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DYNAMICS OF THE CYTOKINE BLOOD PROFILE UNDER THE EFFECT OF WOUND HEALING MEDICINAL PRODUCTS WITH DIFFERENT MECHANISMS OF ACTION IN THE EXPERIMENT

Wound healing depends significantly on the level of proinflammatory cytokines in the lesion. Exactly expression of IL-1 β , IL-8, TNF- α retains the course of the wound process in the stage of persistent inflammation. By affecting the mechanisms of cytokine regulation of the reparative tissue regeneration it is possible to correct the wound healing process. The use of topical agents when treating burns is an important component of the complex therapy.

Aim. To study the effect of 10 % methyluracil ointment and 2 % thiotriazoline ointment on the level of proinflammatory cytokines in the blood serum of rats in the dynamics of the thermal burn development.

Materials and methods. The thermal burn was modeled by the method of Yakovleva L. V. (1999), Fenchin K. M. (1979). Ointments with different mechanisms of action, 10 % methyluracil ointment and 2 % thiotriazoline ointment were used as wound-healing agents.

Results. It has been determined experimentally that the level of IL-1 β , IL-8, TNF- α cytokines in the rat blood correlates with the severity of the wound process and the response to the treatment applied. The application of ointments with the wound healing activity has led to the significant decrease in the level of proinflammatory cytokines in the blood of rats and healing of the burn wound in shorter terms. Moreover, thiotriazoline ointment reveals a marked reparative activity than methyluracil ointment.

Conclusions. The use of 10 % methyluracil ointment and 2 % thiotriazoline ointment with a different wound healing effect in the treatment of burn wound leads to the change in the cytokine profile, and it is accompanied with a positive dynamics of healing processes. By the reparative activity 2 % thiotriazoline ointment exceeds the action of methyluracil ointment.

Key words: thermal skin burn; proinflammatory cytokines; methyluracil ointment; thiotriazoline ointment; healing

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Динаміка цитокінового профілю крові під впливом ранозагоювальних лікарських засобів з різними механізмами дії в експерименті

Загоєння ран значною мірою залежить від рівня прозапальних цитокінів у вогнищі. Саме експресія ІЛ-1 β , ІЛ-8, ФНО- α утримує течію ранового процесу у стадії персистуючого запалення. Впливаючи на механізми цитокінової регуляції репаративної регенерації тканин, можна коригувати процес загоєння ран. Застосування лікарських препаратів місцевої дії в лікуванні опіків є важливим компонентом комплексної терапії.

Мета роботи. Вивчити вплив мазі метилурацилової 10 % і мазі тіотриазолінової 2 % на рівень прозапальних цитокінів у сироватці крові щурів у динаміці розвитку термічного опіку.

Матеріали та методи. Термічний опік моделювали за методом Яковлевої Л. В. (1999), Фенчина К. М. (1979), в якості ранозагоювальних засобів використовували мазі з різним механізмом дії, мазь метилурацилову 10 % і мазь тіотриазоліну 2 %.

Результати та їх обговорення. Експериментально встановлено, що рівень цитокінів ІЛ-1 β , ІЛ-8, ФНО- α в крові щурів корелює з тяжкістю ранового процесу і відповіддю на проведене лікування. Застосування мазей з ранозагоювальною активністю приводило до достовірного зниження рівня прозапальних цитокінів у крові щурів і загоєння опікової рани в коротші терміни. Причому мазь тіотриазолінова проявляла більш виражену репаративну активність, ніж мазь метилурацилова.

Висновки. Застосування мазей метилурацилової 10 % та тіотриазолінової 2 % з різним за механізмом ранозагоювальним ефектом при лікуванні опікової рани приводить до зміни цитокінового профілю, що супроводжується позитивною динамікою процесів загоєння. За репаративною активністю мазь тіотриазоліну 2 % перевищує дію метилурацилової мазі.

Ключові слова: термічний опік шкіри; прозапальні цитокіни; метилурацилова мазь; мазь тіотриазоліну; загоєння

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Динамика цитокінового профіля крові под влиянием ранозаживляющих лекарственных средств с разными механизмами действия в эксперименте

Заживление ран в значительной степени зависит от уровня провоспалительных цитокинов в очаге. Именно экспрессия ИЛ-1 β , ИЛ-8, ФНО- α удерживает течение раневого процесса в стадии персистирующего воспаления. Влияя на механизмы цитокінової регуляції репаративної регенерації тканин, можно корректировать процесс

заживления ран. Применение лекарственных препаратов местного действия в лечении ожогов является важным компонентом комплексной терапии.

Цель работы. Изучить влияние мази метилурациловой 10 % и мази тиотриазолиновой 2 % на уровень провоспалительных цитокинов в сыворотке крови крыс в динамике развития термического ожога.

Материалы и методы. Термический ожог моделировали по методу Яковлевой Л. В. (1999), Фенчин К. М. (1979), в качестве ранозаживляющих средств использовали мази с разным механизмом действия, мазь метилурациловую 10 % и мазь тиотриазолиновую 2 %.

Результаты и их обсуждение. Экспериментально установлено, что уровень цитокинов ИЛ-1 β , ИЛ-8, ФНО- α в крови крыс коррелирует с тяжестью раневого процесса и ответом на применяемое лечение. Применение мазей с ранозаживляющей активностью приводило к достоверному снижению уровня провоспалительных цитокинов в крови крыс и заживлению ожоговой раны в более короткие сроки. Причем мазь тиотриазолиновая проявляла выраженную репаративную активность, чем мазь метилурациловая.

Выводы. Применение мазей метилурациловой 10 % и тиотриазолиновой 2 % с различным по механизму ранозаживляющим эффектом при лечении ожоговой раны приводит к изменению цитокинового профиля, что сопровождается положительной динамикой процессов заживления. По репаративной активности мазь тиотриазолина 2 % превышает действие метилурациловой мази.

Ключевые слова: термический ожог кожи; провоспалительные цитокины; метилурациловая мазь; мазь тиотриазолина; заживление

The problem of burn wounds is always pressing since most burns occur within the domestic conditions that dramatically change the victim's life. As a result of burn injury, functional and metabolic nutritional disorders develop. The frequency and duration of these disorders depend on the severity of the burn and the methods of pharmacological correction. Despite the numerous studies of the pathogenesis and methods of pharmacological correction of the wound process the problem of effective treatment of burn wounds has not been solved yet [1, 2], especially in respect of chronization of the wound process [3, 4].

Solution of the problem of effective treatment of long-term non-healing wounds is associated with detection of the key mechanisms of the wound process; disorders of intercellular interactions play an important role among these mechanisms.

It has been found that healing depends significantly on the level of proinflammatory cytokines in the lesion [5, 6]. According to the data [7] delayed healing is caused by the expression of IL-1 β and TNF- α . These cytokines retain the course of the wound process at the stage of persistent inflammation. IL-8 has the pronounced proinflammatory properties, inducing the expression of intercellular adhesion molecules and enhancing adhesion of neutrophils to endothelial cells and subendothelial matrix proteins. This indicates its primary role in mediating the inflammatory response. Another important pathogenetic mechanism of developing a chronic wound, according to many authors, is hypoxia in the lesion, and the key to successful healing is adequate neovascularization. IL-1 β , TNF- α , IL-8 promote chemotaxis of endothelial cells to the site of inflammation, and it leads to initiation of angiogenesis at the site of injury, promoting the processes of returning the blood circulation in the lesion and the reparation processes [7]. By affecting the mechanisms of cytokine regulation of the reparative tissue regeneration it is possible to correct the wound healing process.

The challenge of treating thermal skin injuries requires both development of new methods and improvement of the existing methods of burn treatment. In this case, the use of topical agents when treating burns is an important component of the complex therapy.

Thus, the aim of the current studies was to determine the levels of proinflammatory cytokines in the dynamics of the experimental burn development, as well as in the treatment with wound healing topical agents having different mechanisms of action.

Materials and methods

The wound healing activity was studied in the experiment on 24 male rats of the WAG population weighing 200-250 g. All studies were conducted in accordance with the legislation of Ukraine (Law of Ukraine No.3447-IV "On the protection of animals against ill treatment" / Verkhovna Rada of Ukraine Vidomosti. – 2006. – No. 26 – P. 230), the rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. – Strasbourg, 1986. – 53 p.). Prior to the modeling of the thermal burn wound, the hair on the outer surface of the animal's thigh was shaved, then the skin was treated with the solution of 70 % ethanol, and the second-degree burn was caused under thiopental anesthesia subjected to conservative treatment [8]. Ointments with different mechanisms of action, 10 % methyluracil ointment and 2 % thiotriazoline ointment were applied as treatment for a thermal burn.

All animals were divided into four groups consisted of 6 rats each: group 1 – intact animals; group 2 – control animals with a thermal burn without treatment; group 3 – animals with a burn and 10 % methyluracil ointment as a treatment; group 4 – animals with a burn and 2 % thiotriazoline ointment as a treatment. The agents were applied in the treatment regimen. They were applied

once a day with a thin layer on the burnt surface immediately after the thermal treatment and during the whole experiment (28 days). Observations of the healing processes of burn wounds were conducted on days 3, 7, 14, 21 and 28. Within these terms the state of the wound was recorded, its size was measured [9], and the level of cytokines IL-1 β , IL-8, TNF- α in the rats' peripheral blood was studied.

The level of cytokines IL-1 β , IL-8, TNF- α in the peripheral blood – the key markers of the wound process – was evaluated by enzyme immunoassay on a "Libline-90" analyzer (Austria) using "eBioscience" reagents (USA).

The results obtained were processed by a "Statistica-6" software package for statistical analysis. Differences were considered to be reliable at a significance level not higher than $p < 0.05$ (Lapach S. N. et al., 2001).

Results and discussion

When observing visually in animals of groups 2, 3 and 4 after the thermal exposure, there was a wound filled with a dry light-brown scab, the edges of the wound were swollen and hyperemic.

The animals of the control group, starting from the third day, had a tendency to softening of the central part of the thick scab. When pressing it a serous-purulent exudate was under the scab. On day 7 the wound was a zone of deep necrosis filled with a serous-purulent exudate. Over the next two weeks of observation (up to day 21) there was a decrease in the necrosis zone in the center of the burn wound and epithelization of the wound defect. By day 28 the burn wound was partially epithelialized with formation of a fine tender scar. There was a decrease in the size of the wound: day 3 – by 8 %, day 7 – by 30 %, day 14 – by 59 %, day 21 – by 85 % and day 28 – by 99 % compared to the baseline size.

Animals from group 3 were treated with methyluracil ointment. Starting from the third day the central part of the thick scab was also softened, however, when pressing it, a serous exudate was predominantly under it. On day 7, the necrosis zone in the wound area was filled with a serous exudate. During the following two weeks of observation (up to day 21) the decrease in the necrosis zone in the center of the burn wound and epithelization of the wound defect occurred faster than in the control group. By day 28 the burn wound was almost completely epithelialized with formation of a tender scar. The size of the wound defect decreased more rapidly than in the group without treatment: day 3 – by 9 %, day 7 – by 39 %, day 14 – by 70 % and day 21 – by 92 %. By day 28 the wound defect was completely closed.

Group 4 was treated with thiotriazoline ointment. Softening of the central part of the scab occurred on the third day, a moderate amount of a serous ex-

udate discharged from it, the swelling and hyperemia of the wound were less pronounced, indicating reduction in the period of wet desquamation. The necrotic zone in the center of the burn wound during the following two weeks (up to day 21) decreased more rapidly, epithelization of the wound defect occurred faster, edema and hyperemia were less pronounced than in the group without treatment. By day 28 the burn wound was almost completely epithelialized, the tender scar was more durable. The animals of this group had the fastest closure of the wound defect: by day 3 the wound size decreased by 10 %, day 7 – by 40 %, day 14 – by 71 %, day 21 by 93 % and day 28 – by 100 %.

Thus, healing of the wound defect in groups 3 and 4 was more favorable than in the group without treatment. The most active healing occurred when using thiotriazoline ointment. This may be not only due to the broad spectrum of the pharmacological activity of thiotriazoline, but also due to the hydrophilic base of the ointment. This ointment base, unlike the baseline base of methyluracil ointment, increases thiotriazoline absorption and, consequently, its therapeutic properties.

Cytokines have a regulatory effect on all processes associated with inflammation and post-inflammatory regeneration. A relatively moderate concentration of cytokines, especially IL-1, IL-8, is a necessary condition for the reparative effects of cytokines in the focus of inflammation.

The study of IL-1 β level in the blood serum of animals in the control group showed its increase during the first three weeks of observation compared to intact animals with the maximum level by 2.5 times on day 14 (Fig. 1).

The use of methyluracil ointment led to a decrease in IL-1 β level compared to the control group. Compared to intact animals cytokine production was increased only during the first week.

By day 14 of the treatment this indicator decreased to the normal value and was 2.5 times lower than in the group of animals without treatment. On day 21 of the experiment the IL-1 β level in the blood was significantly lower: by 1.3 times in relation to the control group. The dynamics of changes in the concentration of IL-1 β in group 4 of the study was similar to group 3: the increase during the first week by 1.4 times compared to intact animals, but by 1.3 times lower compared to the animals of the control group. Further a stable decrease was observed in IL-1 β values in group 4 before up to the normal value during days 14-28. During all these terms the cytokine concentration in the blood was significantly lower than in the group without treatment.

According to the study results it has been found that the IL-8 level in the blood of rats with a thermal burn correlates with the severity of the wound

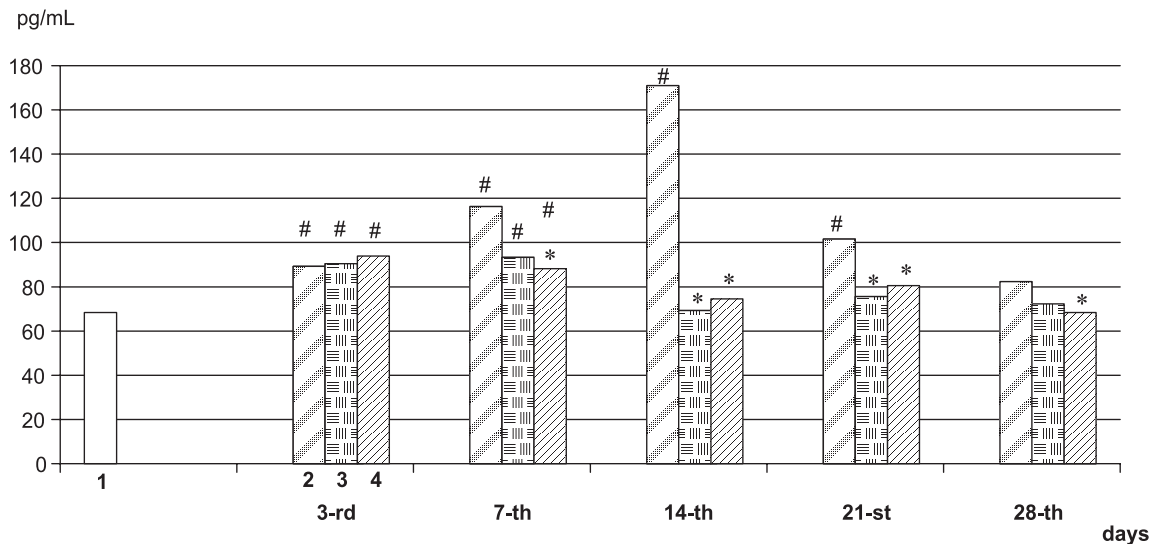


Fig. 1. The dynamics of changes in IL-1 β values in the blood serum of animals with a thermal burn without treatment and with the use of wound healing agents: 1 – intact animals; 2 – control pathology; 3 – treatment with methyluracil ointment; 4 – treatment with thiotriazoline ointment
Note. * significant in relation to the control group of animals ($p < 0.05$); # significant in relation to the intact group of animals ($p < 0.05$).

process and the response to the treatment applied (Fig. 2).

The use of methyluracil and thiotriazoline ointments led to a significant decrease in chemokine to the normal value by day 14, remaining as it was till the end of the observation.

The study of the TNF- α level in the blood of animals of the control pathology group showed its significant increase in relation to the intact group: by 2.1 times on day 3 and by 2.3 times on day 7. The increase in TNF- α values was also observed throughout the study (Fig. 3).

According to Fig. 3 the application of methyluracil and thiotriazoline ointments as a treatment of a burn wound in both groups reduced the TNF- α level to the normal value on day 21 and day 28 of the observation. In the early periods (days 3-14), TNF- α levels in the blood of animals of the both

groups were significantly higher than those of intact rats, but lower than in rats of the pathology control group.

The inflammatory reaction developing in the response to a thermal injury creates conditions for elimination of necrotic cells and conditions for reparative processes. Therefore, regulatory processes at the initial stage of posttraumatic regeneration are aimed at development of inflammation in the lesion. Immunoregulatory processes at the next stage are aimed at creation of the conditions for restoration of damaged tissues [9, 10]. According to the studies conducted it has been determined that the course of a burn injury in rats is accompanied with a significant and prolonged production of proinflammatory cytokines – IL-1 β , IL-8, TNF- α . This may be an important mechanism, which keeps the burn wound in the state of persistent inflammation and

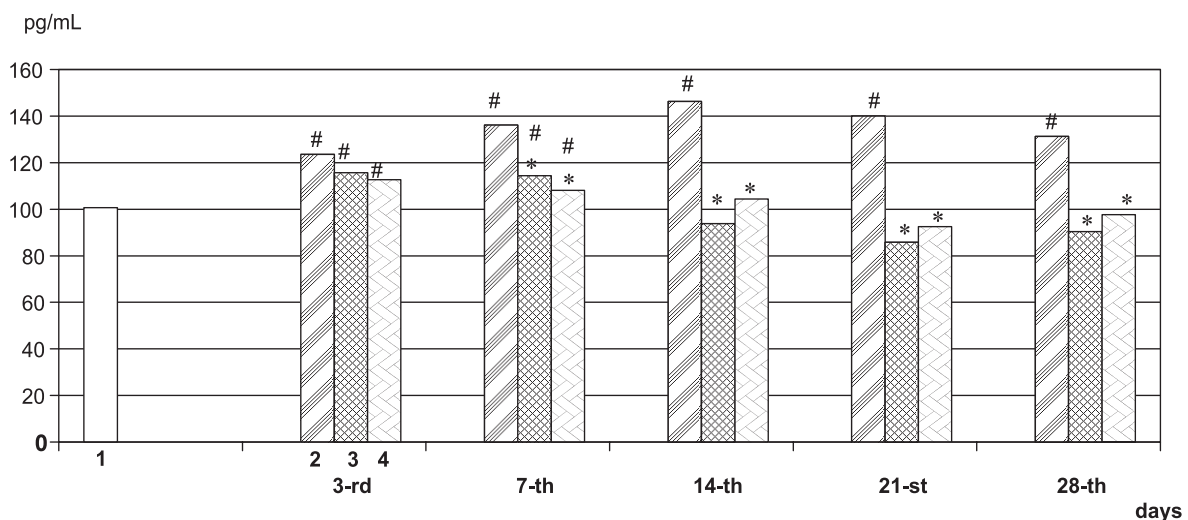


Fig. 2. The dynamics of changes in IL-8 values in the blood serum of animals with a thermal burn without treatment and with the use of wound healing agents. The legend is the same as in Fig. 1

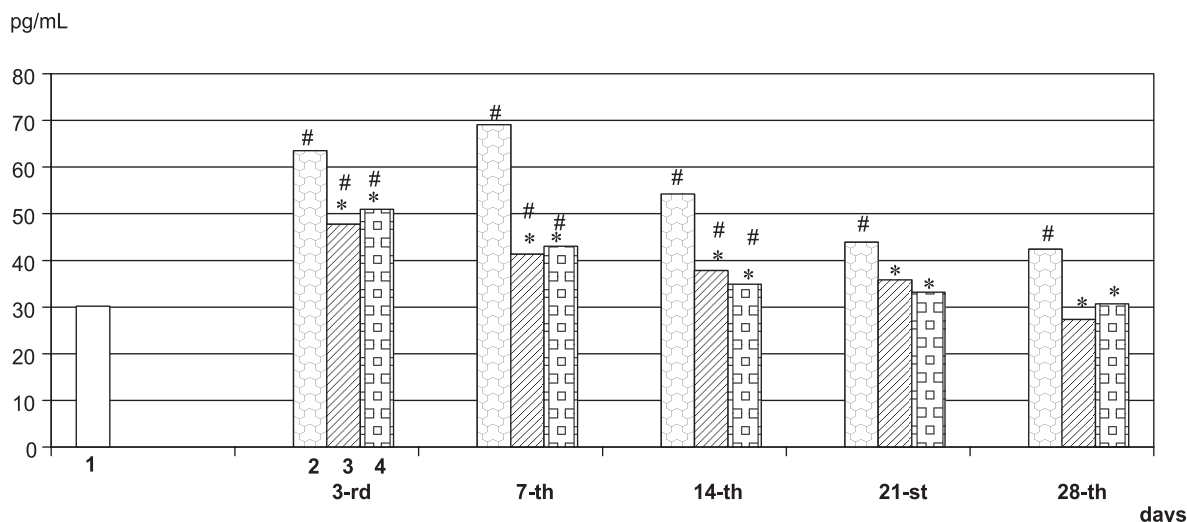


Fig. 3. The TNF- α level in the blood serum of animals with a thermal burn without treatment and with the use of wound healing agents. The legend is the same as in Fig. 1

prevents from normal healing. The application of methyluracil and thiotriazoline ointments for the treatment of burn wounds provides healing of the wound defect in shorter terms.

CONCLUSIONS

1. The course of the wound process in rats with a thermal burn is characterized by a prolonged increase of the level of proinflammatory cytokines (IL-1 β within 3 weeks of observation, IL-8, TNF- α throughout the whole period of observation) in the blood serum. These cytokines regulate the inflammatory process development in the lesion.

2. The use of 10 % methyluracil ointment and 2 % thiotriazoline ointment with a different wound healing effect in the treatment of burn wound leads to the change in the cytokine profile, i.e. to the decrease to the normal value of IL-1 β and IL-8 by day 14, TNF- α – by day 21, and it is accompanied with a positive dynamics of healing processes.

3. By the reparative activity 2 % thiotriazoline ointment exceeds the action of methyluracil ointment.

Conflict of Interests: authors have no conflict of interests to declare.

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