EVALUATION OF WOUND HEALING ACTIVITY OF SILVER SULFADIAZINE EMULGEL (1%) IN THE RAT BURN WOUND MODEL AND ITS SKIN IRRITATION STUDY

ABSTRACT

The study provides a scientific evaluation of the biological and therapeutic properties of new topical formulation of silver sulfadiazine emulgel (1%) as an alternative for the treatment of burn wounds. The solid dispersion was prepared with Poloxomer 407 by melt method and is used for emulgel formulation. The prepared silver sulfadiazine emulgel (1%) was compared with marketed silver sulfadiazine cream on healing of burn wounds in rats. Burned area evaluations on the 4th, 8th, 12th and 16th days showed statistically significant better burn wound healing in silver sulfadiazine emulgel (1%) as compared to marketed silver sulfadiazine (1%) group. Moreover, it showed no irritation when tested in rabbit skin irritation test. In conclusion, application of silver sulfadiazine emulgel may be more effective in healing burn related skin wounds in the rat model.

Keywords: Silver sulfadiazine emulgel, Rat wound healing, Skin irritation

INTRODUCTION

Burn injury is a very most common admission cause for in a hospital and remains a major global concern. Majority of burn the injuries are minor and thus can be managed on outpatient basis. Three zones of injury exist around a burn: a zone of necrosis, a zone of stasis, and a zone of hyperaemia [1]. In a severe burn, bacteria growth is faster due to the loss of the skin layer. High levels of bacteria in the wound can reduce the availability of growth factors, which can decrease the rate of healing process[2]. Among the topical antibiotics, silver sulfadiazine possess good tolerability by the patient, has low toxicity, good penetrability in the scar tissue and a broad spectrum of activity against most microorganisms, particularly the gram-negative micro organisms[3]. Silver sulfadiazine is non toxic and also having properties such as wound healing, antimicrobial and analgesic activities.

The anti-microbial efficacy of silver sulfadiazine is probably the main reason why the use of this agent is so widespread. Moreover, silver sulfadiazine stimulates the activity of macrophages and fibroblasts on the process of wound healing, resulting in a more pronounced formation of granulation tissue[4]. In this study, the emulgel formulation was prepared using the method as given below. Bio adhesive strength of the emulgel was determined. The effect of the silver sulfadiazine emulgel on healing of burn wounds in rats was compared with a marketed formulation silver sulfadiazine (1%). Also, skin irritation study was performed.

MATERIALS & METHOD

Silver sulfadiazine (SSD) was purchased from Anju Drug Chem Private Limited , Indore, India. Poloxamer 407 (Pluronic F127) was purchased from Sigma Aldrich. Lecithin (PHOSPHOLIPON® 85 G) received as gift sample of Lipoid Company. Sepineo P600 received as gift sample from Yasham Bio-Science Pvt Ltd. Mumbai. Tween 80, Span 60, calcium hydroxide, methyl paraben, propyl paraben were purchased from Loba Chemie Mumbai. Coconut oil, Lavender oil were procured from local market. All other chemicals were of pharmaceutical grade and used without further modification.

PREPARATION OF THE SILVER SULFADIAZINE EMULGELS

The solid dispersion of silver sulfadiazine with Poloxamer 407 (1:1) was prepared by melt method and used for the preparation of emulgel. Oil phase of the emulsion was prepared by dissolving (span 60, calcium oleate) surfactant in coconut oil and aqueous phase by dissolving Tween 80 in water. Preservative

(methyl paraben and propyl paraben) and solid dispersion were then added to aqueous phase. Both the oily and aqueous phases were separately heated to 70° to 80°c. Heated aqueous phase was then added to oil phase with constant stirring, cooled to room temperature and then lavender oil was added. Gelling phase was prepared by the dispersing gelling agent (Sepineo P 600) in purified water with constant stirring and the organic phase (coconut oil) with lecithin and both phases were mixed well to form gel. Finally, emulgel was prepared by mixing gelling phase and emulsion phase in 1:1 proportion using mortar and pestle. The pH was adjusted to 5.5 to 6.5 using triethanolamine.

Evaluation parameter

Bio adhesive strength

The bioadhesive strength of the emulgel was determined by means of modified analytical two pan balance. The burn human skin was washed with saline solution to 37^{0}C before use. At the time of testing a section of skin was attached to upper glass vial using a rubber band. One vial with a section of tissue was connected to the balance and the other vial was fixed on a height-adjustable pan. To the lower vial, emulgel was applied. The height of the vial was adjusted so that the gel could adhere to the burn skin of upper vial, after which the upper vial was then connected to the balance. Weights were added at a constant rate to the pan on the other side of the modified balance of the used device until the gel gets detached from skin. The bioadhesive strength, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights required for the detachment using the following equation

$$\text{Bioadhesive Strength} = \text{Weight required (gm)} / \text{Area (cm}^2\text{)}$$

Burn Wound Model for Emulgel

Eighteen male Wistar rats weighing 200-300 gm were used for burn wound model. Rats were acclimatized for five days. The protocol for the study was approved by the institutional animal ethics committee Marathwada Mitra Mandal's College of Pharmacy. The IAEC approval No. is 001/2012. The animals were housed in individual cages with water and food (rodent chow) ad libitum. Each rat was previously anesthetized with (IP) intraperitoneal injection of xylazine (5 to 10mg/kg) with ketamine (20 mg/kg). The dorsum hair on the area to be burned was shaved and then waited 24 hrs before infliction to minimize local inflammation. The burn infliction was made by holding 1.5 cm^2 aluminium square, previously heated at 100°C, over the exposed skin for 2 seconds. Silver sulfadiazine emulgel (1%), silver sulfadiazine marketed formulation and emulgel without silver sulfadiazine (control) were applied topically to respective group. Silver sulfadiazine emulgel (1%) and control were applied every alternate day whereas, silver sulfadiazine marketed formulation was applied every day. Percentage wound contraction and wound healing was observed (Table I).

Wound Contraction

Wound area was measured planimetrically after every four days for each animal. The size of the wound was traced on the transparent paper for measurement[6]. The measured area was then used to calculate the percentage contraction of wound. The initial wound area i.e. 150 mm^2 is considered as 100%. The following equation was used to calculate wound contraction:

$$\text{Percentage of wound contraction} = \frac{\text{Initial Wound Size - Specific Day Wound Size}}{\text{Initial Wound Size}} \times 100$$

Epithelization period (healing time)

Number of days required to fall eschar from the wound surface and exclusively leaving a raw wound behind is considered as epithelization period.[7]

Skin Irritation Test[11]

Three healthy rabbits weighing 1.5 to 2.5 kg were used for conduct of skin irritation study. The hairs on dorsum side of the rabbits were clipped. Area was

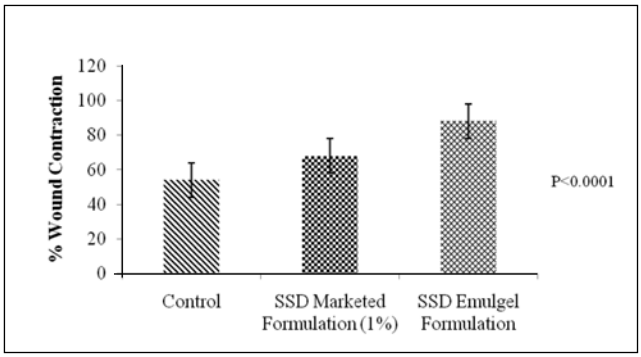

Fig.1: Effect of Formulations on % Wound Contraction in the Burn Wound Model.

shaved by applying hair removal cream. About 200 mg of the SSD emulgel formulation (1%) was applied onto post dorsum side (right). The other side (left side) was applied with 200 mg of emulgel without silver sulfadiazine and it served as the control. Toxicity symptoms if any were observed in the treated skin at pre-selected intervals i.e. 1, 4, 24 hrs and suitable scores were assigned depending on the severity of the reaction for each animal.

RESULTS AND DISCUSSION

Bioadhesive strength[5]

The gelling agent concentration can optimize by finding gelling concentration at body temperature. Bioadhesive strength was found to be 4853.41±0.25 dyne cm^{-1}.

Burn Wound Model for Emulgel

The result was analysed using one way anova, followed by graph pad prism software. The percentage of burn wound contraction in the emulgel formulation was found to be higher and statistically significant as compared to control and silver sulfadiazine on the 8th day, 12th day 16th day[7, 8]. (Table I) The present study shows a significant improvement in burn wound contraction in the rats treated with SSD emulgel formulation 1% though it is applied every alternate day (Fig.1). The formulation contains coconut oil, Poloxamer 407 and lavender oil in formulation which might be to attributed faster healing of wound without complication as compared to SSD marketed formulation which was applied every day. [9, 10]

Skin irritation Test

The primary irritation index of the test article was calculated to be 0.00; no irritation was observed on the skin of the rabbits. This may indicate that the emulgel formulation of silver sulfadiazine is safe when applied to human skin.

CONCLUSION

The topical use of silver sulfadiazine emulgel 1% ameliorated inflammation in burn wounds.

It is concluded that coconut oil with Poloxamer 407, lavender oil and gelling agents Sepineo P 600

Table I: Effect of Silver Sulfadiazine Emulgel on %Wound Contraction and Wound Healing Activity.

Treatment (n=6)		% wound contraction (mean ±S.E.)				wound healing in days
		4th day	8 th day	12th day	16th day	
Group I-control		10.1±3.94	14±1.43	44±2.85	54±2.55	30± 2.14
Group II- SSD Marketed Formulation (1%)		13±0.21	27.3±1.94	50±2.85	68±1.53	28± 2.56
Group III- SSD Emulgel formulation		13±0.21	40± 4.5	60±0.89	88±1.65	25±1.45
one –way anova	F	5.45	80.33	54.82	64.51	35.65
	P	<0.0166	<0.004	<0.001	<0.0001	<0.0001

lecithin may be helpful to develop safe and effective topical formulation of silver sulfadiazine. One of the examples is emulgel formulation which is being discussed in this research article. The SSD emulgel formulation 1% may be more effective than SSD marketed formulation. Since it is applied alternate day and SSD marketed formulation applied every day.

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