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Evaluation of Topical Drug Containing Solcoseryl and Micronomicin on Surgical Wound in Mice

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Abstract—Wound healing and antibacterial effects of solcoseryl-micronomicin combination gel on an open wound were studied in mice. A simple model was designed for assessing the effects. Using the model, we compared the efficacy of a combined topical gel of solcoseryl and micronomicin with those gels of solcoseryl or micronomicin alone. From the results of our experiment, the wound healing effect of open wounds by treatment with the combination gel was significantly enhanced when compared with those by treatment with solcoseryl gel or micronomicin gel alone. And the antibacterial effect of the combination gel was higher than those of solcoseryl gel or micronomicin gel alone.

Keywords □ topical, wound healing effect, antibacterial effect, solcoseryl-micronomicin combination.

Wound healing is restoration of the continuity of living tissue. It is a product of the integrated response of several cell types to injury. It involves platelet aggregation and blood clotting, formation of fibrin, inflammatory response to injury, alteration in the ground substance, endothelial and capillary proliferation, surface covering, regeneration of certain cell types, variable contracture and remodeling. Healing can not be completed until the disrupted surfaces are firmly knit by collagen. And man is not germfree. Even in health, man's environment is one of an ubiquitous bacterial presence. All wounds are to a certain extent "contaminated" if only from the normal skin flora, yet only relatively few actually develop infection.

Experimental and clinical studies show that solcoseryl, protein-free haemodialysate, activates cellular respiration (Jaeger *et al.*, 1965) and increases the energy potential of the cells thus accelerating the healing of tissue lesions (Szekeres *et al.*, 1973). It is well known as an effective and successful agent for treatment of burns, skin transplants, torpid wounds (Brochure of Solco Basle Ltd., 1986), trophic skin ulcers (Wyss and Basti, 1964) and other disorders of the peripheral blood circulation with or without trophic lesions (Krause and Dittmar, 1976). Micronomicin (MCR) is an aminoglycoside antibiotic with a wide antibacterial spectrum against both gram negative and positive bacteria (Goto *et al.*, 1977). MCR exhibits particularly high activity against *Pseudomonas aeroginosa, Proteus, Serratia and Klebsiella pneumoniae* (Sato *et al.*, 1977). With the recent increase in opportunistic infections largely due to gram negative rods, aminoglycoside antibiotics are gaining clinical significance. Even though aminoglycoside antibiotics are known to have ototoxicity and nephrotoxicity as general side effects, MCR is less toxic than gentamicin or dibekacin to the 8th cranial nerve or kidney (Takeda *et al.*, 1977).

In this paper, the well-marked tissue regenerative action of solcoseryl and the effective antibacterial action of MCR led us to try the combination in topical gel of the two components for the treatments of burns, surgical wounds and other skin lesions with primary and secondary bacterial infections. To evaluate the efficacy of the topical drug containing solcoseryl and MCR, we designed a surgical wound model in mice and compared the wound healing effect and the antibacterial effect of the combination with those of solcoseryl or MCR alone.

Materials and Methods

Animals

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Table I. The composition of gel (g per 100 g)

Micronomicin sulfate	0.5
Solcoseryl	10.0
Poloxamer F 127 (Lutrol)	18.0
Propylene glycol	5.0
Sodium chloride	0.9
Methylparaben	0.2
Propylparaben	0.02
D.W.	q.s.[a]

[a]q.s. stands for quantum sufficit. Solcoseryl was obtained from Solco Basle Ltd. (Switzerland). Micronomicin sulfate was purchased from Kyowa Hakko Koqyo Co., Ltd. (Japan). Poloxamer F 127 was from BASF (Germany). All other chemicals were obtained from commercial sources and were reagent grade.

Male ICR mice, 25 ± 1 g, were purchased from Charles River Inc. (Japan). Mice were maintained in temperature-humidity-controlled clean rack system and were allowed to access sterilized food and water *ad libitum.*

Preparation of the Combined Gels

The composition of the gel is shown in Table I. The gels were prepared by Department of Pharmaceutical Science in Yung Jin Pharm. Co., Ltd.

***In vitro* MCR Elution Test**

To measure the rate of release of antibiotics from various bases, MCR elution test was performed *in vitro.* Using this method, an ideal gel to apply to open wounds was selected. *Bacillus subtilis ATCC 6633* was incubated in Müller Hinton Broth at 37℃ for 18 hrs and then, the culture broth was diluted to 1 : 100 with Müller Hinton Agar. The mixture was poured into a bioassay tray (245×245×20 mm) and solidified. New topical gels and standard MCR solution were loaded on the solidified media using paper disc (diameter: 6 mm). After the media were placed at room temperature for 1 hr, the media were incubated at 37℃ for 18 hrs and then the inhibitory diameters were measured. Finally, the released amount of anbtibiotics was calculated from the calibration curve of standard MCR.

$$\text{Release percent } (\%) = \frac{\text{Released amount of antibiotics}}{\text{Loaded amount of antibiotics}} \times 100$$

Experimental Groups

In the experiment to test wound healing effect, experimental groups were divided into control (mice were operated, and non-treated), base (treated only with vehicle), 10%-solcoseryl (solcoseryl in vehicle), 0.5 %-MCR (MCR in vehicle) and 10%-solcoseryl+0.5%-MCR(in vehicle) groups. Each group consisted of 10 mice.

In the experiment to test antibacterial effect, experimental groups were divided into control, base, 10%-solcoseryl, 0.5%-MCR and 10%-solcoseryl+0.5%-MCR groups. Each group consisted of 5 subgroups each containing 10 mice. On the first, second, seventh, tenth and fifteenth days after operations, 10 mice in each subgroup were sacrificed.

Experimental Wound and Infection

On the day of infection, mice were anesthetized by intraperitoneal injection of urethane (1.5 g/kg). The backs of the mice were closely shaved with a fine-tooth electric clipper. One wound (10 mm in diameter) was produced on the back and extended down to the panniculus carnosus using a sterile scissors. The wound was infected with 10^4 cfu of *Staphylococcus aureus* by direct seeding.

Test Organism

S. aureus ATCC SG 511 was used as the test organism. The organism was maintained on Müller Hinton Agar slants stored at 4℃, with transfering to fresh slants monthly.

Treatment of Wounds

The open wounds were treated topically twice a day. Approximately 300 mg of gel was applied over the entire wound. In the experiments to measure wound healing effect, the treatment was performed immediatly after the operation and in that to mesure antibacterial effect, the treatment was performed 24 hrs after the operation.

Quantitation of Bacteria in Wounds

The method of quantitation of bacteria in wounds used in this study was a modification of surface rinse method described by McRipley and Whitney (1976). At predetermined times after infection, mice were sacrificed. The wounds (10 mm in diameter) were excised and the flap of tissue placed into 10 m*l* of saline. They were vigorously blended for 10 mins to extract the bacteria from the excised tissues. Supernatants were appropriately diluted and then, plated on selective media, Staphylococcus media No. 110 (Difco) plates were used to prevent the chances of contamination. And they were incubated for 24 to 48 hrs at 37℃ and then the numbers of colonies were counted.

Assessment

In the experiment to test wound healing effect, results were represented by the ratio of cured wound area against initial wound area.

$$\text{Healing ratio}(\%) = 100 - \frac{TL \times TS}{IL \times IS} \times 100$$

TL: the longest diameter of the treated wound

TS: the shortest diameter of the treated wound
IL : the longest diameter of the initial wound
IS : the shortest diameter of the initial wound

In the experiment to test antibacterial effect, results were represented as the decreased counts of bacterial colonies in wounds.

Statistics

The data were expressed as means± S.E. The results were compared using Student's *t*-test.

Results and Discussion

General Consideration

In the treatment of skin wounds with various bacterial infections, it is generally accepted that the best way is to prevent bacterial infections effectively and accelerate wound healing activity of the wound tissues. Many topical preparations containing antibiotics and/or a wound healing agent have been developed to treat skin wounds. And we think that combination topical preparations have much advantage in treatment of skin wounds, compared with those containing only one of the components of combination topical drug.

With the advent of the bacterial strains causing resistant and opportunistic infections, the combination of effective antibiotics and wound healing agents has been required to heal skin wounds infected by troublesome bacteria. But, most topical drugs contain single component of antibiotics *i.e.* gentamicin, neomycin, fusidic acid, mupirocin and so forth, or wound healing agent such as *Centella asiatica* or solcoseryl. A few combination topical drugs containing antibiotics and a wound healing agent have been developed to take the advantage mentioned above.

Solcoseryl is a deproteinized dialysate of calf's blood containing low molecular weight peptides and nucleic acid derivatives. It is known that solcoseryl stimulates cellular regeneration and thus accelerates tissue repair (Brochure of Solco Basle Ltd., 1986).

Because of this effect, solcoseryl (Brochure of Solco Basle Ltd., 1986) is indicated for the treatment of trophic lesions in the widest sense of this term ; peripheral circulation disorders, disorders of cerebral circulation and metabolism, exogenous tissular damage; burns, radiation damage, torpid wounds, decubital ulcers and retarded wound healing.

MCR is an aminoglycoside antibiotic with a wide antibacterial spectrum. Because MCR exhibits particularly high activity against opportunistic infections, it is gaining clinical significance (Sato *et al.*, 1977).

Based on the effects of solcoseryl and MCR, we made combination topical drugs and studied with the model in mice.

Table II. Release rates of micronomicin from gels[a]

		Release rate[b] (%)			
Concentration of propylene glycol (%)		1	3	5	10
Concentration of NaCl (%)	0.9	100.7	98.5	86.1	78.1
	2.0	73.6	63.9	65.5	57.1
	3.0	59.1	47.5	50.3	60.6

[a]The gels were prepared as described in Materials and Methods.
[b]All values represent the means of three replicates.

MCR Elution Test

Using the fact that the major component of this gel is a kind of antibiotic, MCR, we checked the release rate of MCR by the agar diffusion method. Concentrations of NaCl and propylene glycol, which are important excipients determining the release rate, were changed according to Table II to determine the optimum concentrations.

As shown in Table II, the higher the concentration of NaCl and propylene glycol, the lower the release rate. The highest release rate was shown below 0.9%-NaCl and 3%-propylene glycol. However, since the gel was changed to liquid state below 15℃, we selected 0.9%-NaCl and 5%-propylene glycol.

Wound Model

In an effort to identify and evaluate substances that may be effective as topical agents in humans, many experimental models in laboratory animals have been developed (Glotzer *et al.*, 1970; Gray and Kidd, 1963; McRiply and Whitney, 1976). However, if a model is to provide an evaluation of efficacy to which some degree of confidence can be attached, then it must satisfy several conditions such as (i) the pathogenesis of wound must be known, (ii) the wound should be reproducible, readily controlled and provide some measurable end points, (iii) the model should be relatively simple to produce, (iv) the model must be easily observed and quantitated and (v) it should be easy to evaluate several compounds at one time in an adequate number of animals. The model described in Fig. 1 was established to use as a screening tool for identifying and evaluating substances that may be employed as topical antibacterial and wound healing agents. We modified the McRipley and Whitney's model (1976). And we think that most of the criteria are met in the surgical model we used. Immediately after the operation, there was only minimal bleeding, and it ceased spontaneously. Slight edema and erythema were evident du-

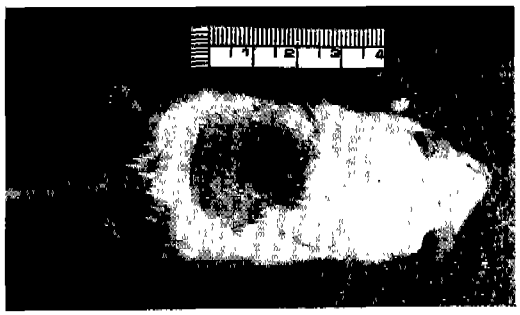

Fig 1. Photograph of surgical wound model.

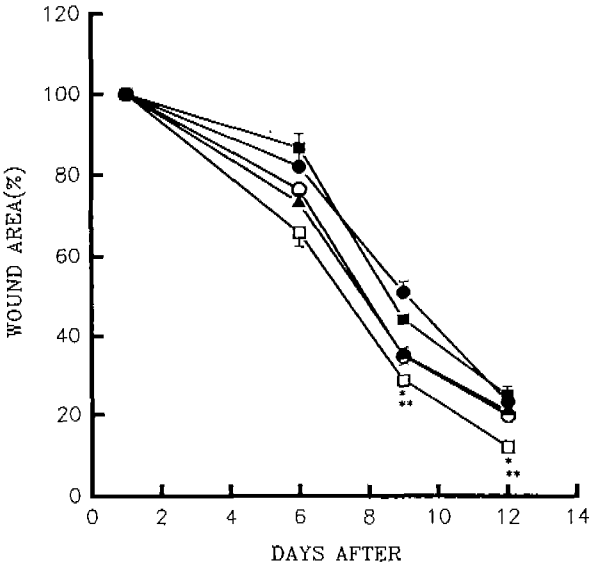

Fig 2. Wound healing effect of combination gel on open wound. ●, Control; ■, Base; ▲, 10%-Solcoseryl; ○, 0.5-Micronomicin; □, 10%-Solcoseryl+0.5%-Micronomicin. Results are expressed as mean± S.E. (n=8~10). Significant difference from 0.5%-micronomicin; *, $p<0.05$ Significant difference from 10%-solcoseryl; **, $p<0.05$.

ring the early stages of wound healing, which processed rapidly to almost complete repair in approximately 3 weeks.

Healing Effect of Gels on Open Wound

As shown in Fig. 2, significant decreases in wound area were observed in the groups treated with the combination topical drug, compared with the control group. Especially, there were significant healing effect in 6, 9 and 12 days in the combination gel-treated group. Furthermore, the combination gel was more effective ($p<0.05$) than topical gels of solcoseryl or MCR alone. Table III shows CT_{50} values that stand for the time for curing 50% of wound by the area. The wound healing period was almost 2 days shorter in the combination gel-treated group than in the control group. And as shown in Table III, significant derease ($p<0.05$) in

Table III. CT_{50} values of various gels on open wound

Gel	CT_{50} (day)[a]
Control	9.05± 0.26
Base only	8.60± 0.09
10%-Solcoseryl	7.92± 0.17
0.5%-Micronomicin	7.95± 0.12
10%-Solcoseryl+0.5%-Micronomicin	7.23± 0.23[b]

[a]CT_{50} stands for Half Closure Time of wound. Results are expressed as mean± S.E. (n=8~10). Significant difference from 10%-solcoseryl or 0.5%-micronomicin.; [b], $p<0.05$.

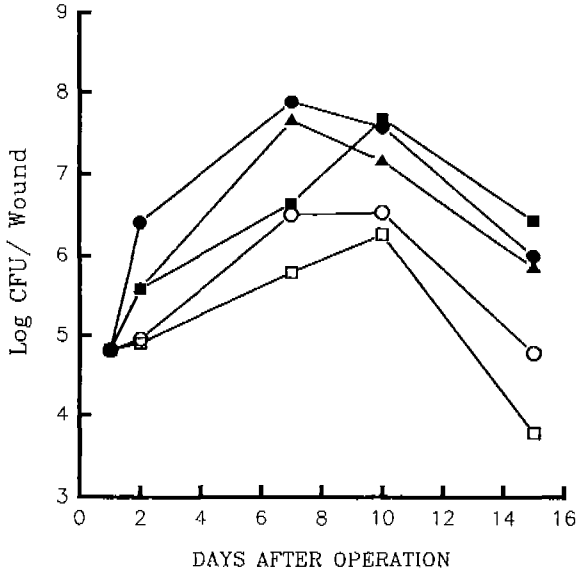

Fig 3. Effect of topical therapy with combination gel twice daily on survival of *S. aureus SG511* in mice skin wound. ●, Control; ■, Base; ▲, 10%-Solcoseryl; ○, 0.5%-Micronomicin; □, 10%-Solcoseryl+0.5%-Micronomicin. Each point represents the mean count from 10 mice.

wound area was observed in the combination gel-treated group, compared with 10%-solcoseryl or 0.5%-MCR group. In conclusion, it was shown that the combination gel was more effective than single component-gel for therapy of surgical wounds.

Antibacterial Effect of the Combination gel

Major problem encountered occasionally in the model infection was contamination from skin, hair and feces. To circumvent potential problems in colony counting that might be caused by contaminants, selective media were employed. As shown in Fig. 3, the bacteria counts were increased over 10^6 organisms/wound 2 days after infection, remaining at this level for up to 2 weeks in control group. Treatment with vehicle only was ineffective, whereas twice daily application of combination gel resulted in great decrease of the *Staphylococci*. Moreover, the combination gel showed a tendency to be more effective than either 0.5%-MCR gel

alone or 10%-solcoseryl gel alone.

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