UDC: 615.262.1.454.1:616–001.17–092.9–07857.083.3

**DYNAMICS OF CYTOKINE BLOOD PROFILE AFFECTED BY VULNERARY MEDICINAL PRODUCTS WITH DIFFERENT MECHANISMS OF ACTION IN THE EXPERIMENT**

***T.I. Iermolenko, O.V. Kryvoshapka, O.I. Pautina***

Kharkiv National Medical University

*Keywords: thermal skin burn; proinflammatory cytokines; methyluracil ointment; thiotriazoline ointment; healing*

The problem of burn wounds is always pressing as 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 the disclosure of key mechanisms of the wound process; violations of intercellular interactions play an important role among these mechanisms.

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

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

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

**Materials and Methods**

Vulnerary activity was studied in the experiment on 24 male rats with a WAG population of 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 European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. – Strasbourg, 1986. – 53 p.). Prior to the modeling of thermal burn wound, the wool on the outer surface of the animal’s thigh was shaved, then the skin was treated with a solution of 70% ethanol, then a second-degree burn was caused under thiopental anesthesia, which is subject to conservative treatment [8]. Ointments with different mechanisms of action were applied as treatment for thermal burn: 10% methyluracil ointment and 2% thiotriazoline ointment.

All animals were divided into four groups consisted of 6 rats each: the 1st one was intact; the 2nd one was control, animals with thermal burn without treatment; the 3rd one was with a burn and 10% methyluracil ointment was applied as treatment; the 4th one was with a burn, 2% thiotriazoline ointment was applied as treatment. The agents were applied in treatment regime. They were applied once a day with a thin layer on the burnt surface immediately after the thermal treatment and during the entire of the experiment (28 days). Observations of the healing processes of burn wounds were conducted on Days 3, 7, 14, 21 and 28. Within these time frames the state of the wound was recorded, its size was measured [9] and the level of cytokines IL-1β, IL-8, TNF-α in rats’ peripheral blood was evaluated.

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

 The obtained results were processed with a software package for statistical analysis, i.e. “Statistica-6”. Differences considered to be reliable at a significance level not higher than p <0.05 (Lapach S.N. and others, 2001).

**Results and Discussion**

Observing visually the 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 serous-purulent exudate. Over the next two weeks of observation (up to 21 days) 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 the 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% % in comparison with the baseline size.

Animals from the third group were treated with methyluracil ointment. Starting from the third day, the central part of the thick scab was also occurred softened, however, when pressing it, serous exudate was predominantly under it. On Day 7, the necrosis zone in the wound area was filled with serous exudate. During following two weeks of observation (up to 21 days), the decrease in the necrosis zone in the center of the burn wound and the epithelization of the wound defect occurred faster than in the control group. By Day 28 the burn wound was almost completely epithelialized with the 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.

The fourth group was treated with thiotriazoline ointment. Softening of the central part of the scab occurred on the third day, a moderate amount of serous exudate emerged from it, the swelling and hyperemia of the wound were less pronounced, that indicates about reduction in the period of wet desquamation. The necrosis zone in the center of the burn wound during the following two weeks (up to 21 days) 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, the healing of the wound defect in the third and the fourth groups was more favourable than in the group without treatment. The most active healing occurred with the use of thiotriazoline ointment. This may be not only due to the broad spectrum of 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 the absorption of thiotriazoline and, consequently, its therapeutic properties.

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

A study of IL-1β level in the blood serum of animals in the control group showed its increase during the first three weeks of observation in comparison with intact animals, with a maximum level on Day 14 by 2.5 times (Figure 1).

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

By Day 14 of the treatment, this indicator decreased to normal value and was 2.5 times lower than in the group of animals without treatment. On Day 21 of the experiment, IL-1β level in the blood was significantly lower: by 1.3 times, respect to the control. Concentration-time pattern of IL-1β in the fourth study group was similar to the third group: increase during the first week by 1.4 times in comparison with intact animals, but by 1.3 times lower in comparison with the animals of the control group. In the future, a stable decrease was observed in IL-1β values in the fourth study group 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.

Figure 1. Dynamic pattern of IL-1β values in the blood serum of animals with 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.

\* Accurately with respect to the control group of animals (p <0.05);

# Accurately with respect to the intact group of animals (p <0.05).

According to the study results IL-8 level in the blood of rats with thermal burn correlates with the severity of the wound process and the response to the applied treatment (Figure 2).

The use of methyluracil and thiotriazoline ointment led to a significant decrease in chemokine to the normal value by Day14, remaining as it was till the end of the observation.

Figure 2. Dynamic pattern of IL-8 values in the blood serum of animals with thermal burn without treatment and with the use of wound healing agents. Legend is the same as in Figure 1

Study of 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. Increase in TNF-α values ​​was also observed throughout the study (Figure 3).

According to Figure 3 the application of methyluracyl and thiotriazoline ointments as treatment of burn wound in both groups reduced TNF-α level to 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.

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

Inflammatory reaction, developing in response to a thermal injury, creates conditions for elimination of necrotic cells and conditions for reparative processes. Therefore, regulatory processes in the initial stage of posttraumatic regeneration are aimed at the development of inflammation in the focus. Immunoregulatory processes at the next stage are aimed at creation of the conditions for restoration of damaged tissues [9]. According to the conducted studies it was established that the course of burn injury in rats is accompanied by a significant and prolonged production of proinflammatory cytokines – IL-1β, IL-8, TNF-α. This may be an important mechanism, which keeps the burn wound in state of persistent inflammation and prevents from normal healing. The application of methyluracyl and thiotriazoline ointments for the treatment of burn wounds ensures the healing of the wound defect in shorter terms.

CONCLUSIONS

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

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

3. As concerns reparative activity, the 2% thiotriazoline ointment is more active than the methyluracil ointment.

REFERENCES

1. Octrovskiy N.V., Petrov V.V., Bystrova A.S., Musatskova M.V. Сравнительная оценка влияния лекарственных средств для местного лечения ран на заживление термических ожогов II-III степени в эксперименте // Fundamental Research. – 2014. – No.63. – P 512-515.

2. Abaev Yu.K. Surgeon’s Reference Book. Wounds and Wound Infection / Yu.K. Abaev. – Rostov-na-Donu: Fenix, 2006. – 427 p.

3. Grinevich Yu.A. Immune and Cytogenetic Effects of Dense and Rare Ionizing Radiation: Monograph / Yu.A. Grinevich. E.A. Demina [edited by A.A. Yerilin]. – K.: Zdorovja. 2006. – 200 p.

4. Vladimirov I.V., Cherdantsev D.V. Современные возможности улучшения результатов лечения ожоговых ран // Current Problems of Science and Education. – 2014. – No.4. URL: https://www.science-education.ru/ru/article/view?id=14160.

5. Kochkina A.V. Влияние пероксиредоксина 6 и паракринных факторов мезенхимальных стволовых клеток на цитокиновый профиль кожи крысы при химическом ожоге // New Medical Technologies Bulletin. – 2017. – No.1. Volume 24. – P. 65–70.

6. Simbirtsev A.S. Цитокиновая система регуляции защитных реакций организма // Cytokines and Inflammation. – 2002. – V. 1, No. 1. – P. 9–16.

7. Khalin I.V. Pharmacotherapeutic Efficacy of Cerebrolysin and Prose in Chronic Wound Process (Experimental Study): Author's Abstract of Thesis for Academic Degree of Candidate of Medicine: spec. 14.03.05 “Pharmacology” / I.V. Khalin. – K.:2008. – 21 p.

8. Patent No.54891 Ukraine, МПК G09B 23/28 (2006.01) Device for Modeling of Thermal Burns / Zviagintseva T.V., Kryvoshapka O.V.; Patentee is Kharkiv National Medical University – No. u201006802; published on 25 November 2010 Bulletin No.22 / 2010.

9. Imasheva A.K., Lazko M.V. Особенности регенераторных процессов кожи при термических ожогах // Fundamental Research. – 2009. – No. 5. – P. 22–24.

10. Zubov D.A., Oksimets V.M. Цитокиновая иммунорегуляция репаративной регенерации костной ткани культивированными мезенхимальными стволовыми клетками // Wound. – 2008. – V.9, No. 2. URL: http://www.dniito.org.ua/pages/journals/Trauma.html

**DYNAMICS OF CYTOKINE BLOOD PROFILE AFFECTED BY VULNERARY MEDICINAL PRODUCTS WITH DIFFERENT MECHANISMS OF ACTION IN THE EXPERIMENT**

***T.I. Iermolenko, A.V. Krivoshapka, O.I. Pautina***

Kharkiv National Medical University

*Keywords:* thermal skin burn; proinflammatory cytokines; methyluracil ointment; thiotriazoline ointment; healing

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

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

**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, ointment methyluracil 10 % and ointment tiotriazolin 2 % was used as wound-healing agents.

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

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

**ДИНАМИКА ЦИТОКИНОВОГО ПРОФИЛЯ КРОВИ ПОД ВЛИЯНИЕМ РАНОЗАЖИВЛЯЮЩИХ ЛЕКАРСТВЕННЫХ СРЕДСТВ**

**С РАЗНЫМИ МЕХАНИЗМАМИ ДЕЙСТВИЯ В ЭКСПЕРИМЕНТЕ**

***Т.И. Ермоленко, А.В. Кривошапка, Е.И. Паутина***

***Харьковский национальный медицинский университет***

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

**Актуальность.** Заживление ран в значительной степени зависит от уровня провоспалительных цитокинов в очаге. Именно экспрессия ИЛ-1β, ИЛ-8,

ФНО-α удерживает течение раневого процесса в стадии персистирующего воспаления. Влияя на механизмы цитокиновой регуляции репаративной регенерации тканей 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 метилурациловая 10 % и мазь тиотриазолина 2 %.

**Результаты и их обсуждение.** Экспериментально установлено, что уровень цитокинов ИЛ-1β, 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.

**ДИНАМІКА ЦИТОКІНОВОГО ПРОФІЛЮ КРОВІ ПІД ВПЛИВОМ РАНОЗАГОЮВАЛЬНИХ ЛІКАРСЬКИХ ЗАСОБІВ З РІЗНИМИ МЕХАНІЗМАМИ ДІЇ В ЕКСПЕРИМЕНТІ**

***Т.І. Єрмоленко, О.В. Кривошапка, О.І. Паутіна***

***Харківський національний медичний університет***

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

**Актуальність.** Загоєння ран значною мірою залежить від рівня прозапальних цитокінів у вогнищі. Саме експресія ІЛ-1β, ІЛ-8, ФНО-α утримує течію раньового процесу у стадії персистуючого запалення. Впливаючи на механізми цитокінової регуляції репаративної регенерації тканин можливо 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 в сироватці крові щурів в динаміці розвитку термічного опіку.

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

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

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

**Відомості про авторів:**

Єрмоленко Т. І.

д. фарм. наук, професор, зав. кафедри фармакології та медичної рецептури

Харківського національного медичного університету

E-mail: ermolenko\_tamara65@ukr.net ORCID – <http://orcid.org/0000-0002-7775-0147>

Кривошапка О. В.

к. мед. наук., ст. викладач кафедри фармакології та медичної рецептури

Харківського національного медичного університету

E-mail: doctorkrivoshapka@ukr.net ORCID – https://orcid.org/0000-0002-4151-7669

Паутіна О. І.

асистент кафедри фармакології та медичної рецептури Харківського національного медичного університету

E-mail: pautinka70@ukr.net ORCID – https://orcid.org/0000-0002-4717-0264

**Information about authors**

Iermolenko T.I.

(Pharm. D) – Professor, Head of the Department of Pharmacology and Prescription writing, Kharkiv National Medical University

E-mail: ermolenko\_tamara65@ukr.net ORCID – <http://orcid.org/0000-0002-7775-0147>

Kryvoshapka O.V.

(PhD) – Assistant at the Department of Pharmacology and Prescription writing, Kharkiv National Medical University

E-mail: doctorkrivoshapka@ukr.net ORCID – https://orcid.org/0000-0002-4151-7669

Pautina O. I.

Assistant at the Department of Pharmacology and Prescription writing, Kharkiv National Medical University

E-mail: pautinka70@ukr.net ORCID – <https://orcid.org/0000-0002-4717-0264>

**Сведения об авторах**

Ермоленко Т. И.

д. фарм. наук, профессор, зав. кафедры фармакологии и медицинской

рецептуры Харьковского национального медицинского университета

E-mail: ermolenko\_tamara65@ukr.net ORCID – <http://orcid.org/0000-0002-7775-0147>

Кривошапка А. В.

к.мед.наук, ст. преподаватель кафедры фармакологии и медицинской

рецептуры Харьковского национального медицинского университета

E-mail: doctorkrivoshapka@ukr.net ORCID – https://orcid.org/0000-0002-4151-7669

Паутина Е. И.

асистент кафедры фармакологии и медицинской

рецептуры Харьковского национального медицинского университета

E-mail: pautinka70@ukr.net ORCID – <https://orcid.org/0000-0002-4717-0264>

**Address for correspondence:**

4, Nauky boul., Kharkiv, 61022, Ukraine

Tel. (57) 702-72-01

Е-mail: farmacologia@ukr.net

Kharkiv National Мedical University