Original Article

Bull Emerg Trauma 2014;2(4):161-165.

Bulletin of Emergency And Trauma

The Effects of Oltipraz on Tissue Regeneration in the Process of Wound Healing: A Stereological Study

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Received: July 27, 2014
Revised: August 10, 2014
Accepted: September 25, 2014

ABSTRACT

Objective: To determine the effects of topical administration of 20% oltipraz solution on histomorphometrical and stereological aspects of skin tissue in full thickness skin wounds in laboratory rats.
Methods: Thirty-six male Wistar portion rats (220±20 g) were randomly divided into three groups (n=12). On the first day of experimentation, a 1-cm^2 circular wound was made on the posterior surface of neck in all rats by removing a full thickness skin piece immediately after induction of anesthesia with ether inhalation. One group was treated with vehicle solution (DMSO alone). The second group was treated daily with 20% oltipraz solution, and the third group, the control group, received no treatment. The wound closure rate was estimated our previously described method. The volume density of collagen bundles, vessels, and hair follicles, the vessels' length density, mean diameter of vessels and also fibroblast population were estimated by using stereological methods.
Results: The oltipraz group indicated a significantly higher improvement (6.26% of the wound surface per day) than control and the vehicle treated groups (p=0.032); furthermore, there was inconsiderable difference between the rate of wound closure in the group treated with vehicle (4.93% per day) and the control group (4.43% per day).
Conclusion: Oltipraz has positive influence on fibroblast proliferation and re-epithelization. A noticeable observation in our study was absence of scar formation in wounds which were treated by oltipraz and can be mentioned as an advantage of this drug.

Keywords: Oltipraz; Wound Healing; Stereology; Rat.

Please cite this paper as:
Noorafshan A, Kardeh S, Ashkani-Esfahani S, Namazi MR, Saleh E. The Effects of Oltipraz on Tissue Regeneration in the Process of Wound Healing: A Stereological Study. *Bull Emerg Trauma*. 2014;2(4):161-165.

Introduction

Skin wounds are one of the most prevalent dermal problems which are still a major concern for scientists to find better and safer treatments for. The wound healing process is consisted of inflammation, activation of fibroblasts and collagen deposition, angiogenesis and increased vascularization, regeneration of epithelial and endothelial tissue, and ultimately tissue remodeling which lead to epithelialization and wound closure [1-3]; however, complications such as disruption of blood vessels,

bacterial infection, advanced inflammation and oxidative reactions might impede the healing process as well [4-6]. Thus, intervening any of the above steps may affect the rate and quality of tissue regeneration. Oltipraz, a drug which is commonly used as a schistosomicide and also an anti-tumor agent, is a dithiole derivate (4-Methyl-5-pyrazinyl-3H-1,2-dithiole-3-thione); the dithiolethiones are a class of organ sulfur compounds, of which oltipraz is the best studied [7,8]. Many biological and pharmaceutical effects of oltipraz such as antioxidative, anti-inflammatory and anti-cancer activities have been previously studied on different tissues and organs including skin and liver [9,10]. As an antioxidant Oltipraz targets Nrf2, an agent that plays a pivotal role in cellular defense against oxidative stress by promoting the transcription of various antioxidant genes [11]. Although several studies have been conducted on the impacts of oltipraz on many skin diseases in which inflammation and oxidative reactions are of their main characteristics, investigations on healing effects of topical oltipraz on skin wounds are still lacking [12,13]. Considering the previously reported potentials of this agent;in this study, we aimed to determine the effects of topical administration of 20% oltiprazsolution on histomorphometrical and stereological aspects of skin tissue in full thickness skin wounds in laboratory rats.

Materials and Methods

Animals

Thirty six Wistar-Albino rats (all males, 5-6 months of age) at Shiraz University of Medical Sciences animal research laboratory, weighing 200 to 250 g, were included in this study. All the animals were housed individually and fed standard food throughout the experiment. The animals were initially evaluated for illness by physical examination and laboratory screening. The animals were purchased from the laboratory animal department of Iran's Pasteur Institute of Pharmacy. The animals lived in cages (one animal per cage) with water and food. They were monitored and acclimated to the new environment for 1 week. All the rats were maintained on standard rat chow and water. They were all housed under controlled standard laboratory conditions (temperature 20-24°C, relative humidity and 12/12 hour light/dark cycle). The study was approved by the institutional review board and ethics committee of Shiraz University of Medical Sciences and the Ethics Committee of Natural Chemotherapeutics Research Laboratory of Iran's Ministry of Health. Study animals were handled in conformity with guidelines for the care and handling of laboratory animals provided by Shiraz Laboratory Animals Center in accordance with global standards for laboratory biosafety guidelines.

Study Protocol

In this experimental study, 36 male Wistar portion rats were randomly divided into three groups (n=12). On the first day of experimentation, a 1-cm^2 circular wound was made on the posterior surface of neck in all rats by removing a full thickness skin piece immediately after induction of anesthesia with ether inhalation. All the wounds were debrided every 24-hourjust after the wounding until the last day of the study, the day in which at least one wound in any of the three groups was completely closed (15th day in our study). One group was treated with vehicle solution (DMSO alone). The second group was treated daily with 20% oltipraz solution, and the third group, the control group, received no treatment. A digital photograph was taken from the wound area of each rat every four days (Figure 1). After sacrificing rats on the 15th day with a high dose of ether, full thickness skin biopsies (1cm×1cm) were dissected from the wound site and fixed in buffered formaldehyde (pH=7.2) for histomorphometrical and stereological evaluations.

Preparation of the Applicable Solution

Oltipraz powder (Sigma-Aldrich ™) was provided and in order to facilitate its application, we prepared a 20%solution by dissolving 20mg of oltipraz in 100cc DMSO. To determine the best effective dose of oltipraz, a pilot study was conducted and the 20% solution was selected as the best dosage that had a considerable solubility and efficacy and also insignificant difference with higher doses.

Histomorphometrical and Stereological Study

The wound closure rate was estimated regarding a previously reported stereological method by Ashkani-Esfahani *et al.*, [14]. The volume density of collagen bundles, vessels, and hair follicles, the vessels' length density, mean diameter of vessels and also fibroblast population were estimated by using stereological methods [15].

Statistical Analysis

The data were collected, analyzed and reported as mean and standard deviation (mean±SD) and proportions as appropriate. Statistical analysis were performed by statistical package for social sciences (SPSS Inc., Chicago, USA)version 16.0.The Mann-Whitney U-test was used to compare the parametric values between groups. A two-sided P-value less than 0.05 was considered as statistically significant.

Results

The mean initial area of the wounds was 105.33±3.21 $mm^2$and with regard to the primary wound areas there were no significant differences among the three groups. Comparing the rate of wound closure in the studied groups, the oltipraz group indicated a markedly higher improvement (6.26% of the wound surface per day) than control (p=0.032) and the vehicle treated (p=0.038) groups; furthermore,

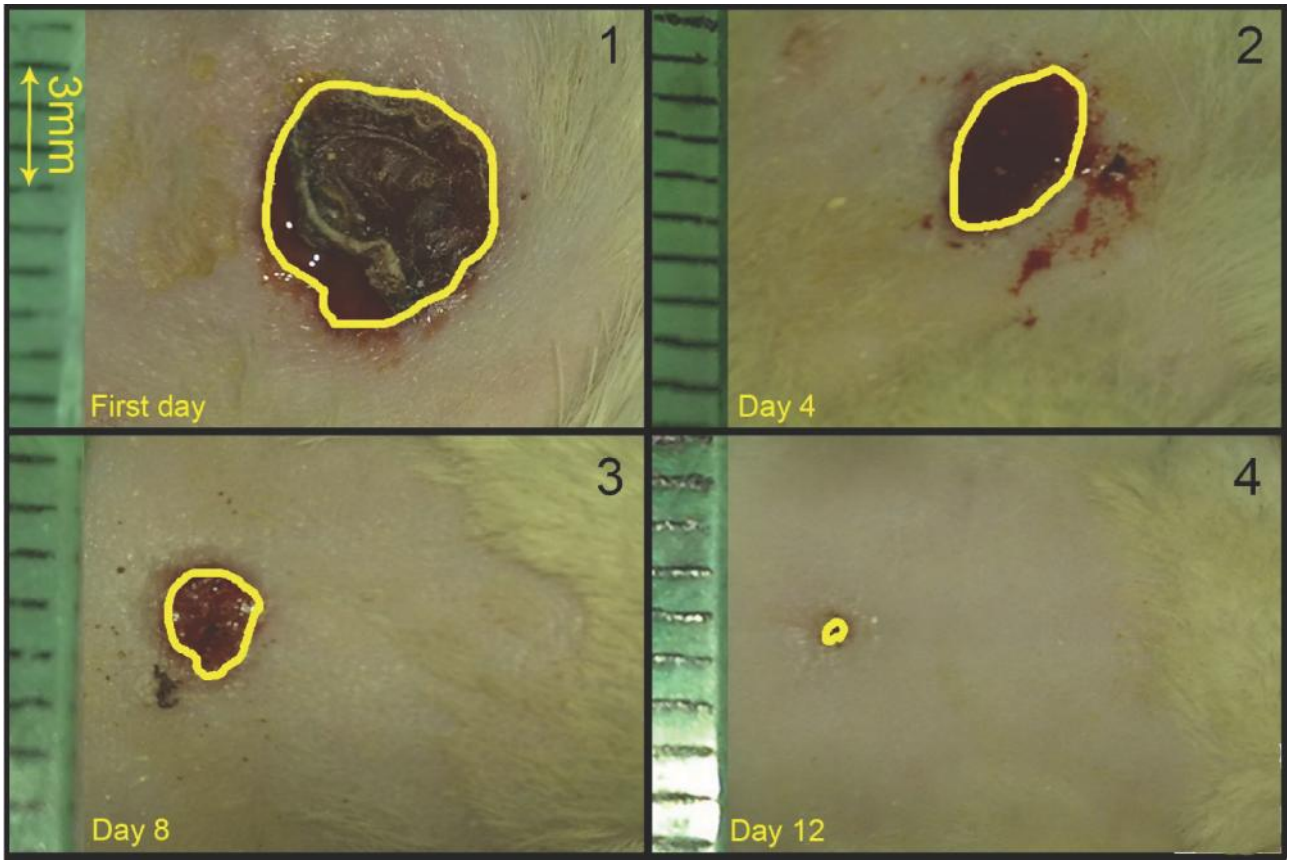

Fig. 1. Digital photographs were captured from the wound surfaces every four days to measure the wound area. A standard ruler was laid beside the wound surface to estimate the degree of magnification. The area within the closed yellow line was measured by stereological software designed in Stereology Research Center of Shiraz University of Medical Sciences.

there was inconsiderable difference between the rate of wound closure in the group treated with vehicle (4.93% per day) and the control group (4.43% per day) (Figure 2).

Fibroblast population in the dermis of the Oltipraz treated group was significantly higher than that of the control and vehicle groups. Fibroblast population in Oltipraz treated group was reported 92.29% higher than the controls (p=0.003) and 104.25% higher than the vehicle group (p=0.003).The volume density of the collagen bundles was 38.03% and 57.75% higher in the oltipraz treated group compared to the control

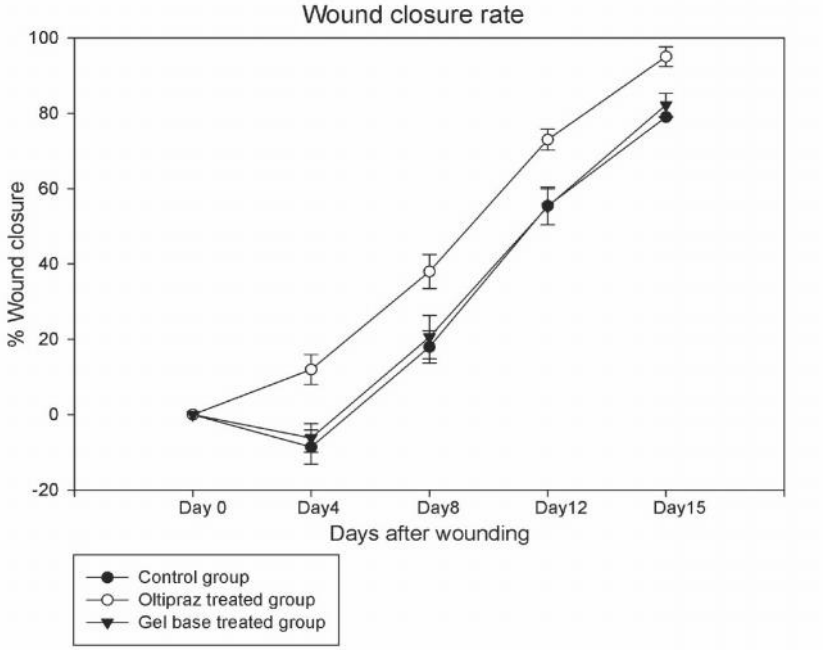

Fig. 2. Comparison of wound closure rate in the three groups of laboratory rats; control group, vehicle treated group and oltipraz treated rats. Each point represents the mean area of closed wounds in any of the treated groups. Wound closure rate was significantly higher in Oltipraz treated rats compared with vehicle treated (p=0.038) and control (p=0.032) groups.

and the vehicle groups, respectively, which both were statistically significant (p<0.001). Consequently, the volume density of hair follicles was higher in the oltipraz group in comparison to the control (66.1%; P=0.043) and the vehicle (210%; p=0.001) groups (Table 1).

Discussion

The significance of skin care and the possible complications of its relateddisordersespecially wound healinghavedrawn the attention of dermatologists for a long time. Hence,searching formethods with more efficacies to ameliorate the process of wound healing has always been a high priority for many researchers. Oltipraz belongs to a class of chemicals, known as the dithiolethiones, which are found in most of the cruciferous vegetables such as broccoli, cauliflower and cabbage [16]. Many studies have been conducted on the antioxidant properties of topical and oral administration of dithiolethiones [17,18]. Antioxidants are postulated to help control wound oxidative stress and thereby accelerate wound healing [19,20]. Oltipraz was also reported to have anti-inflammatory effects [21]. Studies have demonstrated that oltipraz may provoke the induction of phase II detoxification enzymes, conspicuously glutathione-S-transferase (GST), which is remarkably a powerful antioxidantand an enhancer of the early course of wound healing [22-25]. To the best of our knowledge there is currently no published study investigating the effects of topical administration of oltipraz on skin wounds; however, many studies, as mentioned above, have worked on its different potentials, most importantly the anti-inflammatory and antioxidative impacts, which can be influential on various aspects of the wound healing process. As it was reported in this study, oltipraz has positive influence on fibroblast proliferation and re-epithelization and thus plays an important role in regeneration of the skin tissue. A noticeable observation in our study was absence of scar formation in wounds which were treated by oltipraz and can be mentioned as an advantage of this drug.
In conclusion, topical administration of oltipraz was associated with fibroblast proliferation and re-epithelization in laboratory induced wound in experimental model. Absence of scar formation in wounds treated by oltipraz was the most important advantage of the topical oltipraz administration. Further studies and clinical evidence is required to support these findings.

Acknowledgment

This study was financially supported by Shiraz University of Medical Sciences. Animals were provided by the Animal House of Shiraz University of Medical Sciences. Stereological analysis was carried out at Histomorphometry and Stereology Research Center of Shiraz University of Medical Sciences, Shiraz, Iran. Authors wanted to thank the “Scientific writing” group of Student Research Committee of Shiraz University of Medical Sciences and Dr. Bahareh Kardeh for scientific editing of this paper.

Conflict of Interest: None declared.

Table 1. Mean (SD) of the numerical density of the fibroblasts (×10^3 per mm^3), volume densities of the collagen bundles ($Vv_{collagen/dermis}$; %), vessels ($Vv_{vessel/dermis}$; %) and hair follicles ($Vv_{hair\ follicles/dermis}$; %), length density ($mm/mm^3$) and mean diameter (µm) of vesselsin the dermis of the wounded rats treated with 20% Oltipraz solution, vehicle and untreated wounded group (Control).

Groups	Fibroblasts	Collagen bundles	Vessels			Hair Follicles
	Numerical density	Volume density	Volume density	Length density	Mean diameter	Volume density
Control	168.33(22.3)	56% (3.6%)	3.0% (2.7%)	20.2(8.1)	1.76(0.2)	5.8% (2.6%)
Oltipraz	354.21(10.5)[a]	77.3% (8.9%)[a]	3.3% (1.5%)	21.2(9.1)	2.23(0.2)	6.1% (1.2%)
Vehicle	184.20(16.3)	49% (4.7%)	2.4% (2.79%)	17.5(9.1)	2.32(0.3)	4.9% (1.7%)

[a]p<0.05, Oltipraz treated group vs. control group and vehicle treated group

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