

Histopathological Comparison of the Effect of Normal Saline and Silver Sulfadiazine on Scorch Healing in Rats

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Abstract: Skin burn is one of the most common complications throughout the world. Silver sulfadiazine (SSD) is used as a choice treatment in many superficial burn wounds. But, some studies have questioned its efficacy. The main objective of this study was to compare the effects of Normal Saline (NS) and SSD on healing of superficial skin burn wounds in rats. Male Wistar rats were randomly assigned in two different groups including treatment and control, with 20 animals each. After sedation, a type II skin burn wound in the shape of a 1-cm diameter circle was created in the dorsum of the rats with boiled water contact. In treatment group, NS and in control group, SSD was used topically for care. Diameter of the wound as well as the hair growing grade was determined on days 3, 7 and 14 post-intervention. In each step of the experiment, histopathological sections were prepared; thereafter reepithelialization and alleviation of inflammation at the healing site of burn wound were assessed. Statistically, the data obtained were compared between the groups. There were no significant differences between the groups regarding the mean diameter of the wound on days 3 and 14 ($p = 0.089$, $p = 0.495$). The mean diameter of the wound was significantly lower in treatment (NS) group on day 7 ($p < 0.001$). There were no significant differences between the groups regarding the mean hair growth score in different stages of the experiment ($p = 1$, $p = 0.647$, $p = 0.805$). Histopathologically, there were no significant difference between these two experimental groups from the view point of reepithelialization and alleviation of inflammation at the healing site of burn wound. The results of this study showed that NS equals with SSD in improvement of superficial skin burn wounds healing in the rats; and after the randomized clinical trials, it can be used in treatment of superficial skin burn wounds in human bodies.

Key words: Healing, normal saline, rat, scorch, silver sulfadiazine

INTRODUCTION

Scorch as physical and chemical malicious damages are of suffering and mortality agents in all over the world (Demlin, 2003). The aim of common treatments in scorch is faster recovery of skin, which is as first defense barrier of body against body fluids waste as well as development and growth of microbes (Junqueira and Carneiro, 2003). Incidence rate of burn injuries is approximately 1.25 million cases per year in United States (Brigham and McLoughlin, 1996). The prevalence of burning in third world countries is several times than Europe and North America and injuries also are severe (Davis, 1991). One of the criteria in referring to medical centers is depth and extent of the damage. Type II burns which involves more than 10 percent of body surface and type III involving more than 50 percent of body surface, must be refer to medical centers. Burning of vital body compartments, circular and chemical burns and etc. are of cases that must refer to medical centers (Ressources for optimal care of

the injured patient, 1999). Developed burns even in superficial scales are life-threatening (Piccolo-Lobo *et al.*, 1992). Caring of wounds has a long history and from many years ago some of natural substances such as fats, honey, resin and so on, have been used for local treatment of skin burns (Brunner and Suddarth, 1988). One of the common treatments in burns is use of SSD 1% cream (Keswani and Patil, 1985; La *et al.*, 2001; Warden and Heimbach, 1999). Studies have shown that SSD causes rapid healing through stimulating of re-epithelialization, formation of granulation tissue and increase in fibroblasts (Demlin, 2003; Keswani and Patil, 1985; La *et al.*, 2001; Hoekstra and Andrews, 1993). Reports showed that there are other ways for treatment of superficial wounds which have good effects than SSD (Chung and Herbert, 2001). In several studies, natural honey is recommended as effective replacement for SSD in treatment of superficial burns (Subrahmanyam, 1998; Subrahmayam, 1990). In other studies, synthetic replacements such as Duoderm and Biobrane have been used for this purpose (Wuatt

et al., 1990; Gerding *et al.*, 1990; Lal *et al.*, 2000). Application of drugs such as phenytoin and propolis skin cream also recommended (Carmerio *et al.*, 2002; Gregory *et al.*, 2002). Usage of potato crust in treatment of burns also recommended (Keswani and Patil, 1985). Glesinger *et al.* (2004), during a study sought to compare SSD with Biafine and tampons smeary with NS on pig superficial burns and found that there isn't a significant difference among understudying groups considering reduction of healing time (Glesinger *et al.*, 2004). In addition, other studies have shown that silver group drugs are associated with toxic effects and have side effects on fibroblasts growth and proliferation as well as prohibit collagen generation (Fraser *et al.*, 2004). According to above subjects, it is believed that SSD based on traditional opinions in treatment of burns, is not logical. Furthermore, there are evidences that SSD increases the risk of neutropenia, multiform erythema, crystalluria and methemoglobinemia (Subrahmanyam, 1998). Regardless the existence of good replacements for SSD, there are limits for administration of these materials. Considering that NS is a harmless, moisturizing and disinfectant substance and, on the other hand, it is an abundant and available material, therefore this study was under taken to clarify scientific proof of NS efficacy in treatment and healing of burns. In this study we compare SSD and NS effects on healing of superficial skin burns with histopathologic aspects.

MATERIALS AND METHODS

Preparation and maintenance of animals: In this study, we used 40 male Wistar rats with the average body weight of 200 ± 20 g. The animals were provided from laboratory animal breeding and maintenance center of Pasteur Institute. The rats were randomly allocated into 2 treatment and control groups, each group contained 20 animals. Management and nourishing situation for all groups were considered identical with 12/12 h light/dark cycle at $21 \pm 2^\circ\text{C}$. Food and water were provided ad libitum. After one week, study was initiated. All procedures and works on animals were conducted under Animal Rights Monitoring Committee of Pharmaceutical Research Center, Tabriz University of Medical Sciences.

Experiment plan: This study was conducted in Pharmaceutical Research Center, Tabriz University of Medical Sciences during 2010. First, in all rats, ketamine 10% (Alfasan, woerden, Holland) at a dose of 50 mg/kgbw through IM injection and xylazine 2% (Alfasan, woerden, Holland) at a dose of 5 mg/kgbw through IP injection were administrated. Then type II burning was created by 100°C water for 3 second in circular form with 1 cm diameter in the back of rats. Then rats were maintained on chaffy bed in same situations and 12/12 hours light/dark cycle at $21 \pm 2^\circ\text{C}$ was provided. In treatment group NS and in control group SSD (SSD) for local treatment of burns was used. Wound reduction size

Table 1: Histopathological grading of re-epithelialization at healing site of skin burn

1	without squamous re-epithelialization
2	Initiation of squamous re-epithelialization with creating epithelial buds from the wound edges
3	squamous re-epithelialization, as covers about one-quarter of the wound surface
4	squamous re-epithelialization, as covers about one-second of the wound surface
5	squamous re-epithelialization, as that covers about three-quarter of the wound surface
6	irregular full reconstruction of epithelium with inadequate quality and organization
7	regular complete reconstruction of epithelium with adequate quality and organization

Table 2: Histopathological grading of the severity of inflammatory reaction at healing site of skin burn

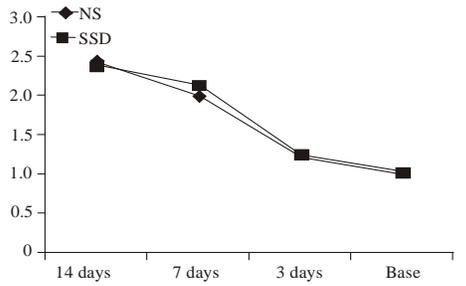
1	Perivascular infiltration of acute inflammatory cells for more than one-second of microscopic field ($\times 10$) along with tissue necrosis
2	Perivascular infiltration of acute inflammatory cells between one-quarter and one-second of microscopic field ($\times 10$) without tissue necrosis
3	Perivascular infiltration of acute inflammatory cells for less than one-quarter of microscopic field ($\times 10$)
4	Partial and diffused inflammatory cell infiltration within connective tissue
5	Without considerable inflammation

and degree of hair growth on days 3, 7 and 14 was measured macroscopically. Wound diameter was measured by caliper. Hair growth rate on days 3, 7 and 14 after initiating of study was assayed with 10 fold magnification in both groups considering this score:

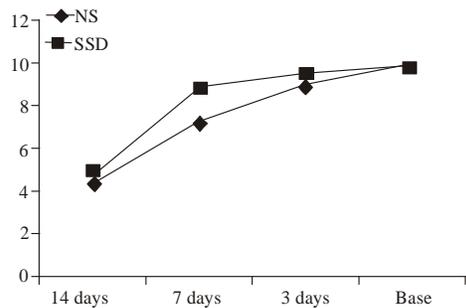
- Low hair growth, hair growth on burned area between zero and 30 numbers
- Medium hair growth, hair growth on burned area between 30 and 70 numbers
- High hair growth, hair growth on burned area more than 70 numbers

For microscopic studies, in each period, 5 rats randomly selected and were euthanized through cervical dislocation and repair areas with a little normal skin from each side were sampled. Tissue samples after fixation in formalin 10% were sent to pathology laboratory of veterinary medicine, Islamic Azad University. From paraffin embedded tissues, pathologic sections with 5μ thickness and hematoxylin-eosin stain prepared and evaluated regarding re-epithelization and inflammation severity in repair area. All slides were observed under light "NIKON" microscope and scored according to Table 1 and 2.

Statistical analysis: We used SPSS software ver. 13 for analyzing the data obtained. Quantitative data rendered as mean \pm Standard Deviation and statistical significance was compared by t-test. Histopathologic data, compared by Mann-Whitney U test and $p < 0.05$ considered as statistical significances.



(a)



(b)

Fig. 1: (a): hair growth score on different times in both group .numbers are based on millimeter, (b): hair growth score on different times in both group. numbers are based on millimeter

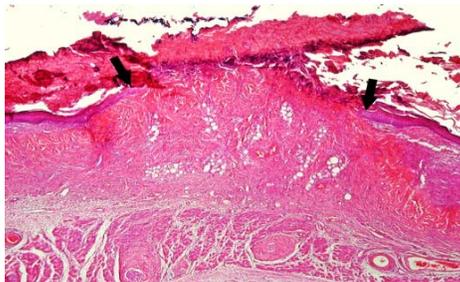


Fig. 2: Microscopic view of healing site from one rat of NS treatment group at day 3 of experiment. Wound surface is covered by a crust consisting blood coagulum, fibrin, inflammatory cells and debris. Wound area is completely occupied by immature granulation tissue. Presence of acute inflammatory cells is mainly limited to upper portion of the wound beneath the clot. Epithelial bud is manifested in the edge of wound expanding underneath the crust toward the wound surface (arrows), H&D $\times 100$

RESULTS

Morphologic findings: The average diameter of the wound on day zero in both groups was 10 mm.

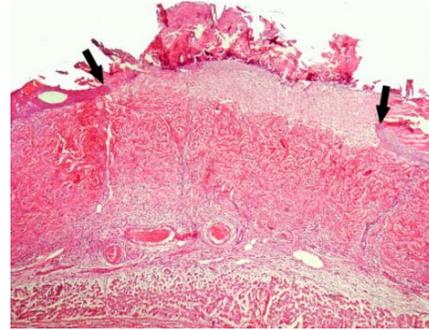


Fig. 2: Microscopic view of healing site from one rat of NS treatment group at day 3 of experiment. Wound surface is covered by a crust consisting blood coagulum, fibrin, inflammatory cells and debris. Wound area is completely occupied by immature granulation tissue. Presence of acute inflammatory cells is mainly limited to upper portion of the wound beneath the clot. Epithelial bud is manifested in the edge of wound expanding underneath the crust toward the wound surface (arrows), H&D $\times 100$

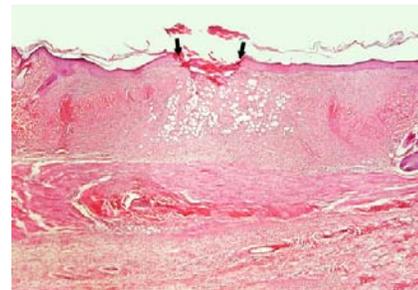


Fig. 3: Microscopic view of healing site from one rat of SSD treatment group at day 3 of experiment. Wound surface is covered by a crust consisting blood coagulum, fibrin, inflammatory cells and debris. Wound area is completely filled with immature granulation tissue. Presence of acute inflammatory cells is mainly limited to upper portion of the wound nearly beneath the clot. There is a prominent hyperemia in hypoderm. Epithelial bud is manifested in the edge of wound expanding underneath the crust toward the wound surface (arrows), H&D $\times 100$

Wound diameter on day 3 in NS and SSD groups were approximately 9.10 ± 0.72 and 9.55 ± 0.94 mm, respectively and there was no statistically significant difference between these groups ($p = 0.089$). Wound diameter on day 7 in NS and SSD groups were 7.30 ± 0.80 and 9.00 ± 1.45 mm, respectively. The average diameter of the wound on day 7 in NS group was significantly lower than SSD group ($p < 0.001$). Wound diameter on day 14 in NS and SSD groups were 4.45 ± 1.66 and 4.90 ± 2.40 mm respectively, and from this view point, there was not

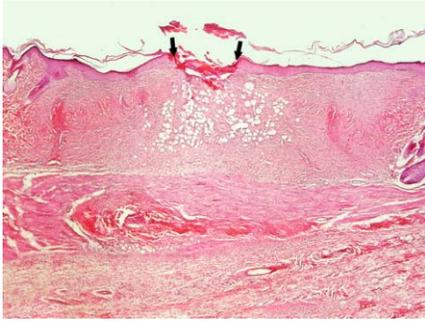


Fig. 4: Microscopic appearance of healing site from one rat of NS treatment group at day 7 of experiment. Squamous epithelial tissue expanding on wound surface has covered main portion of the wound and remained area is still covered by the crust consisting blood coagulum, debris and some inflammatory cells (arrows). Wound area is completely occupied with from immature granulation tissue and adipose tissue. There is an obvious hyperemia in hypoderm, H&D $\times 100$

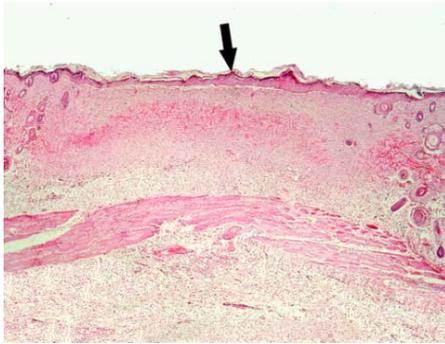


Fig. 5: Microscopic appearance of healing site from one rat of SSD treatment group at day 7 of experiment. Squamous epithelial cells expanding on wound surface has covered main portion of the wound and remained area is still covered by the crust consisting blood coagulum, debris and some inflammatory cells (arrows). Wound area is completely occupied with from immature granulation tissue and adipose tissue. There is an obvious hyperemia in hypoderm, H&D $\times 100$

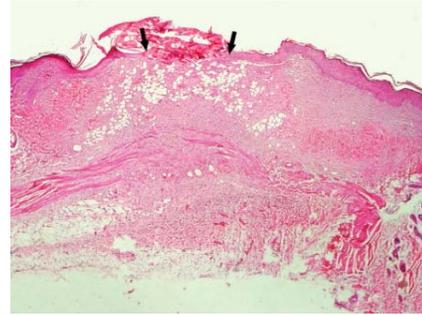


Fig. 6: Microscopic appearance of healing site from one rat of NS treatment group at day 14 of experiment. Relatively regular newly formed squamous epithelial tissue has covered wound surface (arrows). Wound area is completely occupied with mature granulation tissue and fibrous strands. Inflammatory cells are rarely seen. Mild to moderate hyperemia is seen in healing site, H&D $\times 100$

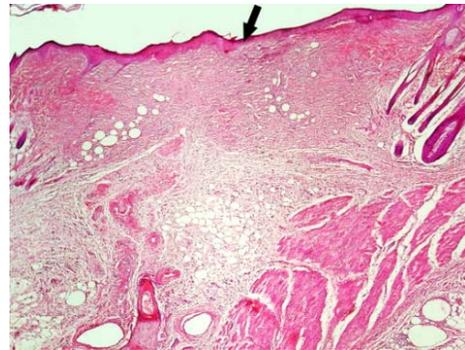


Fig. 7: Microscopic appearance of healing site from one rat of SSD treatment group at day 14 of experiment. Partially well organized newly formed squamous epithelial tissue has covered wound surface (arrows). Wound area is completely filled with relatively mature granulation tissue. Inflammatory cells are seen in some extent. There is mild to moderate hyperemia in in healing site, H&D $\times 100$

statistically significant difference between these experimental groups ($p = 0.495$). The average hair growth score in all groups was 1 at the first of experiment. Hair growth score on day 3 of experiment in NS and SSD groups were 1.25 ± 0.44 and 1.25 ± 0.44 respectively and there was no statistically significant difference between them ($p = 1$). Hair growth score on day 7 of experiment in NS and SSD groups were 2.00 ± 0.79 and 2.10 ± 0.55 respectively and there was no statistically significant difference between these groups ($p = 0.647$). Hair growth score on day 14 of experiment in NS and SSD groups were 2.40 ± 0.60 and 2.35 ± 0.67 , respectively and there was

no statistical difference between these groups too ($P = 0.805$). The average of wound diameter and hair growth score on different times in both group were depicted in Fig. 1.

Histopathologic findings: On day 3 of experiment in understudying groups, wound area was completely occupied by immature granulation tissue consisted of manly fibroblasts and newly formed capillaries accompanied by hyperemia and negligible focal hemorrhages. Simultaneously, Presence of abundant acute inflammatory cells in boundaries and surface of the wound was obvious. Surface of wound also was covered

Table 3: Comparison of re-epithelization and inflammatory response between experimental groups at days 3, 7 and 14 of study

Improvement parameters	Trial period					
	Re-epithelization			Reduction of inflammation		
	Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
Control (SSD 1%)	2.40±0.24	4.20±0.37	6.40±0.24	1.40 [*] ±0.24	2.80 [*] ±0.20	4.20 [*] ±0.37
Treatment (NS)	2.60±0.24	4.60±0.24	6.60±0.24	1.80±0.20	3.00±0.31	4.40±0.24
t-test	p = 0.58	p = 0.39	p = 0.58	p = 0.24	p = 0.60	p = 0.66

Asterisk in superscript indicates significant difference

by a thick crust consist of blood coagulum and fibrin, inflammatory cells and debris. Also, squamous epithelial buds developing from the below of the clots had started to re-epithelialization on wound surface (Fig. 2 and 3). On day 7 of study, wound area was completely filled with immature granulation tissue and mild hyperemia in marginal vessels was also observed. Inflammatory cells were limited to below the clot and surface of the wound. Squamous epithelial cells developing from below the clot was covered about three-quarters of wound surface (Fig. 4 and 5). On day 14 of experiment, wound area was completely filled with mature granulation tissue consisting delicate collagen fibers. In this time, the severity of inflammatory response markedly reduced and newly formed epithelial tissue completely was covered wound surfaces (Fig. 6 and 7). The comparison of treatment parameters between two experimental groups are presented in Table 3.

DISCUSSION

This study carried out to compare beneficial effects of SSD 1% and NS on restoration of superficial burn injuries in an animal model. Although there are many studies in this field, however, the differential aspect of this study with other researches is that here NS was considered as interventional agent and SSD selected as control. In one study that carried out by Shokouhi Sabet Jalali *et al.* (2007) it has been revealed that use of topical honey in treatment of burn in comparison with normal saline not only accelerates the healing process but has valuable antimicrobial effect in promoting the recovery of this kind of wounds (Shokouhi Sabet Jalali *et al.*, 2007). In another study which carried out by them in 2008 it has been showed that topical application of aqueous garlic extract could enhance burn wounds healing process in the dog (Shokouhi Sabet Jalali *et al.*, 2008). In one other study by Yam mun *et al.* (2003) it has been demonstrated that 15 UMF manuka honey is capable of enhancing wound tensile strength and histopathologic changes during healing process in deep partial thickness burn wound inflicted by modified electric solder in rats (Yam mun *et al.*, 2003). In another research by Hosseini *et al.* (2011) he showed that a mixture of honey, vitriol and putty dissolved in olive oil in comparison with silver

sulfadiazine can be a safe therapeutic method to treatment of infected burns (Hosseini *et al.*, 2011). In current study, there wasn't significant difference regarding mean wound diameter between days 3 and 14 of experiment, but in day 7 this parameter was significantly decreased in NS group. From hair growth score view point there were not significant differences between the groups in days 3, 7 and 14. Microscopically, there were not significant differences from view point of re-epithelialization and reduction of inflammatory reaction severity between the groups at different steps of experiment. In sum, it seems that NS causes rapid reduction in wound size than SSD. Glesinger *et al.* (2004) showed that there weren't significant differences among SSD, Biafine synthetic substance and tampons smeary with NS of healing time and reduction in burning surface (Glesinger *et al.*, 2004). Jorsaraei *et al.* (2006) has studied the effects of Myrtle herbal extract on healing of superficial skin wounds in comparison with SSD 1% and NS in white male rats. They indicated that, in some cases, preferences of fibroblasts aggregation belonged to SSD and preferences of angiogenesis belonged to NS. So that, SSD and NS had pros and cons and none of them had complete priority over another (Jorsaraei *et al.*, 2006). On the other hand, in Keswani and Patil (1985) and Hoekstra and Andrews (1993) studies it has been shown that usage of SSD in comparison with control group yields to rapid improvement of burn wounds (Keswani and Patil, 1985; Hoekstra and Andrews, 1993). Contrary, Lee and Moon (2003) reported that application of pure and high amounts of SSD through decrease in fibroblasts yields to slow improvement. They declared that application of SSD is associated with toxic effects and has negative effects on fibroblasts and collagen generation (Lee and Moon, 2003). In our study also this delay was exist but this effect cannot fully be attributed to the SSD.

CONCLUSION

According to the results obtained, beneficial effects of NS in healing of superficial skin wounds in rats is comparable with SSD in rats. Thus, after clinical trials in human, normal saline can be considered as a low cost, easily available and potent topical agent in improvement of type II skin wounds healing.

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