J Surg Med. 2019;3(6):433-436.
DOI: 10.28982/josam.515121

Research article
Araştırma makalesi

Journal of Surgery and Medicine
e-ISSN: 2602-2079

Comparing the effects of silver sulfadiazine and cerium nitrate silver sulfadiazine on burn-wound healing and survival rate of rat animal model

Gümüş sülfadiazin ve seryum nitrat gümüş sülfadiazin'in yanık yarası iyileşmesi ve sıçan hayvan modelinde sağkalım oranı üzerindeki etkilerinin karşılaştırılması

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Ethics Committee Approval: This study was approved by Iran University of medical sciences' ethic committee.
Etik Kurul Onayı: Bu çalışma, İran tıp bilimleri Üniversitesi etik kurulu tarafından onaylandı.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Published: 6/17/2019
Yayın Tarihi: 17.06.2019

Abstract

Aim: Current studies about burn therapy are focusing on survival rate of patients with severe burned. So, here we aim to assess the effects of cerium nitrate silver sulfadiazine (CN+SSD) and compare with SSD ointment on mortality and burn healing in rat animal model.

Methods: Twenty rats were used in this study. After inducing burn wounds on back skin of animals, they were classified into two groups randomly. In one group, SSD was administrated topically immediately after inducing burn wound and in the other the combination of CN+SSD was applied. The treatments in both groups were continued as administration of the ointments two times per day for 28 days. Animal's weight and wound sizes were measured 7, 14, 21 and 28 days after burn induction in all animals. The mortality of rats as well as their wound histopathology was also evaluated.

Results: On day 28, wound's average size was decreased to 25 and 27% of initial area in SSD and CN +SSD groups, respectively. In CN+SSD group, the wound size was not smaller than SSD group significantly. Histological comparison has demonstrated no significant differences between CN+SSD group and SSD treated.

Conclusion: The results of our study did not show any differences between CN+SSD and SSD topical treatments in terms of wound healing and mortality rate in rat animal model significantly.

Keywords: Skin-burn, Cerium nitrate silver sulfadiazine, Wound-healing, Mortality, Animal model

Öz

Amaç: Yanık tedavisi ile ilgili güncel çalışmalar, ciddi yanık hastalarının hayatta kalma oranlarına odaklanmaktadır. Bu nedenle, burada seryum nitrat gümüş sülfadiazin (CN+SSD) etkilerini değerlendirmeyi ve sıçan hayvan modelinde SSD merhem ile mortalite ve yanık iyileşmesi arasındaki karşılaştırmayı hedefledik.

Yöntemler: Bu çalışmada 20 rat kullanıldı. Hayvanların arka derisinde yanık yaraları indüklendikten sonra rastgele iki gruba ayrıldı. Bir grupta SSD, yanık yarasının indüklenmesinden hemen sonra topikal olarak uygulandı ve diğerinde CN+SSD'nin kombinasyonu uygulandı. Her iki grupta da, merhemlerin 28 gün boyunca günde iki kez uygulanmasına devam edildi. Animalin ağırlık ve yara ebatları, bütün hayvanlarda yanma indüksiyonundan 7, 14, 21 ve 28 gün sonra ölçüldü. Sıçanlardaki ölümlerin yanı sıra yara histopatolojileri de değerlendirildi.

Bulgular: 28. günde, yaranın ortalama büyüklüğü sırasıyla SSD ve CN+SSD gruplarında başlangıçtaki alanın %25 ve %27'sine düşürüldü. CN+SSD grubunda yara büyüklüğü SSD grubundan anlamlı derecede küçük değildi. Histolojik karşılaştırma, CN+SSD grubu ile tedavi edilen SSD arasında anlamlı bir fark olmadığını göstermiştir.

Sonuç: Çalışmamızın sonuçları, sıçan hayvan modelinde CN+SSD ve SSD topikal tedavileri arasında yara iyileşmesi ve mortalite oranı açısından anlamlı bir farklılık göstermedi.

Anahtar kelimeler: Deri yanığı, Seryum nitrat gümüş sülfadiazin, Yara iyileşmesi, Mortalite, Hayvan modeli

How to cite / Atıf için: Nur-Ahmad L, Mohammad-Javad FD, Ali-Asghar SK, Azim H. Comparing the effects of silver sulfadiazine and cerium nitrate silver sulfadiazine on burn-wound healing and survival rate of rat animal model. J Surg Med. 2019;3(6):433-436.

Introduction

The new classification of skin burns are including erythema involving merely epidermis (first degree), the epidermis and upper dermis involvement as a superficial thickness and deep partial thickness (both as a second degree), epidermis and dermis involvement with full thickness (as a third degree) and also appendages [1]. Massive burns constitute remarkable complications including mortality and morbidity [2,3]. Burns related-mortalities are classified in early and late deaths. The circulatory shock can cause early mortality [4] which happens in extensive burns and/or acute respiratory failures following inhalational damages [5]. Late deaths are typically attributing to sepsis [6]; however, recent studies have identified that these type of death after burn might be due to immune failure in the lack of documented sepsis [7].

Current studies about burn therapy are focusing on survival rate of patients affecting more than half of their total body surface [8]. The main cause of death in severe burned patients is septic problems. Silver sulfadiazine as a topical antibacterial agent has reduced the risk of septic events but the appearance of resistant strains has restrained the efficacy of them. Silver sulfadiazine ointment has been extensively applied in topical case of burns [7]. However, there is a high failure rate while deep burns exceed 60 percent of body surface. Using silver sulfadiazine ointment in combination with cerium nitrate elevates the rate of healing up to 2.2 fold in large or massive burns cases [7,9].

The best way for preventing the infections covering the extensive burns is just by applying autologous skins which results in definitive covering; nevertheless, healthy skin areas are generally not sufficient to achieve this [8]. Homograft can solve the problem of wound healing temporarily, but in the case of applying both auto graft and homograft, there is the necessity to remove the necrosis tissue before employing the grafts. The postoperative complications including bleeding and risk of infection can limit the excised wound area [1].

Monafo et al. in 1976 proposed that extensive burns can be treated by topical CN as an antiseptic agent [10]. Single applying of cerium nitrate has changed the Gram-negative frequency to the Gram-positive species in the wounds. It can use with silver sulfadiazine (CN+SSD) to diminish burns bacterial contamination. Also, they are used to improve the burn patients' prognosis, particularly in severe cases [11,12]. CN+SSD administration changes the burn eschars' appearance and simulate bound tightly for some months [7]. During clinical studies, no toxic reaction has happened except transient and temperate cases of leucopenia. In these cases leucopenia has induced by silver sulfadiazine, and methemoglobinemia [7,13].

Although primary studies revealed encouraging results [14], there are few studies of using topical CN+SSD to burn care, as well as some reports which demonstrate no differences between therapeutic effects of single silver sulfadiazine (SSD) [15] and combination of (CN+SSD) significantly [7,16,17]. Therefore, to clarify and compare the therapeutic role of CN and SSD, herein, a controlled experimental study on rat burn animal model has conducted.

Materials and methods

This study was performed based on the animal research guidelines (National Institutes of Health). Our study was approved by Iran University of medical sciences' ethic committee.

Animals

Twenty male Wistar rats aged six to eight months (approximately 200–250 gr) were used in our experiment. They were put in plastic cages with food and water ad libitum. With a 12-h light and dark cycle and the temperature was controlled.

Anesthesia

They were anesthetized using single intramuscular xylazine and ketamine hydrochloride injections (6 mg/kg and 85 mg/kg, respectively).

Thermal injury

After anesthetizing the rats, they were shaved and ready with 70% ethanol solution. Then, the skin burns model of full-thickness second-degree by using steel probes, were prepared in a 3.5 cm^2 surface area (about 20% of the whole rat skin surface). For reaching to the thermal equilibrium, the probe was immersed (by boiling in100 °C water). Then, without any pressure the probe was put on the shaved back part of the rats (20 seconds). After that, the animals were immediately resuscitated by using intraperitoneal solution of lactated ringer with dose of 2 ml per 100 g body weight.

Animal experiments

After inducing thermal injury, they were classified into two similar groups randomly (10 animals each). In the first group (SSD group), burned areas were covered with SSD ointment which contains Silverdin, Deva and Silver sulfadiazine with dosage of 10 mg per gram, immediately after burning and twice a day for 4 weeks. The burned areas in the second group (CN+SSD group) were covered immediately after burn with CN+SSD ointment and then twice a day for 4 weeks.

Wound healing and mortality rate follow-up

Regarding to trace the wound healing process, the lesions' sizes (in cm^2) were measured at 7, 14, 21 and 28 days' intervals. Seven days after the burn injury induction, wound area was considered 100% and was compared on the day of injury and on the subsequent days. The rate of mortality was determined at 28 days after burn injury in each experimental group. The animals were weighed at 7, 14, 21 and 28 days during the study.

Histopathological examination

In 2 different time point -14 and 28 days after thermal induction- the samples of small excisions containing part of the wounded area were used for histological evaluations. They were fixed by formalin 10% and preparing the sections which embedded by paraffin and using microtome (5-μm thick) were performed. The prepared tissue sections were stained by H&E (Hematoxylin & Eosin) and also Masson's trichrome for light microscopy examination. The average number of inflammatory cells (×400 magnification) in healed area can determine the severity of inflammation in each group.

Statistical analysis

The data have been reported as means (standard deviation). Statistical differences in two groups (SSD &

CN+SSD) were calculated using T-test. The statistical analyses were done using GraphPad Prism software version 6.01. *P*-value less than 0.05 was considered as statistically significant.

Results

Average weight of animals in SSD and CN+SSD groups were 218 (29) and 221 (26) grams, before beginning of this study, respectively. 7 days after induction of burn injury, the weight of rats in SSD and CN+SSD groups was decreased to 201 and 205 grams, respectively. No significant difference was reported between groups in terms of animal weights during follow-up. At the end of 28-day treatment, the slightly increased in average weight of animals was shown (Table 1).

Two rats died four days after burn induction (one in each group), therefore, no significant difference was reported in mortality rate between SSD group and CN+SSD treatment group.

The surface area of skin lesions was measured in 7, 14, 21 and 28 days after induction of injury. The reduction rate was calculated in comparison with wound size in 7th day. The mean of wound size after 28 days was decreased to 25 and 27 percent of the burned area in 7th day in SSD and CN+SSD groups, respectively (Table 2). No statistical difference was reported between these two groups (P=0.094). Also in terms of wound size on the 28th day of treatment no statistical difference was reported among SSD and CN+SSD groups. The representative image of induced burned areas, 28 days after initiating the treatments is presented in Figure 1.

Histological studies' results demonstrated the wound healing improvement 28th day after treatment. The samples from SSD and CN+SSD treated wounds showed complete inflammatory cell infiltration, epidermis re-epithelialization and dermis fibrosis.

Table 1: Body weights of rats treated with SSD and CN+SSD ointments before and during 7 days after induction of burn injury

Body weights (g)	SSD Group	CN+SSD Group
Before the study	218 g	221 g
Day 7	201 g	205 g
Day 14	198 g	200 g
Day 21	200 g	204 g
Day 28	205 g	211 g

Table 2: Comparison of wound burn size in SSD and CN+SSD treated rats

Wound burn size (mm^2)	SSD Group	CN+SSD Group
Day 7	100%	100%
Day 14	33%	37%
Day 21	29%	32%
Day 28	25%	27%

The wound size area was stated as 100% on the 7th day and the ratio of wound size in following days to 7th day was calculated.

Figure 1: Induced skin burns on rats that treated with SSD (A) and CN+SSD (B) Ointments after 28 days

Discussion

In this animal study, we assessed the effects of SSD and CN+SSD on healing of burn wounds in animal model and compared the results. Generally, our results showed that there is no significant difference between SSD and CN+SSD in terms of enhancing wound healing process and survival rate of rats.

In a prospective investigation which has been done multicenter, has been shown that Ce-SSD was approved but experiments were finished in 2000s incompletely [18]. Ce-SSD is extensively applied in Europe (Flammacerium®) but in the United States and United Kingdom, just was used for the treatment of infrequent diseases [10].

Reduction of 46 percent in deaths following use of CN+SSD has been reported [19]; but these results have been opposed in past years while topical CN or SSD was applied. Previous studies showed that for the treatment of acute facial burns there is no significant difference between CN+SSD or SSD [9]. In burned mice and rats, the effectiveness of CN+SSD in bacterial infection and mortality inhibition has been reported [7,10]. Animals which have been treated with zinc sulfadiazine displayed better and quicker wound healing. Unlike silver and zinc SD, the cerium SD can create an insoluble complex of cerium-DNA [18]. Cerium SD toxicity is less than zinc or silver SD [18]. Both the silver SD's effectiveness and potential wound-healing capacity of zinc SD accompanied by cerium SD's low toxicity could be merge to generate the best topical treatment [20]. In some animal burns studies, CN inhibited death [21], and improved the cellular immunity. It has not been reported with sulfamylon, silver nitrate or SSD [22,23]. The results of this investigation show that infectious organisms are not the only cause of immune suppression induction. In the skin, CN binds to a toxic lipid protein complex manufactured by heat [21]. It can suppress the immunologic responses through the mitochondria and cell membrane [24] and ruffles metabolic function [25].

On the other hand, in a one-year prospective study, CN+SSD ointment (Flammacerium®) was used for a group of children (N=47). They have second to third-degree thermal injuries with five to 60 percent of body surface involvement. They showed the good to very good therapeutic results at the end of study [26].

The effect of CN+SSD ointment was assessed in compare to SSD cream in a randomized study of 60 massive burns patients. In this study none of the treatments have superiority over the other one [17].

Some results showed that there is no inhibition or decreasing in bacterial growth in severe injuries covering more than 50 to 60% of total body surface area by using SSD. First experiments introduced that CN as an inexpensive agent with low toxicity had a wide antibacterial and antifungal spectrum. Applying in burns by wet compresses or using a water soluble cream, the CN merely showed Gram-negative organisms inhibition; but SSD specially prevents Gram-positive one [12]. The clinical trials of administration of topical treatments including combined CN with SSD reported reduced mortality rate up to 50% [11]. But, in two controlled studies applying SSD–CN and single SSD, no significant difference in terms of mortality rate and wound flora modulation was seen between two treatments [16]. The results of other study which was performed in adults, the results were introduced no difference between SSD and SSD–CN [17].

Collectively, these data along with our finding from SSD and CN+SSD groups showed no statistical difference between applying CN+SSD and single SSD in terms of enhancing wound healing process and survival rate of rats. Based on our finding, future studies are recommended.

Conclusion

In conclusion, the results of our study did not show any significant differences in wound healing and mortality rate between CN+SSD and SSD topical treatment in rat animal model.

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The National Library of Medicine (NLM) citation style guide is used in this paper.
Suggested citation: Patrias K. Citing medicine: the NLM style guide for authors, editors, and publishers [Internet]. 2nd ed. Wendling DL, technical editor. Bethesda (MD): National Library of Medicine (US); 2007-[updated 2015 Oct 2; cited Year Month Day]. Available from: http://www.nlm.nih.gov/citingmedicine