Journal of Biomedical Sciences
Research Article Peer-review, Open Access

The Skin Wounds and Effects of Epicyn, Flosteron and Contratubex on il-1 and il-6 Cytokines In the Dynamic of Healing Process

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Abstract

Skin wounds, ranging from minor cuts to severe injuries, are common occurrences in everyday life. The problem of wound healing is multifaceted, involving scar formation, infection risk, and cosmetic outcomes. The wound healing is a complex, dynamic and highly regulated process involving a cascade of cellular and molecular events. Interleukin-6 and interleukin-1 emerge as critical mediators in orchestrating inflammation and tissue repair during wound healing. Understanding the roles of these cytokines provides valuable insights into the mechanisms underlying wound healing and may offer potential therapeutic targets for enhancing the healing process in various pathological conditions. Presented article provides results of investigation carried out on lab. rats designed to study effects of Flosteron, Contratubex and Epicyn on IL-1 an IL-6 cytokines during the skin wound healing process. Excisional, full-thickness skin wounds were aseptically made on the dorsal skin of lab. rats. Contractubex and Epicin creams were applied to the wound surface as a thin layer 2-3 times a day for 4 weeks, Flosteron (0.2 ml) was injected subcutaneously in the wound area once a week during 4 weeks. Proinflammatory cytokines (IL-1, IL-6) were studied by ELISA. **Results** showed that IL-1 on the 7th day of wound healing was increased significantly in all experimental group animals compared to the data of healthy group animals. In subsequent days the IL-1 gradual decrease was detected only in the epycin-treated group animals and by the 21st day of wound healing it was within the normal range. IL-1 in flosteron-treated animals was normalized by 28th day of wound healing, while in control and contratubex-treated animals it was still increased by 35% and 24% respectively. Increase in IL-6 was detected later, on the 14th day of wound healing. Its gradual decrease and normalization was obvious in epicyn- and flosteron-treated animals on the 28th day. There was no significant difference in data of control and contratubex-treated animals and by the 28th day of healing process IL-6

was still increased by 17% and 14% respectively compared to normal value. **Conclusion**: IL-1 and IL-6 are pivotal in skin wound healing, with implications for inflammation modulation and tissue repair. During wound healing, IL-1 and IL-6 exhibit distinct temporal patterns, reflecting their roles in different phases. As wounds progress, IL-1 and IL-6 decline, signaling resolution of inflammation and tissue remodeling. Although, epicyn revealed better wound healing properties, further research is warranted to elucidate the precise mechanisms underlying the effects of flosteron, contratubex, and epicyn on IL-1 and IL-6 dynamics during wound healing. Therapeutic interventions targeting these cytokines may offer promising avenues for enhancing wound healing outcomes.

Key words: Epicyn, Flosteron, Contratubex, IL-1, IL-6, skin wound healing.

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World of Medicine : Journal of Biomedical Sciences Vol .1 No.4 (2024)
https://wom.semanticjournals.org/index.php/biomed

Introduction

The skin, the body's largest and one of the important organs, provides physical protective barrier against pathogens, UV radiation, and mechanical injuries, regulates water loss, electrolyte balance and temperature, plays a crucial role in facilitating sensory perception (touch, temperature, pressure, and pain), contribute the immune responses (Langerhans cells in epidermis) [1,2].

Skin wounds, ranging from minor cuts to severe injuries, are common occurrences in everyday life. Various factors may result in different types of skin injuries. Considering the mentioned functions of the skin, the timely resolution of wound healing is critical. The process of wound healing is a complex and highly orchestrated series of events involving various cells, growth factors, extracellular matrix components and cytokines. Among these, interleukin-6 (IL-6) and interleukin-1 (IL-1) emerge as key players in regulating inflammation, tissue repair and remodeling. Their dysregulation can lead to impaired wound healing or chronic inflammation [3-5].

Several intrinsic and extrinsic factors (age, nutrition, comorbidities and medications) can influence the speed and effectiveness of wound healing process. Understanding the nature of wound healing is essential for developing targeted therapeutic interventions and managing wounds effectively.

Wound healing aimed at restoring tissue integrity and function can be broadly categorized into four overlapping phases:

1. Hemostasis - formation of a blood clot stops bleeding and provides a temporary barrier.

2. Inflammation is a crucial phase characterized by the recruitment of immune cells, primarily neutrophils and macrophages, to the wound site regulating inflammation (IL-6 and IL-1 are key cytokines involved in the inflammatory response);
3. During the proliferation new tissue formation begins as fibroblasts migrate to the wound area and produce extracellular matrix components, such as collagen, to rebuild the damaged tissue. Endothelial cells also proliferate to form new blood vessels (angiogenesis), essential for delivering oxygen and nutrients to the healing tissue.
4. The final phase involves the remodeling of the newly formed tissue. Collagen fibers undergo maturation and reorganization to strengthen the wound site, and excess scar tissue may be gradually broken down and replaced with functional tissue [6-8].

Scar formation is the normal end point of tissue repair, however, excessive scarring can impair normal tissue function [9]. Fibrotic skin tissue covers a spectrum of severity, from flat and pale and relatively static atrophic scars to severe, highly pigmented and rapidly growing pathological hypertrophic and keloid scars [10]. Even the minor normotrophic scar type is dysfunctional. It has decreased sensation, can cause discomfort through itchiness and pain [11] and the tightening of the skin can impair movement and have a detrimental impact on quality of life [12,13]. Scarring also has a psychological toll on patients, due to dissatisfaction with the scar appearance and associated stigma [14].

At present, various treatment methods and drugs are suggested to manage and reduce these complications. Current strategies and emerging technologies aimed at promoting efficient wound healing process involve: steroid Injections (triamcinolone acetonide, are commonly used to reduce inflammation and inhibit collagen synthesis), silicone-based products (gels and sheets are applied to soften and flatten scars by modulating collagen production), pressure dressings or garments are used to apply constant pressure on the scar, reducing collagen production and limiting scar hypertrophy, cryotherapy involves freezing the scar tissue (liquid nitrogen results in controlled tissue destruction and reduces scar prominence), laser therapy (pulsed-dye lasers and fractional lasers, are used to target blood vessels and remodel collagen, reducing scar visibility), surgical excision/surgical removal of hypertrophic scars and keloids (however, recurrence rates can be high, and adjuvant therapies are often used postoperatively), interferon therapy, particularly interferon-alpha, has been used as an adjuvant therapy to reduce collagen production and inhibit scar formation. It's important to note that the effectiveness of these treatments can vary, and a combination of therapies may be used based on the specific characteristics of the scar and patient response [15-18].

Individuals especially suffer at facial skin wounds that prone to hypertrophic scarring and keloid formation, pigmentation changes, or texture irregularities, leading to dissatisfaction with cosmetic outcomes and functional impairment (facial movement, sensation, and expression). Facial wounds can have a significant psychological impact, leading to decreased self-esteem, social withdrawal, and anxiety about appearance.

All of the mentioned emphases the need in selecting methods providing wound healing without complications. Addressing these challenges requires a multidisciplinary approach involving proper wound care techniques, infection prevention strategies, early intervention with appropriate therapies, and consideration of the patient's psychological well-being.

IL-6 and IL-1 are pro-inflammatory cytokines that play multifaceted roles in wound healing, influencing various aspects of the process, including inflammation, cell migration, proliferation, and tissue remodeling. IL-6 is produced by multiple cell types, including macrophages, fibroblasts, and endothelial cells, in response to tissue injury and inflammation. It acts as both a pro-inflammatory and anti-

inflammatory mediator, depending on the context. In wound healing, IL-6 promotes the recruitment of immune cells to the site of injury and stimulates the proliferation and migration of keratinocytes, the predominant cell type in the epidermis. Additionally, IL-6 enhances angiogenesis by inducing the production of vascular endothelial growth factor (VEGF), thereby facilitating the formation of new blood vessels crucial for supplying oxygen and nutrients to the healing tissue [19-21].

Similarly, IL-1 is produced by various cell types, including macrophages, endothelial cells, and keratinocytes, and exists in two isoforms, IL-1α and IL-1β, both of which contribute to the inflammatory response during wound healing. IL-1 promotes the production of other pro-inflammatory cytokines and chemokines, amplifying the inflammatory cascade. It also induces the expression of adhesion molecules on endothelial cells, facilitating the recruitment of leukocytes to the wound site. Moreover, IL-1 enhances fibroblast proliferation and collagen synthesis, promoting tissue repair and remodeling.

Understanding the roles of these cytokines provides valuable insights into the mechanisms underlying wound healing and may offer potential therapeutic targets for enhancing the healing process in various pathological conditions.

At present, several preparations have been used to optimize face skin wound healing outcomes [22-24]. Among them are: flosteron (anti-inflammatory steroid medication), contratubex (topical gel) with three main active ingredients: allantoin, cepae extract (from onions), and heparin help soften, flatten, and reduce the redness of scars over time by promoting skin regeneration and reducing inflammation, and epicyn hidrogel, the antimicrobial wound cleanser forms a protective barrier against physical, chemical and microbial (including bacteria, viruses, and fungi) invasion of the wound, helping to retain moisture, and flatten the scar. Active component hypochlorous acid (HOCl) reduce the risk of infection in wounds.

Coming from the aforesaid we were aimed to study and compare effects of flosteron, contratubex and epicyn on IL-1 an IL-6 cytokines involved in skin wound healing process.

Objects and methods of research: Experiments were carried out on male white lab. Rats with the body weight range 200-250 g. The animals were purchased from the vivarium of Aleksandre Natishvili *Institute of Morphology, Tbilisi, Georgia (*https://www.tsu.ge/en*)*.

All animals were allowed to become acclimatized to laboratory conditions for one week before the experiment. During this period, the animals were kept under constant environmental conditions with a light-dark cycle of 12/12 at a temperature of 23±2°C. They were fed a standard laboratory chow and given free access to water.

For modeling of skin wounds the rats were anesthetized with nembutal (50 ml/kg). After shaving and cleaning with 70% alcohol, excisional, full-thickness skin wounds were aseptically made on the dorsal skin. After, a surgical suture of 5 cm was placed on the skin at 1 cm interval.

All animals were placed in the groups. Each group involved 10 rats.

The group I - intact healthy rats;

The group II - control, untreated rats;

The group III - rats treated with epicyn;

The group IV - rats treated with flosteron;

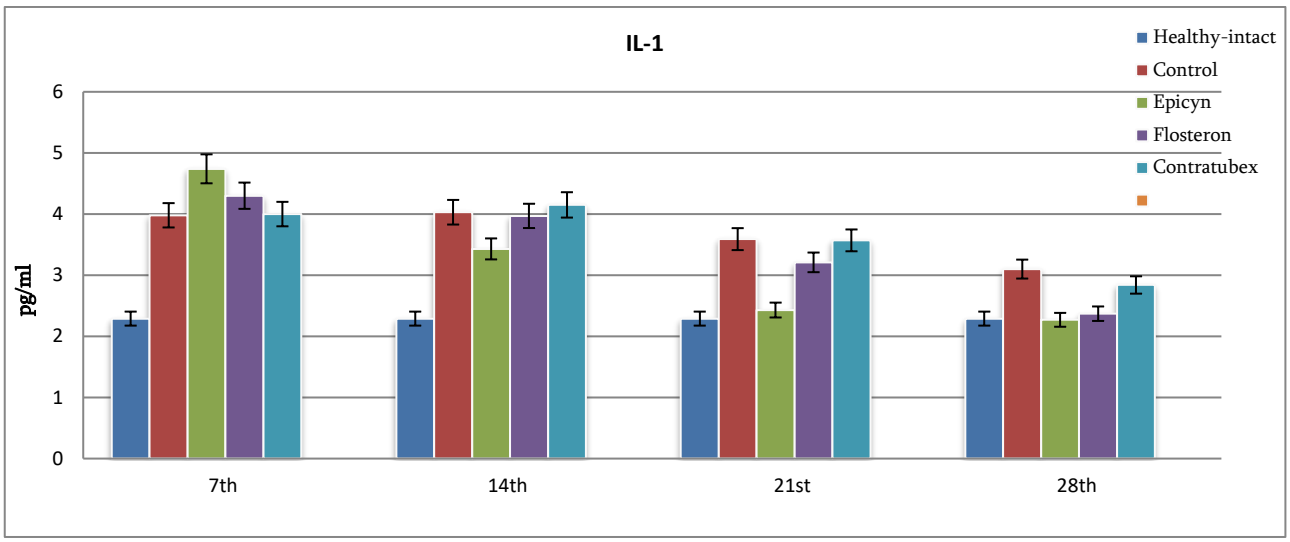
The group V - rats treated with contratubex.

Contractubex and epicin creams were applied to the wound surface as a thin layer 2-3 times a day for 4 weeks in the corresponding group anomals. Flosteron (0.2 ml) was injected subcutaneously in the wound area once a week during 4 weeks.

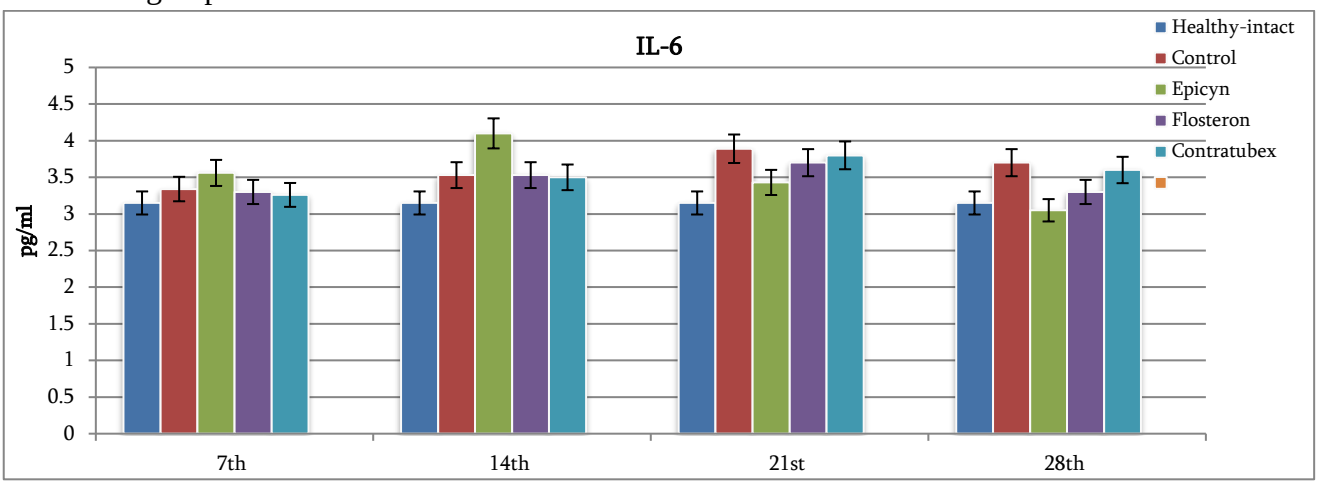
Proinflammatory cytokines (IL-1, IL-6) were studied by ELISA. Blood samples for immunological investigations were collected and studied by the 7th, 14th, 21st and 28th days of experiment.
Results were analyzed statistically. Statistical significance was evaluated by using ANOVA or Mann-Whitney's U test. $P < 0.05$ was accepted as statistically significant.

Results and discussion: Results of experiment are presented on graphs 1-2. Investigations showed that IL-1 concentration on the 7th day of wound healing in the control group animals was increased significantly by 74%, in epicyn group – by 107%, in flosteron group – by 88% and in contratubex group – by 75% (p<0,001) compared to the data of healthy, intact group animals. The slight increase in IL-6 concentration was revealed only in the group III animals. In all other studied groups it was not changed significantly compared to the data of healthy, intact group animals.
On the 14th day of wound healing, IL-1 concentration was increased in control group animals by 76%, in epicyn group – by 50%, in flosteron group – by 73% and in contratubex group – by 81% (p<0.001) compared to the data of healthy, intact group animals. IL-6 concentration was increased in control group animals by 12% (p<0,01) , in epicyn group – by 30% (p<0,001), in flosteron group – by 12% (p<0,01) and in contratubex group – by 11% (p<0,05) compared to the data of healthy, intact group animals.
On the 21st day of wound healing, IL-1 concentration was increased significantly in control group animals by 57%, in flosteron group – by 40% and in contratubex group – by 56% (p<0,001), while in epicyn group animals IL-1 was within the normal range compared to the data of healthy, intact group animals. IL-6 concentration was increased in control group animals by 23% (p<0,01), in epicyn group – by 9% (p<0,05), in flosteron group – by 17% and in contratubex group – by 21% (p<0,01) compared to the data of healthy, intact group animals.
On the 28th day of wound healing, IL-1 concentration was increased in control group animals by 35% (p<0,01), in contratubex group – by 24% (p<0,05) and in epicyn and flosteron groups – it was within the normal range compared to the data of healthy, intact group animals. IL-6 concentration was increased in control group animals by 17% ((p<0,01), in contratubex group – by 14% (p<0,05), in epicyn and flosteron groups – IL-6 was within the normal range compared to the data of healthy, intact group animals.
Results showed that IL-1 on the 7th day of wound healing was increased significantly compared to the data of healthy group animals. In subsequent days the IL-1 gradual decrease was detected only in the epycin-treated group animals and by the 21st day of wound healing it was within the normal range. IL-1 in fosteron-treated animals was normalized by 28th day of wound healing, while in control and contratubex-treated animals it was still increased. Increase in IL-6 was detected later, on the 14th day of wound healing. Its gradual decrease and normalization was obvious in epicyn- and flosteron-treated animals on 28th day. There was no significant difference in data of control and contratubex-treated animals and by the 28th day of healing process IL-6 was still increased compared to normal value.

Graph. 1 IL-1 cytokine concentration in healthy, control and treated with epicyn, flosteron and contratubex group lab. rats.



Graph. 2 IL-6 cytokine concentration in healthy, control and treated with epicyn, flosteron and contratubex group lab. rats.



During the dynamic process of wound healing, the concentrations of interleukin-1 (IL-1) and interleukin-6 (IL-6) undergo distinct changes, reflecting their roles in different phases of wound repair.

Following tissue injury, IL-1 and IL-6 concentrations rise rapidly in the inflammatory phase of wound healing.

Both IL-1 and IL-6 reach peak concentrations during the early stages of inflammation, typically within the first few hours to days after injury [22].

During this phase, IL-1 and IL-6 promote inflammation by recruiting immune cells, such as neutrophils and macrophages, to the wound site. They also stimulate the production of other pro-inflammatory cytokines and chemokines.

As the wound progresses to the proliferative phase, IL-1 concentrations gradually decrease. This decline is indicative of the resolution of the inflammatory response.

In contrast, IL-6 concentrations remain elevated during the early stages of the proliferative phase, although they start to decline as wound healing progresses. IL-6 continues to stimulate fibroblast proliferation, collagen synthesis, and angiogenesis during the proliferative phase. It plays a role in tissue remodeling and the formation of new blood vessels.

As the wound enters the remodeling phase, both IL-1 and IL-6 concentrations return to baseline levels or become undetectable [23,24].

Although IL-1 and IL-6 levels decrease during this phase, they may still play modulatory roles in the final stages of wound repair, influencing tissue remodeling, scar formation, and resolution of inflammation.

Investigations showed a significant increase in IL-1 concentration on the 7th day of wound healing in all treated groups compared to the healthy group, with subsequent gradual decrease observed in the Epicyn and Flosteron groups by the 21st and 28th days, respectively. Contratubex showed a slower normalization of IL-1 levels.

IL-6 concentrations was increased later, on the 14th day, in all groups compared to the healthy group. However, gradual decrease and normalization were observed in the epicyn and flosteron groups by the 28th day, while contratubex-treated animals still exhibited elevated IL-6 levels.

These results suggest that IL-1 and IL-6 play pivotal roles in the wound healing process, with distinct functions during different phases, such as inflammation modulation, immune cell recruitment, fibroblast proliferation, and tissue remodeling.

Epicyn demonstrated better wound healing properties compared to flosteron and fontratubex, as evidenced by more rapid normalization of IL-1 and IL-6 levels. This may indicate that Epicyn promotes a more efficient wound healing process by modulating cytokine dynamics.

Flosteron also showed positive effects on wound healing, with normalization of IL-1 and IL-6 levels by the 28th day, although Contratubex exhibited slower normalization, particularly for IL-1.

These differences in treatment effects underscore the importance of selecting appropriate therapeutic interventions tailored to optimize wound healing outcomes.

Conclusion: In conclusion, IL-1 and IL-6 cytokines play pivotal roles in skin wound healing, with implications for inflammation modulation and tissue repair. Therapeutic interventions targeting and modulating the expression and activity of these cytokines, may offer promising avenues for enhancing wound healing outcomes. Controlling inflammation, promoting tissue repair, and facilitating resolution of inflammation and tissue remodeling could lead to improved wound healing outcomes.

Although, epicyn revealed better wound healing properties, additional research is needed to explore the mechanisms underlying the observed effects of flosteron, contratubex, and epicyn on cytokine dynamics and wound healing outcomes. This would provide a deeper understanding of their therapeutic efficacy and help optimize treatment strategies.

Investigating the long-term effects and potential adverse reactions of these treatments would be important for ensuring their safety and efficacy in clinical practice.

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