DOI 10.26724/2079-8334-2021-4-78-219-224
UDC 618.311.2:616.314.16/17-08:615.27:616-008.9

L.S. Kravchenko, O.L. Appelhans, A.E. Poliakov, O.V. Pasechnyk, I.Yu. Borysiuk, O.V. Honcharenko, Ya.I. Ivanova
Odessa National Medical University, Odessa

EVALUATION OF THE TREATMENT-AND-PROPHYLACTIC COMPLEX EFFICACY DURING INDUCED METABOLIC SYNDROME

e-mail: lyudmila.Kravchenko.52@gmail.com

The efficacy of the treatment-and-prophylactic complex, which includes phytopreparation based on the dihydroquercetin bioflavonoid – a natural capillary protector, an antioxidant that by its pharmacological action belongs to the group of angioprotectors and a microcirculation corrector, used per os, in combination with local therapy of periodontal tissues with a new dental elixir based on propolis and biologically active substances of plant origin adaptogens with ultraphonophoresis was determined in experiment on 48 white male Wistar rats, aged 2–3 months, weighing (238±10) g. Under induced metabolic syndrome in rats, the use of the treatment-and-prophylactic complex neutralized the negative effects of the components of the pathological condition in the form of improved metabolism with normalization of glucose, triglycerides, cholesterol, anti-inflammatory interleukins level in the blood serum, improved liver condition and periodontal tissues. The treatment-and-prophylactic complex on the background of MS simulation rendered a periodontal-protective effect and had positive influence on systems of antioxidant defence and nonspecific resistance both at the systemic level and in the periodontal tissues.

Key words: metabolic syndrome, prevention, inflammation, antioxidant protection, periodontium.

Л.С. Кравченко, О.Л. Аппельханс, А.Є. Поляков, О.В. Пасечник, І.Ю. Борисюк, О.В. Гончаренко, Я.І. Іванова

ОЦІНКА ЕФЕКТИВНОСТІ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНОГО КОМПЛЕКСУ ПРИ МОДЕЛЮВАННІ МЕТАБОЛІЧНОГО СИНДРОМУ

В експерименті на 48 білих щурах-самцях лінії Вістар, віком 2–3 місяці, масою тіла (238±10) г визначена ефективність розробленого лікувально профілактичного комплексу, який включає фітопрепарат на основі біофлавоноїду дігідрокверцетіну – природного капілляропротектора, антиоксиданта, що за своєю фармакологічною дією відноситься до групи ангіопротекторів і коректора мікроциркуляції, застосований per os , у сполученні із місцевою терапією тканин пародонту авторським зубним еліксиром на основі прополісу і біологічно активних речовин адаптогенів рослинного походження із ультрафонофорезом. В умовах моделювання метаболічного синдрому у щурів застосування лікувально-профілактичного комплексу нівелювало негативні наслідки дії компонентів патологічного стану у вигляді покращення обміну речовин з нормалізацією рівнів глюкози, тригліцеридів, холестерину, протизапальних інтерлейкінів у сироватці крові, поліпшення функціонального стану печінки та тканин пародонту. Визначена пародонтопротекторна дія лікувально-профілактичного комплексу на тлі відтворення метаболічного синдрому, позитивний вплив на системи антиоксидантного захисту і неспецифічної резистентності як на системному рівні, так і в тканинах пародонту.

Ключові слова: метаболічний синдром, профілактика, запалення, антиоксидантний захист, пародонт.

The work is a fragment of the scientific work "Development of new therapeutic and prophylactic agents and a pathogenetic justification of their use in inflammatory periodontal diseases against a background of metabolic syndrome", state registration No. 0120U002197.

According to etiological and pathogenetic factors, periodontal pathology can be referred to heterogeneous diseases, the etiology and pathogenesis of which is influenced by a complex of pathological changes in the body. The presence of a somatic pathology blunts the body defences and predisposes to oral tissues diseases.

Analysis of literature data related to the scientific research in the treatment of periodontal diseases shows that the concomitant pathology of the organism and in particular the metabolic syndrome (MS) [11, 13] was not always taken into account. Successful treatment of periodontal diseases requires a comprehensive approach, which involves local therapy along with the mandatory treatment of general somatic pathology. The metabolic syndrome is considered the main cause of inflammatory and dystrophic-inflammatory processes in the tissues of the oral cavity, and the low efficiency of prevention and treatment of inflammatory diseases of the periodontium, oral mucosa under comorbide conditions is explained by underestimation of disorders in MS that affect their occurrence and clinical course [7, 14]. Taking into account the above-said, the study of changes in the state of periodontal tissues at the initial stage of MS for timely prevention and successful pathogenetic therapy is of scientific interest. The signs of the development of inflammatory periodontal diseases on the background of MS require further improvement of prevention and treatment of complications using an appropriate treatment-and-prophylactic complex, including anti-inflammatory, detoxicative agents that lower cholesterol, regulate lipid exchange and improve metabolism.

The purpose of the experiment was to evaluate in the experiment the effectiveness of the developed treatment-and-prophylactic complex for the prevention of periodontal tissue disorders under metabolic syndrome simulation.

Material and methods. The experiment was performed on 48 white Wistar rats aged 2–3 months, weighing 238±10 g, obtained in the vivarium of the Odessa National Medical University. The animals were studied in accordance with the requirements presented in the international guidelines for biomedical research with experimental animals, as well as in accordance with standards the Council of Europe Bioethics Convention 1997, the European Convention for the Protection of Vertebrate Animals. All animals were divided into 5 groups: I – an intact group consisted of 8 rats. The animals in this group received a standard vivarium diet and had free access to drinking water. The rats of group II were simulated MS by introducing 20 % internal lard into the diet, and 10 % fructose solution was used instead of drinking water. The duration of MS simulation was 70 days. The introduction of these substances into the diet were explained by a possibility of creating disorders of metabolism of carbohydrates and lipids of an alimentary nature in the body of animals typical for MS manifestations [1, 6]. The rats of group III on the background of MS simulation, in a week after the start, 5 times a week in the morning were per-orally administered a "Capillaroprotect" aqueous solution (bioflavonoid, antioxidant) produced by "Ekosvit Oil" (Ukraine) at a rate of 135 mg/kg. The rats of group IV, under similar conditions receiving the preparation based on the dihydroquercetin were locally applied on gums a new dental elixir based on bee products and adaptogens of plant origin by 0.5 ml/rat with a tampon, which was held for 5–7 minutes [4].

The rats of group V during MS simulation from the second week were used the preparation based on the dihydroquercetin, the dental elixir topically on gums and physiotherapy. In 70 days after the beginning of the experiment, the animals were removed under the thiopental narcosis (40 mg/kg) by dissection of the major vessels, the blood from which the blood serum was obtained was collected, the gums were cut off, the liver was removed after its preliminary perfusion with chilled saline. The blood serum level of triglycerides (TG) was determined using the enzymatic colorimetric test [3], general cholesterol (Ch) [3], Ch in high-density lipoproteins (HDL) [3], glucose [3], alanine aminotransferase (ALT), aspartate aminotransferase (AST) activity [3].

In the liver and gums tissues, the level of lipid peroxidation (LPO) was determined by the level of malonic dialdehyde (MDA) using the thiobarbituric method [3] and the state of the antioxidant system – by catalase activity [3], the level of antioxidant-prooxidant index (API). Biochemical markers of inflammation were revealed: elastase activity [5], urease activity [5], acid phosphatase [5], insulin resistance – triglycerides level [3], non-specific immunity – lysozyme activity [5].

Statistical processing of digital data was performed using the software STATISTICA 6.0. The values of the arithmetic mean (M) and the mean error (m) were calculated for all indicators. The significance of the difference in values was determined using t-Student test. The changes were considered statistically significant at $p<0.05$.

Results of the study and their discussion. Experimental studies of the treatment-and-prophylactic complex effect on the condition of liver tissues, gums of rats under MS simulation demonstrated a positive effect of biochemical processes of animals. In the serum of animals of the experimental group during MS simulation in 10 weeks, there was a significantly higher concentration of total cholesterol 6.24±0.05 mmol/l and triglycerides 2.18±0.02 mmol/l compared with the intact group (respectively, 4.86±0.04 mmol/l and 1.38±0.02 mmol/l ($p<0.001$)). At the same time, the level of cholesterol in high-density lipoproteins during MS simulation decreased 3 times. Serum glucose in animals with MS was 2.4 times ($p<0.001$) higher than in intact rats. The functional disorders in the liver of rats during MS simulation was proved by a significant increase in the serum transaminases activity. So, ALT activity increased 2.2 times ($p=0.001$), ACT activity – 1.7 times ($p=0.001$) relative to the data in the intact group. Under the action of the dihydroquercetin preparation the main blood serum parameters of rats decreased, which characterize the manifestations of experimental MS – triglycerides level – by 24.8 % and total cholesterol – by 14 %. The level of HDL cholesterol was determined to be 2 times increased, but did not reach the level of the intact group. The level of glucose in the blood serum of rats under the influence of the drug was reduced 2 times, reaching the values of the intact group. The dihydroquercetin preparation improved the functional state of the rats' liver – the activity of ALT in the blood serum was reduced 1.5 times, AST – 1.3 times as compared to rats with MS model.

Under the conditions of MS modeling, LPO processes were activated – the MDA level in the serum of rats increased on average from 2.87±0.70 mmol/l to 4.20±0.50 mmol/l, ie 2 times, in liver tissues from 3.86±0.11 mmol/kg to 7.12±0.60 mmol/kg – 1.8 times, in oral mucosa from 4.64±0.50 mmol/kg to 6.16±0.50 mmol/kg – 1.3 times ($p=0.001$). At the same time, there was a decrease in the activity of

antioxidant protection by the functional activity of the enzyme catalase, having a tendency to decrease by an average of 8–11 % ($p<0.05$). Per os introduction of the dihydroquercetin preparation against a background of MS simulation inhibited increase in MDA, triglycerides level in the liver, prevented decrease in lysozyme and catalase activity.

A more pronounced positive effect on the studied parameters in the tissues of the gums of rats with MS simulation had a combined usage with per os the dihydroquercetin preparation of the new dental elixir with ultraphonophoresis (table 1).

Table 1

The effect of the prophylactic complex on the indicators of microbiocenosis, nonspecific resistance, antioxidant-prooxidant system, insulin resistance, inflammation in the gums of rats during the development of metabolic syndrome (M±m)

Indicators	Investigation groups				
	Intact group n=8	MS model, n=10	MS model + dihydroquercetin preparation, n=10	MS model + dihydroquercetin preparation + dental elixir, n=10	MS model + dihydroquercetin preparation +dental elixir +physiotherapeutic procedure, n=10
Urease activity, μ-cat/kg	0.82±0.06	1.78±0.10	1.18±0.12	1.06±0.10	0.92±0.08
p		<0.05	<0.05	<0.05	>0.05
p_1			<0.05	<0.05	<0.05
Lysozyme activity, units/kg	386±19	168±12	296±15	330±12	342±14
p		<0.05	<0.05	<0.05	>0.05
p_1			<0.05	<0.05	<0.05
Elastase activity, μ-cat/kg	28.3±1.3	39.6±1.8	30.6±1.4	28.8±1.2	27.9±1.5
p		<0.05	<0.05	>0.05	>0.05
p_1			<0.05	<0.05	<0.05
Acid phosphatase activity, ncat/g	14.8±0.40	24.6±0.60	20.2±0.50	16.8±0.49	15.2±0.58
p		<0.05	<0.05	<0.05	>0.05
p_1			<0.05	<0.05	<0.05
Catalase activity, μ-cat/kg	9.12±0.10	7.70±0.40	8.42±0.11	8.82±0.12	8.99±0.10
p		<0.05	<0.05	>0.05	>0.05
p_1			>0.05	<0.05	<0.05
MDA level, mmol/kg	8.64±0.40	12.60±0.90	10.12±0.70	8.97±0.80	8.88±0.60
p		<0.05	>0.05	>0.05	>0.05
p_1			<0.05	<0.05	<0.05

Notes: 1. p – the probability of difference relative to the intact group; 2. p_1 – the probability of difference relative to the group with metabolic syndrome.

According to the obtained data, the simulation of MS resulted in metabolic processes disorders in the tissues of rats' gums: an increase in elastase activity by 63.5 % ($p=0.001$), an increase in MDA by 45.8 % ($p=0.001$), an increase in urease activity by 65.8 % ($p=0.001$), triglyceride level by 133.4 % ($p=0.001$) with a decrease in catalase activity by 15.6 % ($p=0.001$) and lysozyme activity by 57.0 % ($p=0.001$). The conducted biochemical analysis determined an increase in contamination of the opportunistic pathogen, LPO activation and inflammation against a background of reduced nonspecific and antioxidant protection of gums tissues during the development of metabolic syndrome.

The use of the prophylactic complex effectively prevented the MS simulated disorders. The use of per os the dihydroquercetin preparation in the morning with local application to the tissues of the periodontal mucosa of the dental elixir by applying a wet swab in the amount of 0.5 ml with simultaneous exposure to ultraphonophoresis at a frequency 830 kHz, intensity 0.4 W/cm^2 in a pulse mode for 5 minutes, once a day, five times a week inhibited the occurrence of periodontal complications during the period of MS simulation. Pathogenic microflora reduction was determined in the gums tissues (almost 2 times reduction of urease activity) ($p<0.05$), the inflammatory process (1.4 times decrease in elastase activity ($p=0.001$), 1.4 times – MDA level ($p<0.05$), 1.6 times – acid phosphatase activity ($p<0.05$). Under conditions of MS simulation, the treatment-and-prophylactic complex led to 1.8 times decrease in triglycerides, had antioxidant properties, keeping on a high level of non-specific protection – catalase activity, API index and lysozyme activity. The activity of lysozyme in the gums of rats with the prophylactic use of the complex 2 times exceeded the value in rats with MS, the activity of catalase, API in the gums were near those in intact animals.

Changes in the level of pro- and anti-inflammatory cytokines in animals with simulation of MS and the comparative effect of the prophylactic complex on them were studied (table 2).

Table 2

Influence of the prophylactic complex on indicators of the cytokine status of animals at induced metabolic syndrome (M±m)

Indices	Groups of animals		
	Intact, n=10	Metabolic syndrome, n=10	Metabolic syndrome + preventive complex, n=10
IL-2, pg/ml p p_1	4.10±0.10	5.86±0.20 =0.001	4.52±0.20 >0.05 =0.001
IL-6, pg/ml p p_1	0.30±0.08	1.42±0.30 =0.001	0.58±0.10 >0.05 =0.005
IL-4, pg/ml p p_1	0.38±0.07	0.18±0.06 <0.05	0.34±0.08 >0.05 <0.05
IL-10, pg/ml p p_1	1.20±0.24	0.42±0.06 =0.005	0.98±0.08 >0.05 =0.001

Notes: 1. p – the probability of difference relative to the intact group; 2. p_1 – the probability of difference relative to the group with metabolic syndrome.

The level of interleukin IL-2 in animals of the intact group was 4.10 pg/ml, while this figure in rats with a simulated MS increased 1.4 times to 5.86 pg/ml. When using the prophylactic complex on the background of MS simulation, the level of IL-2 decreased by 28.9 % to 4.52 pg/ml, slightly exceeding the level of the intact group.

The level of pro-inflammatory interleukin IL-6 in the blood serum of rats with MS increased 4.4 times. The prophylactic complex caused 2.4 times decrease in IL-6 level ($p<0.05$).

The average level of anti-inflammatory IL-4 in rats with MS simulation decreased 2.1 times as compared with intact animals. In the course of preventive measures against the background of MS simulation, the studied indicator in rats increased on average 1.9 times and did not differ significantly from intact animals.

Anti-inflammatory IL-10 in rats with MS decreased 2.8 times as compared with intact animals ($p<0.05$). When using the prophylactic complex, its level with MS simulation in rats increased 2.3 times.

So, with the development of MS in the blood serum of animals, the level of pro-inflammatory increased and the level of anti-inflammatory interleukins decreased. The literature data gives contradictory data on the dynamics of changes in the level of pro- and anti-inflammatory cytokines [8, 10, 12]. The introduction of the components of the prophylactic complex in the scheme of MS simulation gives a positive effect confirmed by improvement of indicators.

The obtained data indicate that in animals against a background of MS simulation, cellular mechanisms of cytokine regulation are disrupted, which can be both a consequence and a cause of metabolic, humoral and structural disorders. The use of the treatment-and-prophylactic complex gave a positive effect in general. The difference with untreated animals in increasing the level of anti-inflammatory interleukins and decreasing the level of pro-inflammatory interleukins when using the treatment and prophylactic complex was significant.

The obtained results prove that under conditions of experimental MS simulation with a diet rich in saturated fats and simple carbohydrates there are systemic disorders in the body: reduced nonspecific antimicrobial protection (lysozyme activity), increased microbial contamination (urease activity), intensification of lipid peroxidation (MDA level), the development of inflammation (elastase activity) and hepatotoxicity (ALT activity). In the liver of rats, simulation of pathology caused activation of LPO, excessive accumulation of fat (triglycerides) and a decrease in nonspecific resistance (lysozyme activity). These data confirm the results of other authors on the development of metabolic syndrome with a high-calorie diet and the significant role of LPO processes in lysosome damage with the release of lysosomal enzymes [8, 9, 15]. It is known that lysosomes accumulating LPO products are damaged by releasing enzymes, causing the destruction of subcellular structures [6, 9]. At the same time there is increase in pathogenic microflora in gums, development of inflammation against a background of decrease in nonspecific immunity and activity of antioxidant protection. Our data are proved by researches of scientists. [1, 2, 9].

Prophylactic administration of the proposed dihydroquercetin preparation to animals in the process of simulation of MS significantly inhibits the established disorders, positively affecting the biochemical parameters of the blood serum, liver tissue, periodontium, reducing triglycerides, total cholesterol, glucose

level, restoring the state of non-specific resistance, lipid metabolism, preventing inflammation and hepatosis, as well as contamination with pathogenic microflora.

The capillary-protective action of the applied drug is realized in 2 directions: indirectly and directly. The indirect way is associated with the prevention of atherosclerotic plaques formation in the arteries by reducing the level of cholesterol and triglycerides in the blood, as well as inhibiting the inflammatory reaction in the vascular wall. The direct mechanism is related to the protection of the most important chain of the vascular system – capillaries, through which there is an exchange of oxygen and nutrients between blood and cells. The drug which contains dihydroquercetin protects cell membranes, normalizes their permeability and elasticity, improves capillary function, restores blood microcirculation, normalizes metabolism at the cellular level. It is known that by improving microcirculation with increasing reserve capacity of the capillary bed, the agent reduces trophic disorders [2, 7, 14]. The experiment revealed an increase in cell sensitivity to insulin under the dihydroquercetin preparation action. Biologically active components of the drug have vasodilatory, antihypoxic, anti-edematous properties that contributes to the mechanism of its anti-inflammatory action, improves cellular metabolism, stimulates the immune system, activates regenerative processes.

A combined topical application of the new dental elixir based on propolis and biologically active substances of plant origin adaptogens with ultraphonophoresis on the oral mucosa areas in combination with the dihydroquercetin preparation provides rapid arrest of inflammation, anti-edema effect, correction and improvement of structural and functional condition of periodontal tissues.

Conclusions

1. The research have shown that the proposed treatment-and-prophylactic complex, which includes the the dihydroquercetin preparation, used per os in combination with local therapy of periodontal tissues with a tooth elixir based on propolis and biologically active substances of plant origin adaptogens with ultraphonophoresis under induced metabolic syndrome significantly removed the negative effects of its most important components in the form of normalization of glucose level, triglycerides, total cholesterol, anti-inflammatory interleukins in the blood serum, improving the functional state of the liver and periodontal tissues.

2. A regular administration of the the dihydroquercetin preparation against a background of MS simulation prevented the nonspecific immunity decrease in the liver of rats (lysozyme activity), antioxidant protection (catalase activity) and inhibited the increase in triglycerides, MDA level.

3. A topical application of the treatment-and-prophylactic complex in the process of MS simulation in rats led to a positive effect on the biochemical parameters of periodontal tissues, significantly reducing triglycerides level, providing anti-inflammatory and antioxidant effects, activating local functional activity of antioxidant enzymes, nonspecific resistance.

4. The created treatment-and-prophylactic complex at MS simulation improved metabolism, reduced signs of oral cavity inflammation, demonstrating periodontal-protective properties, that allows to offer it for clinical approbation in patients with MS for the purpose of prevention of periodontal diseases.

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Стаття надійшла 18.12.2020 р.

DOI 10.26724/2079-8334-2021-4-78-224-228
UDC 616.136.6-091.8-02:616-001.17]-092.9

V.V. Kulbitska, Z.M. Nebesna, S.B. Kramar, I.B. Hetmaniuk
Ivan Horbachevsky Ternopil National Medical University, Ternopil

SUBMICROSCOPIC CHANGES OF THE HEMOCAPILLARIES IN THE ADRENAL GLAND CORTEX IN DYNAMICS AFTER EXPERIMENTAL THERMAL INJURY

e-mail: kulbitska@tdmu.edu.ua

The purpose of the study was to establish submicroscopic changes in the hemocapillaries of the adrenal gland of white rats in the dynamics after experimental thermal injury. The experiment was performed on 36 mature white male rats. Burns of 2B degree were simulated under thiopental-sodium anesthesia. Submicroscopic changes were studied on days 1st, 7th, 14th and 21st from the beginning of the experiment. Small pieces of adrenal cortex were taken for electron microscopic examination. Tissue processing was performed according to generally accepted methods. Ultrathin sections were studied in an electron microscope PEM-125K. In the early period after experimental thermal trauma there are initial destructive changes in the components of the blood capillaries wall of the organ, which were overfilled with blood, there were manifestations of excessive edema, destruction of the cytoplasm in the endothelial cells and pericytes, deformation of nuclei, uneven thickening of the basement membrane. In the late post-burn period, significant destructive-degenerative changes in the wall of hemocapillaries of the adrenal cortex were found. They were accompanied by uneven thickening and homogenization of the basement membrane, pyknosis of nuclei, destruction and fragmentation of organelles in the cytoplasm of endothelial cells, violation of fenestration and disappearance of micropinocytic vesicles, which lead to insufficiency of transendothelial metabolism in the body.

Key words: adrenal gland, hemocapillary ultrastructure, experimental burn injury.

В.В. Кульбіцька, З.М. Небесна, С.Б. Крамар, І.Б. Гетманюк

СУБМІКРОСКОПІЧНІ ЗМІНИ ГЕМОКАПІЛЯРІВ КОРИ НАДНИРКОВИХ ЗАЛОЗ У ДИНАМІЦІ ПІСЛЯ ЕКСПЕРИМЕНТАЛЬНОЇ ТЕРМІЧНОЇ ТРАВМИ

Метою даного дослідження було встановити субмікроскопічні зміни гемокапілярів надниркових залоз білих щурів в динаміці після експериментальної термічної травми. Експеримент проведено на 36 статевозрілих білих щурах-самцях. Опік IIб ступеня моделювали під тіопентал-натрієвим наркозом. Субмікроскопічні зміни вивчали на 1, 7, 14 та 21 добу від початку експерименту. Для електронномікроскопічного дослідження забирали невеликі шматочки кори надниркових залоз. Обробку тканин проводили згідно загальноприйнятих методик. Ультратонкі зрізи вивчали в електронному мікроскопі ПЕМ-125К. В ранні терміни після експериментальної термічної травми відбуваються початкові деструктивні зміни складових елементів стінки кровоносних капілярів органу, що проявлялось їх надмірним кровонаповненням, набряком та деструкцією цитоплазми ендотеліоцитів, перицитів, деформацією ядер, нерівномірним потовщенням базальної мембрани. В пізні терміни після опіку, встановлено значні деструктивно-дегенеративні зміни в стінці гемокапілярів кори надниркових залоз, що супроводжувалось нерівномірним потовщенням та гомогенізацією базальної мембрани, пікнозом ядер, деструкцією і фрагментацією органел в цитоплазмі ендотеліоцитів, порушенням фенестрації та зникненням мікропіноцитозних пухирців, що призводить до недостатності трансендотеліального обміну в органі.

Ключові слова: надниркова залоза,ультраструктура гемокапіляра, експериментальна опікова травма.

The work is a fragment of the research project "Peculiarities of structural reorganization of the nervous, digestive, endocrine systems, hematopoietic and immune organs under conditions of thermal injury and the use of corrective factors", state registration No 0120U104152.

Burn injuries are common in everyday life and at work, as well as in the modern military conflicts. According to the WHO, the number of victims of thermal burns is constantly increasing, and the consequences of such injuries are a serious medical and economic problem for all countries [1, 11]. Deep burns cause destructive changes in the skin and significant morphological and functional changes in all organs and systems of the body [7]. The most common cause of death in burn patients are burn shock and intoxication, the source of which is a burn wound. From it toxic substances spread