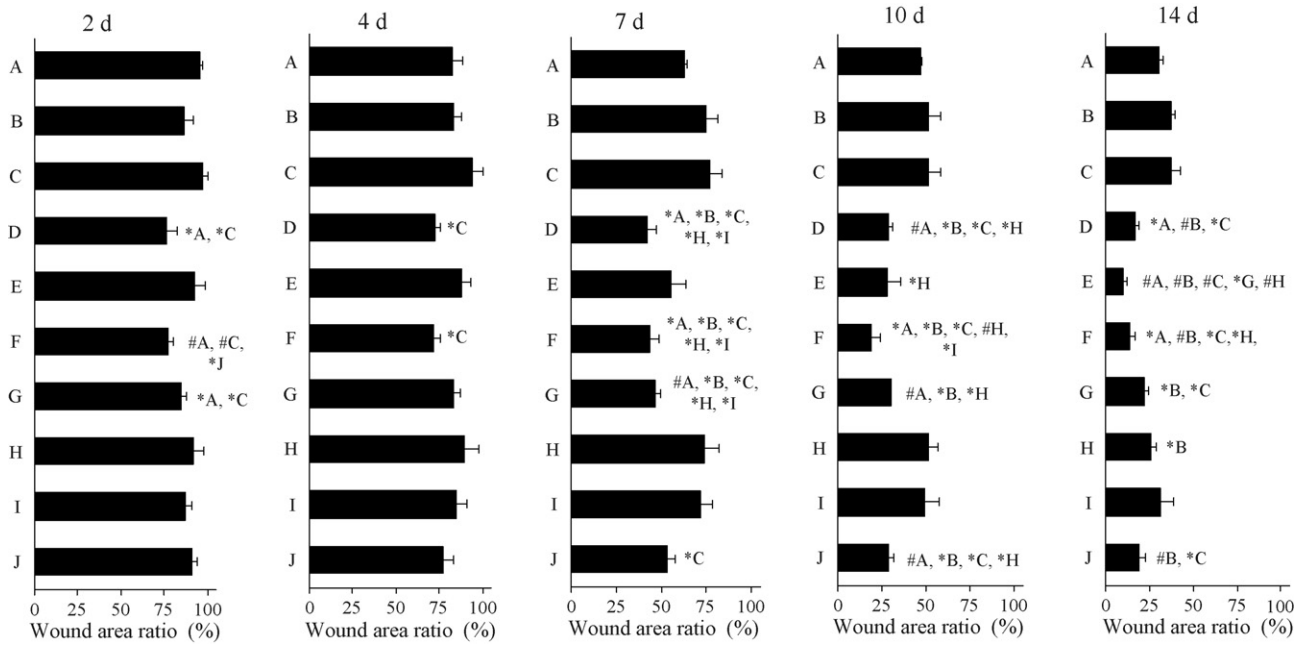

Fig. 8. Change in the wound surface area after the application of each preparation to the burn wound. The results are expressed as the mean ± S.E. ($n = 3$). For comparison between two groups in each observation day: $^{\#}$X, significantly smaller than X group ($P < 0.01$); *X, significantly smaller than X group ($P < 0.05$). A, gauze alone; B, CH83-MH2-N; C, CH67-MH2-N; D, CH83-MH2-A; E, CH67-MH2-A; F, CH83 film; G, CH67 film; H, Beschitin W; I, MH ointment; J, Geben cream.

wound surface after the application of CH-MH-N. CH-MH10-N retarded wound healing to a great extent. For CH-MH10-N and CH-MH2-N, minocycline was retained on and in the wound for a long time even after changing to gauze, the wound became black, and this status was prolonged. The long retention of minocycline on and in the wound appeared to inhibit wound healing. Another reason for retardation of healing was considered to be that complete sealing by Tegaderm prevented the removal of exudate. In particular, the wound status was inferior in CH-MH10-N. Although the amount of MH was reduced to 2 mg (CH-MH2-N), this situation did not improve sufficiently. Namely, for CH-MH2-N, the wound also became black and was not good in comparison with the control (gauze alone), though CH83-MH2-N and CH67-MH2-N were not significantly different from the control (gauze alone) ($P > 0.05$) (Fig. 7).

Fig. 8 shows the change in the wound surface area for the control and each formulation, which were examined in three experiments. Beschitin W was similar to the control (gauze alone), suggesting that its healing effect was slight for this severe burn wound. CH-MH2-A and CH film showed good reduction of the wound area ratio. In particular, CH83-MH2-A, CH83 film and CH67 film showed a good healing effect (Fig. 8), which appeared to be related to the good wound status (Table 1). On the whole, CH83-MH2-A and CH83 film were excellent for wound healing. In CH-MH2-A, the wound became pale yellow, and neither blackening nor wound deterioration was observed.

Although MH ointment and Geben cream are clinically available formulations, they appeared not as effective as CH83-MH2-A and CH83 film. MH ointment showed no healing effect, while Geben cream displayed good effects in the later stage. As shown in Table 1, when MH ointment was applied, less exudate was eliminated, probably leading to lower effectiveness. In Geben cream, the absorption of exudate was fairly good; however, CH83-MH2-A and CH3 film tended to be better than those commercially available formulations.

These results suggested that CH83-MH2-A and CH83 film were the best formulation for severe burn wounds. They were considered to keep the wound in a good condition because of wound protection, appropriate absorption of exudate and antibacterial function. One possible reason for the little difference between CH83-MH2-A and CH83 film is that the effect of minocycline may not have been observed markedly because the in vivo experiments were performed in a clean room; that is, chitosan itself might work well as an antibacterial agent under these conditions (Seo et al., 1992). Animal experiments in less clean conditions may elucidate the difference in efficacy between CH83-MH2-A and CH83 film; however, the elimination of pus appeared to be somewhat better with CH83-MH2-A than CH83 film, which might be associated with the effect of MH (Table 1). Furthermore, further examinations including an increased number of in vivo experiments are considered important for more detailed evaluation of CH83-MH2-A.

4. Conclusion

A novel wound dressing made of CH film and MH using Tegaderm backing film showed gradual drug release, which was dependent on the type of CH and the amount of MH. The use of 10 mg of MH and complete sealing with Tegaderm film had a negative effect on wound healing. MH ointment did not show a good effect, but Geben cream was fairly effective. On the whole, CH83-MH2-A and CH83 film exhibited an excellent

healing effect. Furthermore, CH83-MH2-A and CH83 film had the advantage that there was no need to wipe or wash the wound surface when changing the formulations. Considering the elimination of pus, CH83-MH2-A appeared better than CH83 film. CH83-MH2-A is suggested as a possibly useful formulation for healing severe burn wounds. Further examination including an increased number of in vivo experiments may be needed for more detailed evaluation of CH83-MH2-A.

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References

Fukawa, K., Iwadate, K., Ito, Y., Ohbayashi, S., Saita, O., Irino, O., Sawabe, T., 1982. Studies on a rat model of decubitus: therapeutic effects of a powder preparation of aluminum chlorohydroxy allantoinate for external use on the established experimental decubitus. Pharmacometrics 23, 999–1011.

Holland, K.T., Davis, W., Ingham, E., Gowland, G., 1984. A comparison of the in vitro antibacterial and complement activating effect of 'OpSite' and 'Tegaderm' dressings. J. Hosp. Infect. 5, 323–328.

Honnorat-Benabbou, V.C., Lebugle, A.A., Sallek, B., Duffaut-Lagarrigue, D., 2001. Stability study of tetracyclines with respect to their use in slow release systems. J. Mater. Sci. Mater. Med. 12, 107–110.

Imamura, S., et al., 1989. The clinical effect of KT-136 (sugar and povidone–iodine ointment) on decubitus ulcers—a comparative study with lysozyme ointment. Jpn. Pharmacol. Ther. 17, 255–281.

Katsuura, M., Ikeda, K., Mikajiri, K., Oishi, M., Arakawa, Y., Kataoka, K., Kurokawa, N., 2002. Preparation of a hospital dosage form 0.2% minocycline ointment and accumulation of its drug information. Med. Drug J. 38, 1067–1074.

Machida, Y., Yamamoto, N., Furuya, K., Takahashi, Y., Iwata, M., Shirotake, S., Onishi, H., 1997. Preparation of a novel patch for the treatment of deep wounds and evaluation of the therapeutic effect on rats. Yakuzaigaku 57, 57–63.

Niimura, M., et al., 1990. Clinical evaluation of DT-5621 in patients with chronic skin ulcer: multicenter, placebo-controlled double blind study. Jpn. Pharmacol. Ther. 18, 2757–2770.

Orti, V., Audran, M., Gibert, P., Bougard, G., Bressolle, F., 2000. High-performance liquid chromatographic assay for minocycline in human plasma and parotid saliva. J. Chromatogr. B: Biomed. Sci. Appl. 738, 357–365.

Oshima, Y., et al., 1987. The clinical study of chitin wound dressing (Beschitin W) on skin wound caused by collection of split-thickness graft. Nishinihon J. Dermatol. 49, 721–726.

Pawelczyk, E., Matlak, B., 1982. Kinetics of drug decomposition Part 74. Kinetics of degradation of minocycline in aqueous solution. Pol. J. Pharmacol. Pharm. 34, 409–421.

Seo, H., Mitsuhashi, K., Tanibe, H., 1992. Antibacterial and antifungal fiber blended by chitosan. In: Brine, C.J., Sandford, P.A., Zikakis, J.P. (Eds.), Advances in Chitin and Chitosan. Elsevier Applied Science, London, pp. 34–40.

Shigeyama, M., 2004. Preparation of a gel-forming ointment base applicable to the recovery stage of bedsore and clinical evaluation of a treatment method with different ointment bases suitable to each stage of bedsore. Yakugaku Zasshi 124, 55–67.

Shigeyama, M., Ohgaya, T., Kawashima, Y., Takeuchi, H., Hino, T., 1999. 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. Chem. Pharm. Bull. 47, 744–748.

Shigeyama, M., Ohgaya, T., Kawashima, Y., Takeuchi, H., Hino, T., 2000. Modification of the physicochemical properties of minocycline hydrochloride ointment with cyclodextrines for optimum treatment of bedsore. Chem. Pharm. Bull. 48, 617–622.

Shigeyama, M., Oogaya, T., Yoneyama, T., Futamura, M., Murakawa, T., Shibata, H., Takeuchi, H., Kawashima, Y., 2001a. Preparation of a gel-forming ointment base applicable to the recovery stage of bedsore and clinical evaluation of a treatment method with different ointment bases suitable to each stage of bedsore. Yakugaku Zasshi 121, 441–450.

Shigeyama, M., Ohgaya, O., Takeuchi, H., Hino, T., Kawashima, Y., 2001b. Formulation design of ointment base suitable for healing of lesions in treatment of bedsores. Chem. Pharm. Bull. 49, 129–133.

Sone, K., Nakamura, Y., Ikeda, T., Handa, Y., 1984. Use of granulated sugar to pressure sores. J. Nippon Hosp. Pharm. Assoc. 10, 315–322.

Susuki, T., et al., 1983. Clinical studies on 1% silver sulfadiazine cream (T-107)—results in the 10 medical facilities in the Kansai district. Clin. Rep. 17, 3827–3836.

Tachihara, K., Onishi, H., Machida, Y., 1997a. Evaluation of films of chitin, chitosan and chitin–chitosan mixture as dressings for dermal burn wounds. Yakuzaigaku 57, 40–49.

Tachihara, K., Onishi, H., Machida, Y., 1997b. Preparation of silver sulfadiazine-containing spongy membranes of chitosan and chitin–chitosan mixture and their evaluation as burn wound dressings. Yakuzaigaku 57, 159–167.

Yamamoto, S., et al., 1990. The clinical study of L-300 ointment on skin ulcers. Nishinihon J. Dermatol. 52, 1222–1229.

Yamashita, S., Ohmi, A., Hamada, Y., Oishi, K., Yano, M., Kuroki, S., Fujii, Y., Akamatsu, H., Kobatake, H., 1989. Effects of ointment containing collagenase derived from achromobacter iophagus (ACR-59 ointment) upon burn, decubital ulcer, open and cut wounds studies with experimental rat models. Pharmacometrics 37, 313–327.