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STUDY OF THE INTENSITY OF MICROBIAL CONTAMINATION AND NONSPECIFIC IMMUNITY UNDER THE EXPERIMENTAL DIABETES AFTER USING DIFFERENT WAYS OF FIXING THE WOUND EDGES

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Abstract

Diabetes mellitus and its complications are one of the most important causes of death. As a consequence of hyperglycemia in patients with diabetes there is an increased risk of concomitant diseases, and one of the important consequences of diabetes is impaired ability to repair. Healing disorders in diabetes are the result of complex pathophysiology involving vascular, neuropathic, immune and biochemical components

Aim of research: was to analyze changes in inflammation markers, microbial contamination and nonspecific immunity in skin homogenate of rats with diabetes after using sutures and skin glue.

Material and research methods. Experimental studies were performed on 130 white outbred adult male rats, weight from 240 to 320 g. Diabetes mellitus was induced by using streptozotocin (Sigma, USA) (intraperitoneally - 65 mg / kg) with previous (15 minutes) injection of nicotinamide (intraperitoneally - 230 mg / kg). On the background of obesity, which was caused by 4 weeks of keeping animals on a high-fat diet. The development of diabetes mellitus 2 was confirmed by determining the concentration of glucose in the blood using a glucometer BAYER Contour Next (Germany). Animals of all groups (I-IV) under thiopental anesthesia (40 mg / kg body weight of rats) were made full-layer rectilinear

incisions, 2 cm long, in the anterior-lateral region of the abdomen. All animals were divided into 4 experimental groups: I group (30 rats) – healthy rats, wound edges were sutured with Vicryl 5/0 thread (ETHICON, Inc. and Johnson & Johnson company (USA); II group (30 rats) – healthy rats, fixing of the wound edges was performed by applying skin glue Dermabond (ETHICON, Inc. and Johnson & Johnson company (USA)); III group (30 rats) – rats with diabetes mellitus, wound edges were sutured with Vicryl 5/0 thread; IV group (30 rats) – Rats with diabetes mellitus, fixing of the wound edges was performed by applying skin glue Dermabond. For control, all the results were compared with those of intact animals (10 rats). Animals were removed from the experiment on 3, 7, 28 days after surgery under thiopental anesthesia (90 mg / kg body weight of rats).

The degree of contamination of the wound with opportunistic and pathogenic microflora determined by the level of urease activity using Nessler's reagent. Lysozyme activity was determined by bacteriolytic method, using as a substrate a suspension of bacteria Micrococcus lysodeicticus.

Results. The obtained results prove that the wound process under conditions of streptozotocin diabetes mellitus in rats leads to a significantly greater contamination of the wound with pathogenic and opportunistic microflora and a decrease in nonspecific immunity, compared with healthy animals. Also, different effects of suture materials on the indicators of microbial contamination and immune protection were found: significantly larger deviations from the norm were found in groups of rats, where we used surgical sutures to fix the wound edges. When comparing the intensity of microbial contamination and nonspecific immunity in the long term (28 days) in the homogenate of the skin of rats with diabetes mellitus and the use of skin glue, the level of urease was 27.3% lower and the level of lysozyme 7.0% lower than in III group where we used surgical sutures.

Changes in the activity of the antimicrobial enzyme lysozyme allow to assess the state of nonspecific immunity in the studied tissues. Many authors note a decrease in lysozyme activity in various bioliquids and tissues in diabetes mellitus. The results of our studies prove a significant decrease in the activity of lysozyme in the homogenate of postoperative skin wounds of rats with diabetes.

Conclusion. Wound process in rats with experimental diabetes mellitus leads to reliable contamination of the wound with pathogenic and opportunistic microflora, as evidenced by increased urease activity, and reduced nonspecific immunity, characterized by reduced lysozyme activity in the homogenate of postoperative wound/scar tissue in relation to intact animals during all the terms of observation.

In the remote period (28 days) in the skin homogenate of rats with diabetes where skin glue was used for wound closure, the level of urease was 27.3% lower and the level of lysozyme was 7.0% lower than that of rats with diabetes, where surgical sutures were used.

Key words: streptozotocin diabetes; wound healing; scars; lysozyme and urease activity

ИССЛЕДОВАНИЕ ИНТЕНСИВНОСТИ МИКРОБНОЙ КОНТАМИНАЦИИ И НЕСПЕЦИФИЧЕСКОГО ИММУНИТЕТА ПРИ РАЗЛИЧНЫХ СПОСОБАХ ФИКСИРОВАНИЯ КРАЕВ РАНЫ НА ФОНЕ САХАРНОГО ДИАБЕТА

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Сахарный диабет и его осложнения – одна из важнейших причин смерти. Вследствие гипергликемии у пациентов с диабетом повышается риск сопутствующих заболеваний, и одним из важных последствий диабета является нарушение способности к восстановлению. Нарушения заживления при диабете являются результатом сложной патофизиологии, включающей сосудистые, невропатические, иммунные и биохимические компоненты.

Цель исследования: проанализировать изменение маркеров воспаления, микробной контаминации и неспецифического иммунитета в гомогенате кожи крыс с сахарным диабетом после наложения швов и кожного клея.

Материал и методы исследования. Экспериментальные исследования проведены на 130 белых беспородных взрослых крысах-самцах массой от 240 до 320 г. Сахарный диабет моделировали стрептозотоцином (Sigma, США) (внутрибрюшинно - 65 мг / кг) с предшествующим (15 минут) введением никотинамида (внутрибрюшинно - 230 мг / кг). На фоне ожирения, вызванного 4 недельным содержанием животных на высокожировой диете. Развитие сахарного диабета 2 подтверждено определением концентрации глюкозы в крови на глюкометре BAYER Contour Next (Германия). Животным всех групп (I – IV) под тиопентальным наркозом (40 мг / кг массы тела крыс) выполняли полнослойные прямолинейные разрезы длиной 2 см в переднебоковой области живота. Все животные были разделены на 4 экспериментальные группы: I группа (30 крыс) – здоровые крысы, края раны зашиты нитью Викрил 5/0 (ETHICON, Inc. и компания Johnson & Johnson (США); II группа (30 крыс) – здоровые крысы, фиксацию краев раны проводили с помощью кожного клея

Dermabond (ETHICON, Inc. и Johnson & Johnson company (США)); III группа (30 крыс) – крысы с сахарным диабетом, края раны зашивали Викрилом 5 / 0; IV группа (30 крыс) – крысы с сахарным диабетом, фиксацию краев раны проводили с помощью кожного клея Dermabond. Для контроля все результаты сравнивали с результатами интактных животных (10 крыс). Животных выводили из эксперимента на 3, 7, 28 сутки после операции под тиопентальным наркозом (90 мг / кг массы тела крыс).

Степень заражения раны условно-патогенной микрофлорой определяли по уровню активности уреазы с помощью реактива Несслера. Активность лизоцима определяли бактериолитическим методом, используя в качестве субстрата суспензию бактерий Micrococcus lysodeicticus.

Результаты. Полученные результаты доказывают, что раневой процесс в условиях стрептозотоцинового сахарного диабета у крыс приводит к значительно большему загрязнению раны патогенной и условно-патогенной микрофлорой и снижению неспецифического иммунитета по сравнению со здоровыми животными. Также обнаружено различное влияние шовных материалов на показатели микробной контаминации и иммунной защиты: значительно большие отклонения от нормы были обнаружены в группах крыс, где для фиксации краев раны использовались хирургические швы. При сравнении интенсивности микробной контаминации и неспецифического иммунитета в отдаленном периоде (28 дней) в гомогенате кожи крыс с сахарным диабетом и применении кожного клея уровень уреазы был на 27,3% ниже, а уровень лизоцима - 7,0. % ниже, чем в III группе, где использовались хирургические швы.

Изменения активности антимикробного фермента лизоцима позволяют оценить состояние неспецифического иммунитета в исследуемых тканях. Многие авторы отмечают снижение активности лизоцима в различных биожидкостях и тканях при сахарном диабете. Результаты наших исследований подтверждают достоверное снижение активности лизоцима в гомогенате послеоперационных кожных ран крыс с сахарным диабетом.

Вывод. Раневой процесс у крыс с экспериментальным сахарным диабетом приводит к достоверному заражению раны патогенной и условно-патогенной микрофлорой, о чем свидетельствует повышение активности уреазы и снижение неспецифического иммунитета, характеризующееся снижением активности лизоцима в гомогенате послеоперационной раны / рубцовой ткани по отношению к интактные животные в течение всех сроков наблюдения.

В отдаленном периоде (28 дней) в гомогенате кожи крыс с диабетом, где для закрытия ран использовали кожный клей, уровень уреазы был на 27,3% ниже, а уровень лизоцима на 7,0% ниже, чем у крыс с диабетом, где использовались хирургические швы.

Ключевые слова: стрептозотоциновый диабет; лечение раны; шрамы; активность лизоцима и уреазы

Introduction

Diabetes mellitus and its complications are one of the most important causes of death [1]. According to some authors, the presence of diabetes is associated with increased mortality from infections, cardiovascular disease, stroke, chronic kidney disease, chronic liver disease and cancer [2]. As a consequence of hyperglycemia in patients with diabetes there is an increased risk of concomitant diseases [3], and one of the important consequences of diabetes is impaired ability to repair [4]. Healing disorders in diabetes are the result of complex pathophysiology involving vascular, neuropathic, immune and biochemical components [5]. Hyperglycemia correlates with the stiffness of blood vessels, which leads to slow blood circulation and microvascular dysfunction, causing a decrease in tissue oxygenation [6]. The changes in blood vessels observed in patients with diabetes also explain the decrease in the migration of leukocytes into the wound, which becomes more vulnerable to infections [5]. Timely prevention of infectious complications provides an easier course of wound healing significantly lower chance of developing severe septic processes and scar formation [7].

Aim of research: was to analyze changes in inflammation markers, microbial contamination and nonspecific immunity in skin homogenate of rats with diabetes after using sutures and skin glue.

Materials and research methods. Experimental studies were performed on 130 white outbred adult male rats, weight from 240 to 320 g, which were kept in the same vivarium. All animals were divided into 4 experimental groups. For control, all the results were compared with those of intact animals (Table 1).

Table 1

Distribution of groups of experimental animals

Group	Observation group	Number of animals
Інтактні щурі		10
I group	Healthy rats, wound edges were sutured	30
II group	Healthy rats, fixing of the wound edges was performed by applying skin glue	30
III group	Rats with diabetes mellitus, wound edges were sutured	30
IV group	Rats with diabetes mellitus, fixing of the wound edges was performed by applying skin glue	30
Total number		130

Diabetes mellitus was induced by using streptozotocin (Sigma, USA) (intraperitoneally - 65 mg / kg) with previous (15 minutes) injection of nicotinamide (intraperitoneally - 230 mg / kg). On the background of obesity, which was caused by 4 weeks of keeping animals on a high-fat diet. The development of diabetes mellitus 2 was confirmed by determining the concentration of glucose in the blood using a glucometer BAYER Contour Next (Germany). Animals of all groups (I-IV) under thiopental anesthesia (40 mg / kg body weight of rats) were made full-layer rectilinear incisions, 2 cm long, in the anterior-lateral region of the abdomen. To fix the edges of the surgical wound, the animals of the I and III experimental groups were sutured with Vicryl 5/0 thread (ETHICON, Inc. and Johnson & Johnson company (USA). In animals of the II and IV experimental groups, the edges of the surgical wound were fixed by applying Dermabond skin glue (ETHICON, Inc. and Johnson & Johnson company (USA)). Animals were removed from the experiment on 3, 7, 28 days after surgery under thiopental anesthesia (90 mg / kg body weight of rats).

The degree of contamination of the wound with opportunistic and pathogenic microflora reflects urease - an enzyme that is absent in human and animal cells. Since urease is produced by almost a third of all bacteria, the urease method has significant advantages over sowing methods for determining microbial contamination. It is possible to determine the presence of no more than 1-3% of bacteria by using another methods [8]. In addition, the determination of urease activity by hydrolysis of urea is carried out easily and quickly using Nessler's reagent [9]. Lysozyme activity was determined by bacteriolytic method, using as a substrate a suspension of bacteria Micrococcus lysodeicticus [8].

The obtained digital material was subjected to statistical analysis using Student's t-test [10]. We calculated the arithmetic mean, the standard (M), the standard deviation (sigma Σ),

the standard error (m), the probability criteria (t). Differences were taken into account in the probability at p≤0.05. Statistical results for which the probability of error was less than 5% (P≤0.05) were considered reliable.

Findings and discussion

The degree of contamination of the wound with pathogenic and opportunistic microflora is determined by the level of urease activity. The increase in this indicator was found in all groups of experimental animals on day 3: 6.3 times in group I, 6 - in group II, 8 - in group III and 7.7 in group IV. On the 7th day after modeling the wound process, the excess of normal urease activity was also detected in the homogenates of skin wounds of all experimental animals and was 3.5 times in healthy rats, which were sutured and 3 times when applying biological glue. In the case of diabetes, such excesses were 5.3 (III group) and 4.7 times (IV group). On the 28th day of observation in healthy animals, urease activity did not differ significantly from that of intact skin of rats. Under conditions of streptozotocin diabetes, the excess was 2.2 times when using surgical sutures to fix the wound edges and 2 times when applying Dermabond glue (Fig. 1).

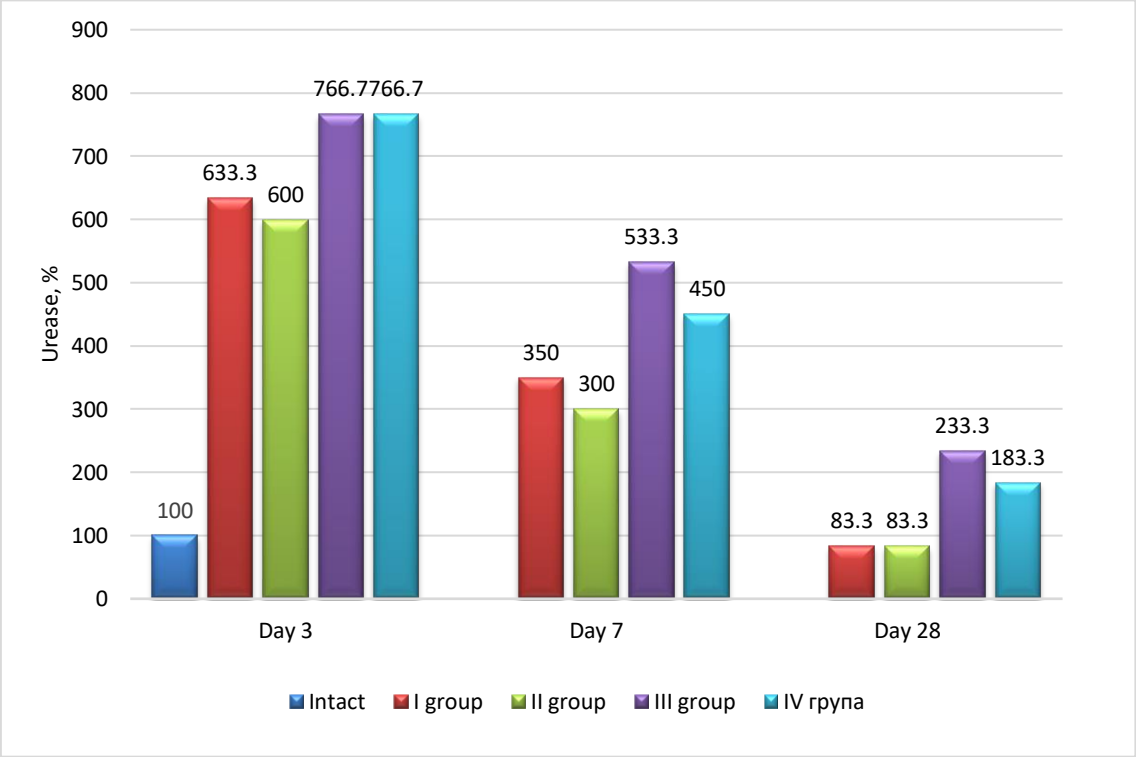

Fig. 1. Dynamics of urease content in skin homogenate under the condition of using threads and skin glue in rats with diabetes mellitus

Decreased lysozyme activity was observed on day 3 after surgery in all experimental groups of rats: 29.3% in group I, 27.2% in group II, 39.4% in group III and 38.7% in group

IV. A linear increase in this indicator was observed in both healthy and diabetic animals throughout the experimental period. Achievement of normal activity was detected in groups I and II of rats on the 28th day of the experiment. Under conditions of streptozotocin diabetes, the decrease in lysozyme activity is 14.6% when applying nodal sutures to the wound and 13% when applying biological glue (Fig. 2).

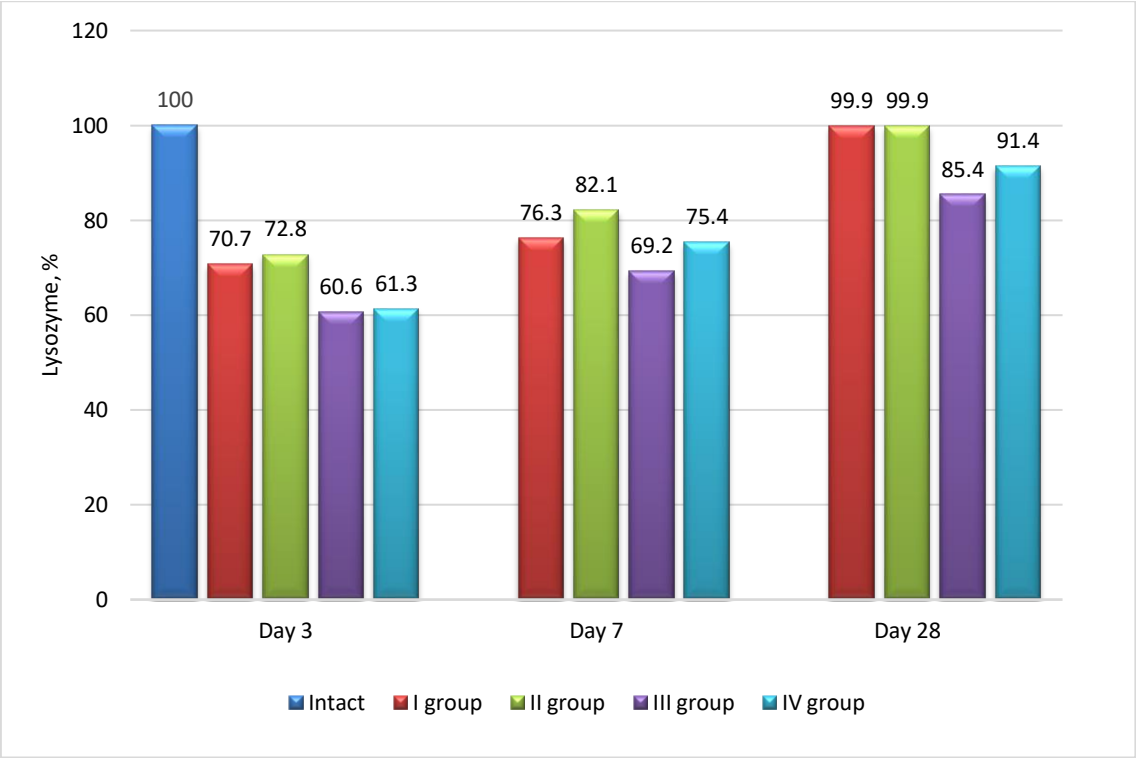

Fig. 2. Dynamics of lysozyme activity in skin homogenate under the condition of using threads and skin glue in rats with diabetes mellitus

The obtained results prove that the wound process under conditions of streptozotocin diabetes mellitus in rats leads to a significantly greater contamination of the wound with pathogenic and opportunistic microflora and a decrease in nonspecific immunity, compared with healthy animals (Table 2). Also, different effects of suture materials on the indicators of microbial contamination and immune protection were found: significantly larger deviations from the norm were found in groups of rats, where we used surgical sutures to fix the wound edges. When comparing the intensity of microbial contamination and nonspecific immunity in the long term (28 days) in the homogenate of the skin of rats with diabetes mellitus and the use of skin glue, the level of urease was 27.3% lower and the level of lysozyme 7.0% lower than in III group where we used surgical sutures.

Table 2

Indicators of the intensity of microbial contamination and nonspecific immunity, (M ± m)

Index	Urease, μcat / kg			Lysozyme, units / kg		
Intact rats (n=10)	0,06±0,01			136,42±0,79		
	Day 3	Day 7	Day 28	Day 3	Day 7	Day 28
I group (n=10)	0,38± 0,05*	0,21± 0,04*	0,05± 0,01	96,48± 0,40*	104,09± 1,81*	136,28± 1,17
II group (n=10)	0,36± 0,03*	0,18± 0,01*	0,05± 0,01	99,36± 0,87*	112,00± 1,44*	136,34± 1,11
III group (n=10)	0,46± 0,04*	0,32± 0,01*	0,14± 0,01*	82,64± 2,28*	94,41± 2,40*	116,48± 2,75*
IV group (n=10)	0,46± 0,02*	0,27± 0,01* #	0,11± 0,01*#	83,58± 2,07*	102,84± 1,79*#	124,63± 2,69*#
Notes: * - the difference is significant to the data of the intact group # - the difference is significant between III and IV experimental groups within one day ($p \leq 0.05$)						

Numerous studies have examined the causes of reduced resistance of patients with diabetes to purulent infection [11-13]. Despite the fact that clinical signs of wound suppuration in animals with streptozotocin diabetes were not observed in our own studies, the excess of urease was detected during all periods of the experiment. Such results indicate the constant excessive contamination of the wound with pathogenic and opportunistic microflora, which is an unfavorable condition for its healing.

Changes in the activity of the antimicrobial enzyme lysozyme allow to assess the state of nonspecific immunity in the studied tissues. Many authors note a decrease in lysozyme activity in various bioliquids and tissues in diabetes mellitus [14-16]. The results of our studies prove a significant decrease in the activity of lysozyme in the homogenate of postoperative skin wounds of rats with diabetes.

Conclusion. Wound process in rats with experimental diabetes mellitus leads to reliable contamination of the wound with pathogenic and opportunistic microflora, as evidenced by increased urease activity, and reduced nonspecific immunity, characterized by reduced lysozyme activity in the homogenate of postoperative wound/scar tissue in relation to intact animals during all the terms of observation.

In the remote period (28 days) in the skin homogenate of rats with diabetes where skin glue was used for wound closure, the level of urease was 27.3% lower and the level of lysozyme was 7.0% lower than that of rats with diabetes, where surgical sutures were used.

Prospects for further research. In the future, it is planned to analyze the reparative capacity of the skin of postoperative wound/scar tissue of rats with streptozotocin diabetes.

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