



The Effects of Topical Dimethyl Sulfoxide on Second-Degree Burn Wound Healing in Dogs

Ayman Atiba and Alaa Ghazy

Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, El Geish street, 33516, Egypt.

Key words

ABSTRACT:

DMSO, Dog, Burn, Wound, Healing

Burn injury is a major cause of death and disability worldwide. Healing of burn wounds still remains a challenge to modern medicine. The aim of this study was to investigate the effects of Dimethyl sulfoxide (DMSO) on a second-degree burn wounds and compare it with that of silver sulfadiazine (SSD) 1% cream in dogs. A standard deep second-degree burn wound was produced, five dogs, each dog have three groups, DMSO, SSD and control (untreated). The efficacy of treatment was assessed based on the healing percentage of the wound, time to complete wound healing and the degree of inflammation. Wound contraction was higher in the DMSO group than both SSD and the control group. It was significantly higher in the DMSO group than the control group on days 21 and 24 ($P < 0.05$). The mean times for wound complete closure were 24.4 ± 2.23 and 25.7 ± 2.31 days for DMSO and SSD, respectively, being shorter for DMSO but not significantly ($P > 0.05$). Clinically, inflammatory reaction was less in DMSO group than the control group. Topical application of DMSO has significant positive effects on the healing of burn wounds in a dog model.

Corresponding Author: Ayman Atiba: atiba_2003@yahoo.co.uk

1. INTRODUCTION

Burns are one of the most important wounds in animal. Most of home thermal injuries in dogs caused by a variety of house hold items, chemicals, hot stove, and thermal as open flam (Pavletic and Trout, 2006, and Quist et al., 2012).

Burn injuries are one of the most complex and painful physical injuries to treat and manage with high health care cost (Khorasani et al., 2009). The pathophysiology and histopathology of thermal burns in animals is very similar to that in humans (Geiser and Walker, 1984). Burns are classified by the depth of injury: first degree burns involve only the most superficial layers of the epidermis; second-degree burns involve the entire epidermis and can be superficial or deep; third-degree burns are characterized by loss of the epidermal and dermal components; and fourth-degree burns involve all the skin and underlying muscle, bone and ligaments (Hanson 2005). The current practice of burn care in human is focused on antimicrobial control of wound infection and analgesia (Muller et al., 2001), Silver Sulfadiazine (SSD) 1% cream is the mostly used topical treatment of burns in both human and animals (Hanson 2005, Pavletic and Trout, 2006, and

Khorasani et al., 2009) The antimicrobial efficacy of SSD is probably the main reason for the usage of this agent. Although SSD is considered a gold standard for treatment of burn injuries, it has been suggested that SSD cream itself may delay healing (Cho Lee et al., 2005 and Atiyeh et al., 2007). Several adverse reactions and side effects have been reported, such as resistance to SSD, renal toxicity and leukopenia, thus confirming that this topical cream should not be used for long periods on extensive wounds (Atiyeh et al., 2007 and Hosseinimehr et al., 2010). For this reason, looking for an agent with minimal side effects is indicated.

Dimethyl sulfoxide (DMSO) is an organ sulfur compound with the formula $(\text{CH}_3)_2\text{SO}$. It is a colorless liquid derived as a by-product from wood pulp in the production of paper (Capriotti and Capriotti, 2012). Since the 1860s, DMSO has been extensively studied in the chemical literature. Use of DMSO in medicine dates from around 1963, when an Oregon Health & Science University Medical School team, headed by Stanley Jacob, discovered it could penetrate the skin and other membranes without damaging them and could carry other compounds into a biological system. In medicine, DMSO is

predominantly used as a topical analgesic, a vehicle for topical application of pharmaceuticals, as anti-inflammatory, and an antioxidant (Duimel-Peeters, 2003). DMSO has local anesthetic effects and thus would improve ischemic injury by effects on cell excitation (Shimizu et al., 1997). DMSO is often used as a cream or ointment applied to the skin to reduce pain, decrease swelling (Solit et al., 2007). Moreover, it exerted a marked inhibitory effect on a wide range of bacteria and fungi (Basch and Gadebusch, 1968, Rndhawa, 2006, Tarrand et al., 2012 and Hassan, 2014).

Wound healing is another potential area of interest for use of DMSO (Capriotti and Capriotti, 2012). Several studies have documented that DMSO is effective as an anti-inflammatory and analgesic agent with positive effects on wound healing (Lawrence et al., 1983, Cruse et al., 1989, Alberts and Dorr, 1991, Lubredo et al., 1992, and Duimel-Peeters, 2003). Applying DMSO cream during early stages of pressure ulcers leads to a decrease in pressure ulcer occurrence among high-risk patients (Lasher et al., 1985). A systematic review performed by Duimel-Peeters et al. 2003 stated that the effects of topical DMSO on wound healing were beneficial, both for wound healing and analgesia. The most frequent outcome measures were reduction of erythema and rapid healing of ulcers, along with decreased signs of inflammation. These findings suggest that the DMSO may be effective for the treatment of burn wound healing.

To our knowledge, the application of DMSO in the treatment of second-degree burn has not yet been reported in the literature. Therefore, the aim of this study was to investigate the effects of DMSO on a second-degree burn wounds and compare it with that of SSD in a dog model.

2. MATERIALS AND METHODS

2.1. Chemicals and Drugs

Dimethyle Sulfoxide 99% (Oxford Laboratory, Mumbai, India). Silver Sulfadiazine 1% topical cream (El-Nasr Pharmaceutical Chemicals Co. Cairo, Egypt).

2.2. Animals

Five healthy male mixed breed dogs, mean body weight of (10-14) kg, a mean age of 13-15 month-old,

were used for this experiment. Food, but not water, was withheld 12 hours before procedures. All dogs were handled according to the ethical principles for animal experiments of the international council for animal protection.

2.3. Skin Burn Wound model

Skin burn wounds were performed under general anesthesia with 0.2% ketamine HCl by intravenous drip method after premedication with xylazine (5 mg/kg). Each dog was then placed in the prone position and prepared for aseptic surgery. Deep second-degree burns were created as described by Durmus, et al. 2012 and Atiba et al. 2014 (Fig.1.a.). Briefly, equal sets of standardized deep, second-degree burn wound were created with a hot iron plate (diameter: 2×2 cm). The plate was immersed in boiling (100 °C) water until thermal equilibrium was reached and it was then placed without pressure for 20 seconds on the backs of the dogs. The skin burn wounds of the five dogs were randomly assigned in three groups (10 wounds each), each dog had six burn wounds, 2 wounds in the DMSO group, 2 wounds in the SSD group and 2 wounds in the control group (untreated group). After removal of necrotized tissues (Fig.1.b.), all wounds were covered with a non-adherent occlusive bandage. The bandage was changed every 3 days and DMSO and SSD cream were reapplied every 3 days. Scabs that covered the wound were gently removed, to allow the assessment of the wound.

The primary outcome criterion was the percentage wound healing. For assessment of wound healing, digital photography was taken every 3 days. The photographs are then assessed by NIH Image J analyzer software (downloaded from <http://imagej.nih.gov/ij/>). The percentage of healing was determined as follows: Percent of wound healing = [initial wound area – unhealed wound area / initial wound area] × 100. The secondary outcome criterion was the time period between the inflictions of the burns to complete wound closure.

The clinical course of skin lesions by burns was evaluated for 27 consecutive days based on the following aspects: redness, swelling, crust, bleeding, and secretion. The intensity of clinical signs was scored (0-3) as 0: absent, 1: mild, 2: moderate, 3: strong.

2.4. Data analysis

Data were collected, analyzed and reported as mean and standard deviation (Mean±S.D.). Statistical comparisons between groups were carried out by using SPSS software (Version 16.0, Chicago, IL, USA). One-way ANOVA followed by Tukey's post test were used to analyze the data. P 0.05 was considered as statistically significant.

3. RESULTS

On day 6 post-burn, after debridement of necrotic tissue had been performed (Fig.1.b.), the wound size was stated as 100% and wound size on other days calculated compared to the day 6 post-burn. After day 6 post-burn, wounds were initiated to promote healthy granulation tissue in both DMSO and SSD groups with a degree more than that of the control group.

Wound contraction was higher in the DMSO group than both SSD and the control group, but no significant differences observations in healing were

made between the groups before 18 days of the wound induction (Fig. 2). DMSO group was significantly higher than the control group at 21 and 24 days post-burn ($P < 0.05$) (Fig. 2 and Fig. 3), while not significantly higher than the SSD group ($P > 0.05$) (Fig. 2 and Fig. 3). The wound size percent at 6, 9, 12, 15, 18, 21, 24 and 27 days post-burn are shown in Fig. 3.

The mean times for wound complete closure were 24.4 ± 2.23 and 25.7 ± 2.31 days for DMSO and SSD, respectively, being shorter for DMSO but not significantly ($P > 0.05$). On day 27 post-burn, 8 wounds were complete healing and 2 wounds were incomplete healing in the control group (Fig.1.c).

Clinically, Wounds in the control group displayed a greater degree of inflammation on the basis of the clinical signs of the inflammatory process: redness, swelling, crust, bleeding, and secretion, which appeared to be less in wounds treated with SSD and DMSO. Also, there were no adverse outcomes (such as infection) noted in any of the groups.

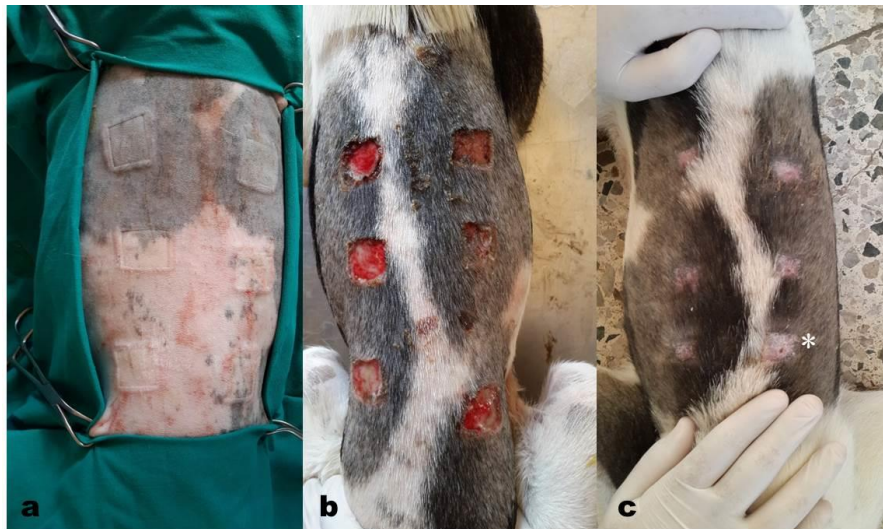


Fig.1: (a) Second-degree burn wounds on the back of the dog at day 0. (b) Debridement of necrotic tissue had been performed at day 6 post-burn. (c) Complete wound healing in DMSO and SSD groups but incomplete in control (*) group on day 27 post-burn.



Fig.2: Visualization of burned wounds from DMSO, SSD and control groups on days 18, 21 and 24 post-burn.

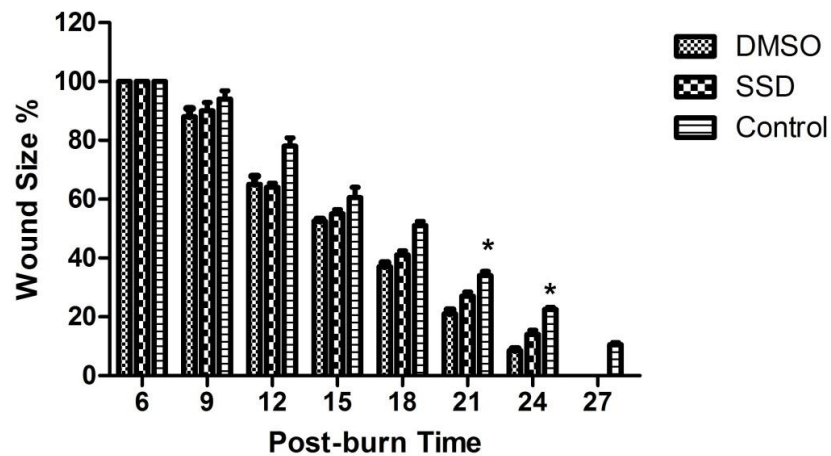


Fig.3: Comparison of wound burn sizes in the DMSO, SSD and control groups. The wound size was defined as 100% on day 6 post-burn and then calculated and compared with day 6 thereafter.

4. DISCUSSION

DMSO has also been reported harbour beneficial properties with regard to wound healing (Manjunath and Shivaprakash, 2013). It applied to the skin to reduce pain, decrease swelling and promotes healing in wounds and burns (Solit et al., 2007). Healing of ischemic ulcers of fingertips was achieved with topical application of DMSO (Scherbel AL, McCormack LJ, Poppo MJ 1965). DMSO was found to be dramatically effective for healing of severe skin necrosis caused by accidental extravasation of the anticancer drug mitomycin C during intravenous administration (Alberts and Dorr, 1991). In addition, the healing effects of DMSO in ischemia-reperfusion damage have been the subject of many experimental studies in recent years (Kilincaslan, 2013). Assessment of wound healing rate was employed for evaluating efficacy of topical DMSO in the treatment of burn injuries in dogs. Our results indicated that DMSO is able to accelerate the rate of wound healing and shortens the healing time, compared with that of SSD and control untreated group.

The mechanism of action of DMSO is not fully understood. It is a free radical scavenger and thus may reduce oxidative stress after ischemia (Shimizu et al., 1997, Alsup and DeBowes, 1984, and Carpenter, 1994). DMSO has local anesthetic effects and thus would improve ischemic injury by effects on cell excitation (Shimizu et al., 1997). One of the main limitations of this study is the histological examination to support our findings. Further studies were required to understand the mechanism underlining the effect of topical DMSO on wound healing after burn in dogs.

Previous studies have revealed that DMSO has anti-inflammatory and analgesic effects (Capriotti and Capriotti, 2012). Gorog and Kovacs demonstrated that 70 % DMSO exerted potent anti-inflammatory effects on contact dermatitis, allergic eczema, and calcification of the skin in rats (Gorog and Kovacs 1969). The favorable results of DMSO are reduction of erythema and healing of ulcers, analgesic effects or pain relief, and positive effects on one or more inflammation symptoms, such as inflammation, redness, pain, and swelling (Duimel-Peeters et al., 2003). In the current study, there was a decrease in inflammatory signs following topical application of DMSO compared to other groups. DMSO reduces

inflammation by several mechanisms. It is an antioxidant, a scavenger of the free radicals that gather at the site of injury.

Several reports demonstrated that the effect of DMSO differed according to its concentration (Duimel-Peeters et al., 2003). From 70 to 90 % has been found to be the most effective strength across the skin. A combination of 10% alpha-tocopherole acetate and 90% DMSO applied topically prophylactically, after extravasation into tissue of antineoplastic agents but before ulceration, has been found to universally prevent severe ulceration and tissue breakdown (Ludwig et al. 1987). DMSO ability to do varies proportionally with its strength up to 100% solution. Gautam et al. 2014 reported that, 100% DMSO could be useful in the treatment of acute pain resulting from tissue injuries such as burns. In the present study, 99 % DMSO was also effective to promote burn wound healing in dogs. Although, the human skin sensitivity to topical application of DMSO usually is greater with higher concentrations of DMSO (70%~100%) (Sulzberger et al. 1967), but no negative effects upon using 99 % DMSO have been observed in dogs.

In conclusion, the finding of the present study showed that topical application of DMSO had anti-inflammatory and healing effects in deep second-degree burn in dogs and more than that of control untreated group. However, further studies are certainly needed to remove the mystery for healing mechanism of topical DMSO in burn wounds.

5. ACKNOWLEDGMENT

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