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Dimethyl Sulfoxide (DMSO) Does Not Affect Epidermal Wound Healing (41561)

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Abstract. We studied the effect of 95% DMSO on dermal/epidermal healing and microbiol flora in partial-thickness wounds. Wounds of 0.3 mm were made in the skin of Yorkshire pigs with a keratome and treated daily with either 95% DMSO, water, or they were left untreated. Wounds were excised on Days 2–7 and the dermis was separated from the epidermis. The dermis was assayed for collagen biosynthesis (by measuring the production of [^{14}C]hydroxyproline (HP) and amount of radioactive peptides released after collagenase digestion) and absolute HP (by spectrophotometric analysis). The epidermis was evaluated macroscopically for resurfacing. Aerobic bacteria from unwounded and wounded skin were identified and quantitated. There were no significant differences between treatment groups in HP incorporation or absolute collagen content from Days 2–6 after wounding. HP incorporation in the total protein fractions and in the collagenase digestible fractions were analogous. Collagen biosynthesis was similar in both unwounded, untreated, and unwounded DMSO-treated skin. Epidermal healing did not differ between treatment groups. There were no differences in the number or types of bacteria in wounds between treatment groups. These results indicate that topical DMSO is neither beneficial nor harmful in the healing of superficial wounds.

Dimethyl sulfoxide (DMSO), an industrial solvent and by-product of paper manufacture was first reported to have pharmacologic activity in the early 1960s (1). Early human clinical trials with DMSO indicated beneficial anti-inflammatory and analgesic effects in musculoskeletal injuries, arthritis, and bursitis (2). The Food and Drug Administration has prohibited human use of DMSO for any condition except interstitial cysitsis, an inflammatory disease of the bladder, because of reports of lens changes and teratogenicity in experimental animals administered DMSO. Today DMSO is self-administered for conditions such as arthritis, other musculoskeletal disorders (3), and minor skin injuries (Jacob SW: personal communications).

Since Jacob's original report of the pharmacologic activity of DMSO (1), many investigators have studied the effects of topically applied DMSO on various parameters of wound healing. Huu and Albert (4) found that full-thickness wounds in rabbits treated with 70% DMSO had greater breaking strength after 7 days than wounds from untreated animals. Geever *et al.* (5) showed 95% DMSO, when applied to full-thickness wounds in guinea pigs had no effect on breaking strength, hydroxyproline content, or histologic healing of wounds compared to control animals. Gries *et al.* (6) showed that the hydroxyproline content of cotton pellet granulomas in rats treated with 100% DMSO over a 20-day period did not differ significantly from untreated controls. Kovac and Suckert (7) found that 90% DMSO decreased the weight of granulation tissue in full-thickness wounds in rats after 7 days compared to control animals.

In the adult newt, Slattery and Schmidt (8) demonstrated that immersion of the newts in 2% DMSO led to earlier wound closure of amputated forelimb stumps when compared to untreated controls. Uncontrolled studies with animals and humans have reported beneficial effects of DMSO in the healing of wounds (9, 10).

These conflicting results with DMSO and the availability of a new, simple animal model to study epidermal wound healing (11), led us to investigate the effects of topically applied DMSO on the healing of partial-thickness wounds. We have expanded our original model in order to evaluate the epidermal, dermal, and microbiological aspects of wound repair. We report here that topically applied 95% DMSO does not affect epidermal re-epithelialization, dermal collagen synthesis, der-

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mal hydroxyproline content, and the number and types of bacteria during the repair of partial-thickness skin wounds in domestic pigs. Domestic pigs were used because of the similarity of their skin to human skin (13).

Methods. Ten white domestic pigs weighing 8.0–12.3 kg were shaved with standard animal clippers and any remaining hairs were shaved with a pair of fine barber's clippers. The skin on both sides of the animal was prepared for wounding by washing with sterile saline.

Each pig was anesthetized with sodium pentobarbital (60 mg/2.3 kg) ip and approximately 100–125 rectangular wounds measuring 7 mm wide, 10 mm long, and 0.3 mm deep were made in the paravertebral and thoracic areas with a Castroviejo keratome (Storz Instruments, St. Louis, Mo.) fitted with a razor blade modified so that the cutting edge was 7 mm wide. The wounds were separated from one another by at least 15 mm. The animals were housed separately after wounding.

Treatment. The wounds on six animals were randomly divided into three treatment groups and treated topically as follows: 0.1 ml 95% DMSO (certified ACS[2] DMSO, Fisher Scientific, Pittsburgh, Penn.); 0.1 ml distilled water, and internal control (untreated).[3] Application of DMSO and water to the wounds were made shortly after wounding, Day 0, and each day thereafter. In order to rule out the possibility that wounds made in different anatomical regions healed differently, the regions used for each treatment varied for each animal. At the same time, four additional animals, wounded in the same manner as the six previous animals, were used as an external control and were not treated with DMSO.

Sampling and direct evaluation. Two days after wounding and each day thereafter for 7 days, the animals were anesthetized and several wounds with surrounding normal skin were excised from each treatment group. The excisions were made with a Castroviejo keratome fitted with a 22-mm-wide blade, set to cut at a depth of 0.5 mm. After incubating the specimens in 0.5 *N* sodium bromide for 20 hr, the epidermis was separated from the dermis, the crust, if still present, was removed, and the epidermal sheet was assessed for re-epithelialization according to Eaglstein and Mertz (11). Briefly, this method consists of a macroscopic examination of the epidermal sheet for defect. Defects are visualized as holes in the separated epidermal sheet or as a lack of epidermal continuum in the area that contains the wound. The wound is considered re-epithelialized if there are no defects in the epidermis and not re-epithelialized if there is one or more defects.

Collagen biosynthesis. Collagen biosynthesis in the dermal specimens from unwounded, untreated and unwounded, DMSO-treated skin (0.2 ml DMSO/70 mm^2) from two animals, and dermal specimens from excised wounds of all treatment groups from three animals were assayed for collagen biosynthesis by measuring the incorporation on [^{14}C]proline into [^{14}C]hydroxyproline as described by Uitto (14) and by bacterial collagenase digestion using a modification of the method described by Peterkofsky and Diegelmann (15).

The dermal specimen was finely minced and placed in a 25-ml flask with 5 ml of phosphate-free Krebs–Ringer medium containing 20 m*M* HEPES (pH 7.4), 20 m*M* glucose, 0.05 mg/ml ampicillin, and 5 μCi (^{14}C) proline.[4] After a 3-hr labeling period 30 m*M* disodium EDTA, 10 m*M* *N*-ethylmaleimide, and 0.3 m*M* phenylmethylsulfonylfluoride were added in order to inhibit protease activity. The incubation mixture was then homogenized (Tekmar Tissuemizer) and treated with protease-free ribonuclease (15 μg/ml, 5 min, 37°C) to cleave radioactive prolyl transfer RNA. The homogenate was cooled to 4°C and TCA was added to give a final concentration of 5%. The precipitate was centrifuged at 10,000*g* for 5 min, and the supernatant containing unincorporated radioactive proline was discarded. This washing procedure was repeated three times. TCA was then extracted by two ethanol ether (3:1, v/v) washes.

[2] Abbreviations used: ACS, American Chemical Society; HEPES, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfanic acid; EDTA, ethylenediaminetetraacetate; TCA, trichloroacetic acid.

[3] All chemicals unless specified were purchased from Sigma Chemical Company, St. Louis, Mo.

[4] [^{14}C]Proline specific activity 285 mCi/mmole was obtained from Amersham.

The precipitated protein was dried slowly (under vacuum) to a powder. The protein powder substrate was dissolved in 1 ml 0.2 *N* NaOH at 37°C (20 mg/ml). A 0.2-ml aliquot of the dissolved substrate was transferred to a 3-ml conical tube and neutralized with 0.15 ml 0.1 *N* HCl. The mixture (0.5 ml) was then buffered with HEPES (100 μM, pH 7.4). Collagen in the substrate was digested by purified bacterial collagenase (25 μg) (Advanced Biofactures) in 0.05 *M* Tris–HCl buffer (pH 7.5) containing 0.25 μM $CaCl_2$, 10 m*M* *N*-ethylmaleimide and 1 m*M* phenylmethylsulfonylfluoride. Collagenase digestion was carried out for 2 hr at 37°C and stopped by the addition of 0.5 ml of 10% TCA–0.5% tannic acid. Collagen released in the supernatant fluid was analyzed by liquid scintillation as described previously (15).

Hydroxyproline content. The hydroxyproline content of dermal specimens from excised wounds of all treatment groups from two animals were determined by a modification of the procedure of Bergman and Loxley (16). Dermal specimens were weighed and homogenized in 5 ml of phosphate-buffered saline. The homogenate was hydrolyzed with 5 ml of 12 *N* HCl at 120°C overnight. For decolorization, 1 g of activated charcoal was added and the mixture was centrifuged at 1000*g* for 20 min. One milliliter of the supernatant was transferred to a 15-ml conical tube and neutralized with 10 *N* KOH using phenol red indicator. For neutralization, 1 *N* HCl was used to titrate back until a citron yellow color was observed. The volume was adjusted to 2.5 ml with distilled water and up to 2.5 ml with isopropanol. After gentle agitation, 0.5 ml of the oxidant solution (7% aqueous Chloramine T) was added and kept for exactly 5 min at room temperature. Five milliliters of Ehrlich's regeant was added and incubated overnight at room temperature. Appropriate standards and samples were diluted to assayable ranges and were read on a Bausch and Lomb Spectronic 710 spectrophotometer at 558 nm.

Microbiologic studies. The technique of Krizek and Robson (17) was used to determine the number and types of microorganisms in normal skin prior to wounding and in wounds from each treatment group on Days 2, 4, and 6 after wounding in two experimental animals. Biopsy homogenates were plated on nonselective growth media by means of an automatic spiral plater (Spiral Systems Inc., Bethesda, Md.) for quantitation, isolation, and identification of microorganisms. These studies were limited only to fungi and aerobic gram-positive and gram-negative bacteria.

Serum concentrations of DMSO and $DMSO_2$. Serum concentrations of DMSO and a metabolite, dimethyl sulfone ($DMSO_2$), were determined by gas chromatography (18) on Days 2, 4, and 6 after wounding in one experimental animal treated with DMSO.

All data were analyzed statistically using the Student's *t* test (19).

Dermal protein assay. The method of Hartree (20) was used to determine total tissue protein in TCA precipitates before collagenase digestion.

Results. *Epidermal healing.* Six experimental animals each had two groups of wounds treated separately with 95% DMSO and distilled water, and one group of untreated wounds (Fig. 1). Wounds from six animals were evaluated on Days 2–6 after wounding and from five animals on Day 7 after wounding (Fig. 1). The results of epidermal re-epithelialization did not differ significantly between DMSO-treated, water-treated, and internal control (untreated) wounds.

Four additional experimental animals had wounds which did not receive DMSO or water. Wounds from these four animals were evaluated on Days 3–6 after wounding. Re-epithelialization of these untreated wounds did not differ significantly when compared to any of the three treatment groups.

The time needed for 50% of the wounds in each treatment group and the external control to heal (HT_{50}) was determined from the data in Fig. 1 by plotting the percentage of wounds re-epithelialized against days after wounding. These HT_{50} values and the relative rate of healing are listed in Table I. The HT_{50} for each treatment group and the external control were similar.

Dermal healing. The effects of 95% DMSO on the production of [^{14}C]hydroxyproline in total protein and collagenase digestible protein is shown in Fig. 2A and 2B. There were no significant differences between treatment

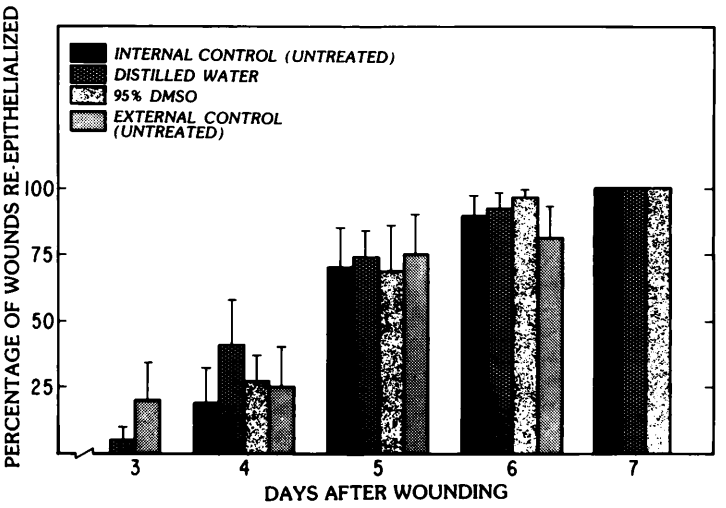

FIG. 1. The effect of 95% DMSO on epidermal re-epithelialization of partial-thickness skin wounds in domestic pigs. Values represent percentage of wounds re-epithelialized ± SEM for each treatment group on Days 3–7 after wounding. Data for Day 2 (all treatment groups) and Day 3 (untreated and 95% DMSO) is not represented since none of these wounds were healed. No specimens were available from the external control on Day 7. Results are based on the pooled data of 484 epidermal specimens from 10 experimental animals. Between 20 and 35 (mean of 36) wounds were evaluated from each treatment group daily.

groups in dermal collagen biosynthesis and dermal hydroxyproline production from Days 2–6 after wounding. Dermal collagen biosynthesis also was similar in unwounded, untreated and unwounded, DMSO-treated skin. However, collagen biosynthesis in wounded skin was significantly greater than in unwounded skin. Dermal hydroxyproline content of wounded skin is shown in Fig. 3. Dermal hydroxyproline content did not significantly differ ($P > 0.5$) between treatment groups throughout the healing process.

TABLE I. TIME NEEDED FOR 50% OF THE WOUNDS TO HEAL (HT_{50}) AND RELATIVE RATE OF HEALING COMPARED TO INTERNAL CONTROL

Treatment	HT_{50} (days)	Relative rate of healing compared to control[a]
Internal control (untreated)	4.6	0
Distilled water	4.3	+6%
95% DMSO	4.5	+2%
External control (untreated)	4.5	+2%

[a] Relative rate of healing

$$= \frac{\text{Internal control } HT_{50} - \text{Treatment group } HT_{50}}{\text{Internal control } HT_{50}} \times 100\%.$$

Microbiologic studies. The number of colony-forming units per gram of tissue (CFU/g tissue) in wound biopsies are listed in Table II. There were no significant differences between treatment groups in the number of bacteria in wound biopsies on Days 2, 4, and 6 after wounding. The number of bacteria in wounds of all treatment groups was greater than the number of bacteria in normal skin prior to wounding.

The normal flora of pig skin, prior to wounding, consisted mainly of gram-positive, coagulase-negative cocci, either *Staphylococcus epidermis* or *Micrococcus species*. These microorganisms were always isolated from all wounds biopsied as the predominant microflora. Occasionally *S. aureus*, *Streptococcus species*, and gram-negative bacteria were identified, but their numbers were minimal.

Serum concentrations of DMSO and $DMSO_2$. The serum levels of DMSO and $DMSO_2$ on Days 2, 4, and 6 after wounding were undetectable (less than 0.2 g/ml for DMSO and less than 16.7 g/ml for $DMSO_2$).

Discussion. To our knowledge, the effect of DMSO on wound re-epithelialization has not been previously studied in a controlled ex-

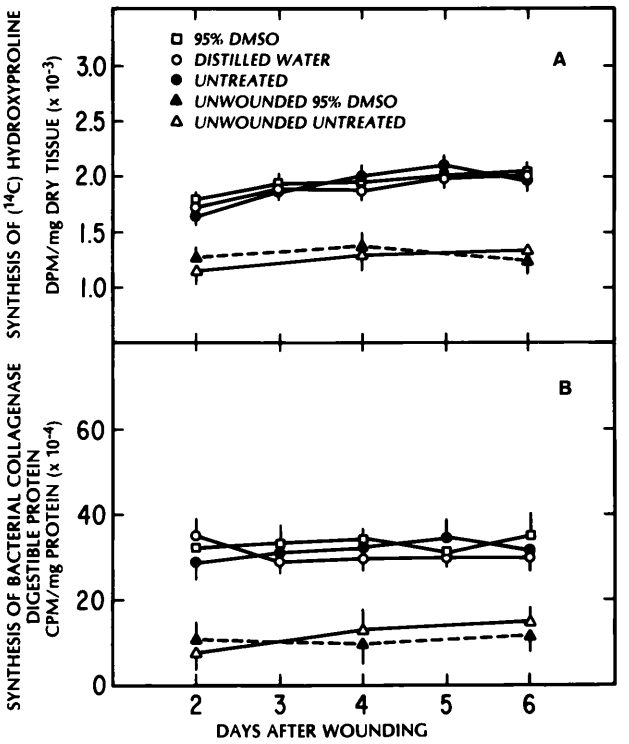

FIG. 2. (A) Effect of DMSO on the incorporation of [^{14}C]hydroxyproline in unwounded skin and healing wounds. Values from wounded skin represent the mean ± SEM of four observations from each of three animals. Values from unwounded skin represent the mean ± SEM of three observations from each of two animals. (B) Effect of DMSO on collagen biosynthesis in unwounded skin and collagen biosynthesis during wound healing. Values from wounded skin represent the mean ± SEM of three observations from each of three animals. Values from unwounded skin represent the mean ± SEM of three observations from each of two animals.

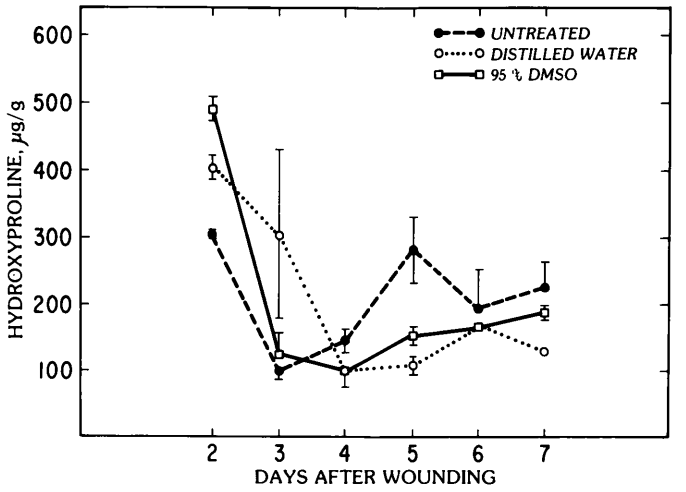

FIG. 3. Effect of DMSO on dermal hydroxyproline concentrations during wound healing. Values represent the mean ± SEM of four observations from each of two animals. Hydroxyproline values are expressed as μg/g tissue weight.

TABLE II. QUANTITATIVE MICROFLORA OF WOUNDS[a]

Treatment	Day 0	Day 2	Day 4	Day 6
Unwounded skin	3.75			
Untreated		5.8	6.6	6.5
Distilled water		6.2	6.8	6.2
95% DMSO		6.4	5.4	6.2

[a] Values are the log geometric mean of triplicate counts for each of two experimental animals.

periment. Kligman (21) observed that 95% DMSO did not affect the re-epithelialization of second-degree burns in humans. Krizek *et al.* (22) has shown that infected second-degree burns in rats treated with 90% DMSO re-epithelialized faster than controls. However, there were no differences in mortality between the two groups of rats. Although the healing of second-degree burns occurs much more slowly than the healing of partial-thickness excision wounds, our results agree with Kligman's findings. It has been shown *in vitro* that concentrations of DMSO greater than 4.5% in the incubation medium inhibited epidermal migration of skin explants (23). We found the DMSO did not inhibit wound re-epithelialization which depends to a large degree upon the migration of epidermal cells across the wound surface (24).

To our knowledge, the effects of DMSO on collagen biosynthesis in healing wounds or in normal skin has also not been studied before. A correlation was observed in the amounts of radioactive hydroxyproline between the total protein fractions and the collagenase-digestible fractions. This indicates that the majority of the hydroxylated proline in both wounded and unwounded pig dermis is incorporated into collagen and not noncollagenous protein. Our observations on the effect of DMSO on dermal hydroxyproline content in partial-thickness wounds are in agreement with two previously described studies (5, 6). Fleischmajer (25) observed that 70% DMSO did not affect the hydroxyproline concentration of guinea pig skin over a 3-week period. In an unpublished experiment, we found that 95% DMSO did not affect the dermal hydroxyproline concentration of unwounded pig skin over a 6-day period compared to unwounded, untreated skin (93 μg/g for 95% DMSO and 97 μg/g for untreated). Since DMSO did not influence dermal collagen biosynthesis or absolute dermal hydroxyproline content in wounded and unwounded skin, it is likely that it does not affect collagen degradation.

Several reports (21, 26–28) have described the *in vitro* bacteriostatic and bactericidal activity of DMSO. Kligman (21) has reported a 90% reduction of axillary flora in humans after topical application of 90% DMSO three times daily for 3 days. Although the organisms present in the human axilla, namely aerobic staphylococci and diphtheroids, are similar to those in partial-thickness wounds in our pigs, we did not find any antibacterial activity of DMSO.

It is well documented that after topical application in human, DMSO is readily absorbed into the circulating blood, and partially metabolized to dimethyl sulfone ($DMSO_2$) (29). Under our experimental conditions, we did not detect either DMSO or $DMSO_2$ in the serum of a DMSO-treated experimental animal. When extractions of epidermal and dermal tissue from a DMSO-treated experimental animal were analyzed chromatographically, significant levels of both DMSO and its metabolite were detected. We conclude that the majority of the DMSO administered topically was sequestered by the skin.

In a preliminary experiment we studied the effect of 20, 50, and 100% topical DMSO on wound re-epithelialization and dermal hydroxyproline content. We found that 20, 50, and 100% DMSO had similar effects on epidermal resurfacing. The relative rate of healing compared to untreated control wounds was +2%, −1%, and +1% respectively. The dermal hydroxyproline concentrations were also similar (185 μg/g for untreated, 197 μg/g for 20%, 179 μg/g for 50%, and 191 μg/g for 100%) on Day 3 after wounding. We then chose to study 95% DMSO concentrations. Although we saw no effect of DMSO on epidermal healing, dermal healing, and the microflora of wounds, it is possible that significantly different results may have occurred with more frequent application of DMSO.

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