The role of silver alginate, epithelial growth factor, and platelet rich plasma on wound healing in burn model.

Mucahit Kapci[1], Buket Demirci[2], Nesibe Kahraman Cetin[3], Bekir Daglı[1], Ali Duman[1], Mucahit Avcil[1], Ayhan Akoz[1], Kenan Ahmet Turkdogan[1*]

[1]Department of Emergency Medicine, Medical Faculty, Adnan Menderes University, Aydin, Turkey

[2]Department of Medical Pharmacology, Medical Faculty, Adnan Menderes University, Aydin, Turkey

[3]Department of Pathology, Medical Faculty, Adnan Menderes University, Aydin, Turkey

Abstract

The aim of this study is to compare the promoting effects of Platelet Rich Plasma (PRP), Silver Alginate (AgS), and intralesional Epidermal Growth Factor (EGF) in burn wound healing through the use of 56 rats equally divided into four groups. The control group was given only saline (SF). Except for AgS, all of the drugs were administered by the intralesional route on the first, third, and fifth days of the experiment. Half of the rats in each group were euthanized on the 14th day and the remaining rats were kept for 21 days to observe the wound healing process. The excised specimens were fixed in a 10% neutral buffered formalin solution for pathological evaluation. In the PRP group, fibroblastic activity and neoangiogenesis decreased between 14 and 21 days while Collagen Deposition (CD) increased. In the EGF group, Fibroblast Proliferation (FP), CD, and neoangiogenesis increased between 14 and 21 days. The correlation was found to be directly proportional to this increase. In the Ag sulfadiazine group, FP and neoangiogenesis did not increase between 14 and 21 days while CD increased slightly. All three drug groups induced wound healing. While Ag sulfadiazine showed uncontrolled wound healing, PRP stimulated more efficient healing in the acute phase and EGF stimulated a more slowly progressing wound healing.

Introduction

The healing of wounds has been an important clinical endeavor for centuries. Currently, more wound care products are being produced to treat specific types of wounds. However, the main phases of healing are similar for almost all wounds. Homeostasis, inflammation, proliferation, and remodeling (resolution or maturation) are the main phases of wound healing [1,2]. Because of these phases, many factors can affect tissue repair. As a result, most wound care products are targeted to stimulate one or more of these phases, especially products used for improving delayed tissue repair or stimulating the healing of chronic wounds [3]. The basic events are constructed in the proliferative phase, just behind the inflammatory process. In this phase, dermal growth is started with neoangiogenesis by stimulating the capillary growth with fibroblastic activity and endothelial cell migration [2,4]. Thereafter, collagen formation and deposition provide tissue granulation. The wound bed is composed with fibroblasts by producing major elements of the extracellular matrix, such as collagens, glycosaminoglycans, and proteoglycans [2,4].

Burns are challenging wounds that require critical care and further support for optimizing functional recovery due to tissue loss and exaggerated systemic responses. Furthermore, skin destruction and protein denaturation lead to impaired tissue repair and facilitate microorganismal penetration [5,6]. Thus, wound care products have more importance for these types of wounds [5]. Burns are also investigated in experimental wound care studies due to their complex nature. This study aims to compare the curative and supportive effects of Silver Alginate (AgS), Platelet Rich Plasma (PRP), and intralesional Epidermal Growth Factor (EGF) in experimentally created burn wounds in rats.

Material and Method

Animals

In this study, the weight of the rats was 220-250 g. We applied an iron 1.5 cm in diameter to the shaved back of each rat for 15 seconds to form a 2nd degree burn model. Following the approval of the Animal Ethics Committee (64583101/2015/133), the experimental study was conducted

by using 56 three- to four-month-old female rats. During the study period, the rats were kept in individual cages to avoid harming each other, at room temperature (20 ± 2 °C) in 12-hour daylight/dark environments, fed with standard food pellets, and allowed free access to water.

Table 1. Design of study.

Groups	n	Treatment of route on 1st day	Treatment of route on 3th day	Treatment of route on 5th day	Specimens taken day
Control group n=14	n=7	intralesional	intralesional	intralesional	14th day
	n=7	intralesional	intralesional	intralesional	21th day
AgS group n=14	n=7	transdermal	transdermal	transdermal	14th day
	n=7	transdermal	transdermal	transdermal	21th day
EGF group n=14	n=7	intralesional	intralesional	intralesional	14th day
	n=7	intralesional	intralesional	intralesional	21th day
PRP group n=14	n=7	intralesional	intralesional	intralesional	14th day
	n=7	intralesional	intralesional	intralesional	21th day

Experimental protocol

In the first day of study, the rats were randomly divided into four main groups of 14: control group, AgS-treated group (Silverdin® 1%, Deva, Istanbul, Turkey), EGF-treated group (Heberprot-P®, Heber Biotec, S.A. La Habana, Cuba), and PRP-treated group. Under the ketamine/xylazine (50 mg/kg+5 mg/kg, intraperitoneally, respectively) anesthesia, the dorsa of the rats were shaved and a preheated hot iron bar was applied for 15 seconds.

After a preheated hot iron bar was applied, the control group was given only saline (SF) on the first, third, and fifth days of the experiment. SF, EGF, and PRP were applied through an intralesional route on the first, third, and fifth days of the experiment. AgS was applied through the transdermal route on the first, third, and fifth days of the experiment. Half of the rats in each group were euthanized on the 14th day under ketamine/xylazine (50 mg/kg+5 mg/kg, intraperitoneally, respectively) anesthesia and the remaining rats were kept for 21 days to observe the wound healing process (Table 1).

PRP was freshly prepared as it was previously. Blood samples were withdrawn from the other two rats into anticoagulant-tubes with acid citrate dextrose (ACD). When the tubes were centrifuged at low rpm (200/mins, 20°C, 10 mins), three parts were identified. Buffy-coat and platelet-poor plasma, taking place at the top, were re-centrifuged together (300/mins, 20°C, 15 mins) and PRP was obtained.

Table 2. The comparison of median scores (25-75 percentile) of the study groups according to Ehrlich Hunt scale.

	Control Group	Ags Group	EGF Group	PRP Group 4	P value
14th day					
Inflammatory infiltration	1.00 (0.00-2.00)	1.00 (0.00-1.00)	1.00 (0.75-1.25)	1.00 (0.75-2.00)	0.684
Fibroblastic activity	0.00 (0.00-1.00)	1.00 (0.00-1.00)	1.00 (0.75-1.25)	1.00 (1.00-2.00)[a]	0.035
Collagen deposition	2.00 (1.00-3.00)	3.00 (2.00-3.00)	2.00 (2.00-2.00)[b]	2.00 (1.75-2.00)[b]	0.022
Neo-angiogenesis	1.00 (1.00-2.00)	1.00 (0.00-1.00)	1.00 (0.75-1.25)	2.00 (1.00-2.00)[b]	0.022
Epitelial rejenerasyon	2.00 (0.00-2.00)	1.00 (1.00-2.00)	1.00 (1.00-2.00)	1.00 (0.75-1.25)	0.745
21th day					
Inflammatory infiltration	0.00 (0.00-1.00)	1.00 (0.00-1.00)	1.00 (0.00-1.00)	0.00 (0.00-1.00)	0.538
Fibroblastic activity	1.00 (1.00-2.00)[c]	1.00 (0.00-1.00)[c]	2.00 (2.00-2.00)	1.00 (1.00-1.00)[c]	0.001
Collagen deposition	1.00 (2.00-2.00)[b]	4.00 (3.00-4.00)	2.00 (2.00-2.00)[b]	2.00 (2.00-3.00)[b]	0.001
Neo-angiogenesis	1.00 (0.00-1.00)[c]	1.00 (1.00-1.00)[c]	2.00 (2.00-2.00)	1.00 (1.00-1.00)[c]	0.004
Epitelial rejenerasyon	2.00 (1.00-2.00)	2.00 (2.00-2.00)	2.00 (2.00-2.00)	2.00 (2.00-2.00)	0.256

Abbreviations: Ags; Ag sulfadiazine, EGF: Epidermal Growth Factor, PRP: Platelet Rich Plasma.

[a]p<0.05 vs Control

[b]p<0.05 vs AgS

[c]p<0.01 vs EGF

Histopathologic evaluation

The excised specimens were fixed in a 10% neutral buffered formalin solution for pathological evaluation.They were

Statistical evaluation

Statistical analysis of the data was performed by using Kruskal-Wallis and Chi-square tests. The level of significance was considered as p<0.05. When significant differences were found among groups, pairwise comparisons were performed between groups in order to detect the groups from which the differences originated.
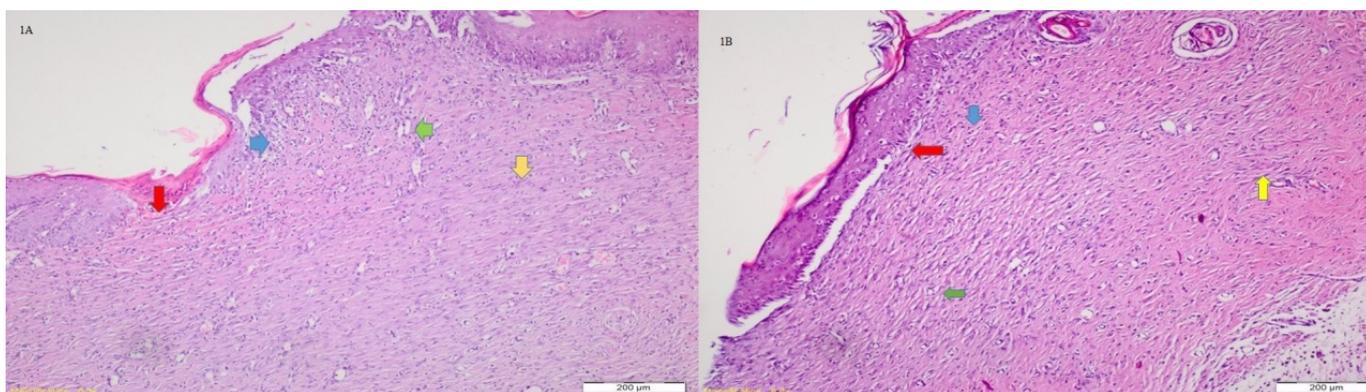
Figure 1. *Photomicrograph of histopathology from control group (A) and EGF group (B): displaying distinctive fibroblastic proliferation (Yellow arrow), marked inflammatory infiltration (Blue arrow) and neo-angiogenesis (Green arrow), impaired epithelial regeneration (Red arrow) (H-E × 200).*
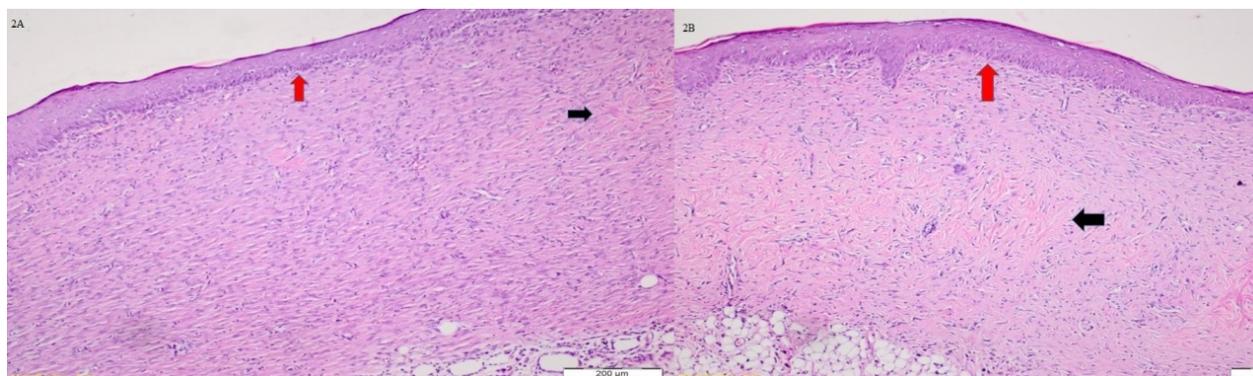
Figure 2. *Photomicrograph of histopathology from PRP group (Figures 2A) and Silver Alginate group (Figure 2B) displaying less fibroblast proliferation, collagen deposition (Black arrow) and good epithelial regeneration (Redarrow) (H-E x 200).*

Results

Evaluation of specimens taken on 14th day

There was no significant difference between groups in terms of inflammatory infiltration findings when the specimens were examined. FP was higher in all three drug groups compared to the control group, but the statistical difference was between the control group and the PRP group (p=0.035). The Ag sulfadiazine group had the highest collagen deposition and the difference between the Ag sulfadiazine and the EGF and PRP groups was statistically significant (p=0.035 and p=0.022, respectively). The highest neoangiogenesis was observed in the PRP group and the difference between the PRP and the Ag sulfadiazine groups was statistically significant (p=0.022) (Table 2).

Evaluation of specimens taken on 21st day

When the specimens were examined, there was no significant difference between the groups in terms of inflammatory infiltration findings. The highest FP was determined in the

EGF group and there was a significant difference between the EGF group and the control group, The Ag sulfadiazine group and the PRP group were compared (p=0.026, p=0.004, p=0.001, respectively) (Figures 1A and 1B). The Ag sulfadiazine group had the highest collagen deposition and there was a significant difference when the Ag sulfadiazine group was compared to the control, EGF, and PRP groups (p=0.001, p=0.001, p=0.007, respectively). The highest neoangiogenesis was observed in the EGF group and a significant difference was found when the EGF group was compared to the control, Ag sulfadiazine, and PRP groups (p=0.011, p=0.004, p=0.026, respectively) (Figures 2A and 2B) (Table 2). Macroscopic images of the samples on the 21st day are shown in Figure 3.
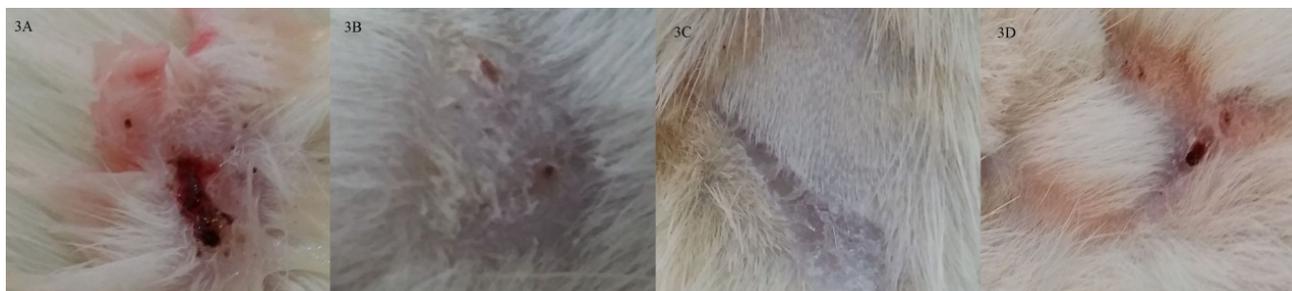
Figure 3. Macroscopic image of the burn wound healing samples (A: control group; B: Silver Alginate group; C: EGF group; D: PRP group) on 21th day.

Discussion

This is a unique study that compares the promoting effects of intralesional PRP, intralesional EGF, and transdermal AgS. In the PRP group, FP and neoangiogenesis decreased between the 14th and 21st days, while collagen deposition increased. In the EGF group, FP, CD, and neoangiogenesis increased between the 14th and 21st days. A correlation was found to be directly proportional to this increase. In the Ag sulfadiazine group, FP and neoangiogenesis did not increase between the 14th and 21st days, while CD increased slightly more.

In the study conducted by Yenilmez et al., highly damaged and intensely inflamed tissue was observed in the AgS group after treatment for seven days. After 14 days, the damage was still obvious; however, formation of granulation tissue and incomplete reepithelialization was seen. On the 21st day, formation of the granulation tissue and increased collagen fiber formation was evident, as well as incomplete epithelialization [7,8]. In the Ag sulfadiazine group, FP did not increase between the 14th and 21st day, and highly increased CD also indicates an uncontrolled wound healing in our study. The failure of increasing neoangiogenesis also supported this finding.

Platelet-Derived Growth Factor (PDGF) was investigated in 18 patients with 26 lower extremity wounds for a duration of at least eight weeks. Only 78% of the patients were diabetic. Over the 12-week study period, the researchers did not find any improvement in wound healing with the use of PDGF [9]. While this study was limited by a small sample size, its results suggested that the treatment of chronic wounds with PDGF is no better than traditional therapy. In another study, they stated that fistulization did not develop and wound healing was complete in the PRP group for experimentally-prepared pharyngocutaneous wound healing [10]. They showed that PRP was beneficial for acute wound healing in both studies. Results obtained in our study were correlated with the literature. In our study, collagen deposition increased while fibroblastic activity decreased between the 14th and 21st days in the PRP group. This indicates that PRP lost influence on chronic wound healing while stimulating acute wound healing. The decrease of neoangiogenesis in the 21st day also supports this.

In one study, Hardwice et al. found that EGF helped in the healing of acute wounds, but it had a limited effect in the healing of chronic wounds [11]. But, JV Pai-Dhungat et al. said that a shortage of growth factors impairs wound healing, which leads to chronic non-healing wounds. Ischemic foot diabetic ulcer is the most difficult to treat and confers the highest amputation risk. Injecting epidermal growth factor deep into the wound bottom and contours encourages an effective pharmacodynamic response in terms of granulation tissue growth and wound closure [12]. The fact that FP and CD increased between the 14th and 21st days in the EGF group in this study showed that the wound was healing quite well. An increase of neoangiogenesis also supported this. This also indicated that wound healing was active in both acute and chronic periods. While FP and CD were correlatively higher in wound healing, collagen deposition increased and fibroblastic activity decreased between the 14th and 21st days in the PRP group in our study. This showed us that PRP's effect lost in chronic wound healing by stimulating acute wound healing. A decrease of neoangiogenesis on the 21st day also supported this. The fact that FP and CD increased between the 14th and 21st days in the EGF group showed that the wound was healing quite well. An increase of neoangiogenesis also supported this. In the Ag sulfadiazine group, FP did not increase and CD increased more between the 14th and 21st days, which also indicated an uncontrolled wound healing. The failure of increasing neoangiogenesis also supported this.

Consequently, all three drug groups induced wound healing. While Ag sulfadiazine showed uncontrolled wound healing, PRP stimulated a more active healing in the acute phase, and EGF stimulated a slowly progressing wound healing. In the chronic period, neoangiogenesis increased only in the EGF

group. We think that EGF may provide an additional benefit when applied to advanced burns due to this effect.

Limitation

The major limitation of our study was that we had no fourth week (28th day) group. This situation makes it hard to reveal the group demonstrating the best organization in complete wound healing. However, this pilot study may lead to further studies that will examine this issue in the future. The second limitation was that the study was designed on an animal model. Thus, our results need to be verified on humans. The third limitation was that the animals couldn't be treated on the seventh day. These limitations may clarify whether collagen deposition will occur in the AgS group when compared with other groups.

Acknowledgement

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*Correspondence to:

Kenan Ahmet Turkdogan

Department of Emergency Medicine

Medical Faculty

Adnan Menderes University

Turkey