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THE CHANGES OF WOUND MACROPHAGES IN PATIENTS WITH DIABETES

ЗМІНИ РАНОВИХ МАКРОФАГІВ У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ
Резюме: Гнійно-некротичні ураження кінцівок в 30-50% випадків вимагають ампутації. Серед всіх випад-ків ампутації нижніх кінцівок 50-70% пов'язані з діабетом. Причому 5 з 6 ампу-Тацій, не пов'язаних з травматичним пошкодженням кінцівки, виконуються хворим са-Харне діабетом. Смерт-ність серед пацієнтів з діабетом, які перенесли ампутацію, коливає-ся від 28 до 40%, а 5-річне спостереження становить всього 10-25%. При дослідженні уль-траструктурних змін макрофагів на 3-й день лікування в цитоплазмі макрофагів були об-дуть виявлені маси хаотично розташо-ваних фібрилярних структур, зрідка мають пови-шенную електронну щільність. Це явище спос-терігалося на гнійно-некротичних ділянках м'яких тканин у пацієнтів основної групи в порівнян-ні з контрольною групою. У всіх случаях мітохондрії були збільшені в розмірах, набрякли, ма-ли світлий матрикс і містили зменшена кількість крист. Крісти деформовані і вкорочені. Набря-клий матрикс в мітохондріях привів до утворення на їх місці вакуолей з дрібнозернистим вміс-том. Ядро звичайної форми і розмірів з наявністю поодиноких інвагінацій. Хроматин був пере-важ-громадської сконцентрований у вигляді твердих електронно-щільних мас або рівномірно розбраті-діловий по ядру. Були ядра з частковою дисперсією хроматину. Вміст ядер включало зер-ність, фібрилярний і дрібнодисперсний вакуолярного матеріал. Складання ядерної мем-Брани істотно не коливалося. Складки не покривали всю поверхню ядра. У деяких областях ін-вагінат були представлені продовженням тільки перинуклеарной простору. Спостерігаються пори ядерної оболонки, які з'єднують вміст цитоплазми і нуклео-плазми. Цитоплазма між зонами пластинчастого комплексу була зайнята дрібними мітохон-дріямі, одиночними полісомаль-нимі розетками та цистернами гранулярного ендоплазматіче-ського ретикулума, який був пред-ставлений протяжними внутрішньоклітинними каналами і ва-куолярнимі утвореннями. Гладка ендоплазматична сітка переважно розташовувалася в центральній частині. Озонотерапія стиму-лює функціональну активність ранових макро-фагів, так як викликає деструктивні зміни в цих клітинах без некротичних поврежде-ний. Внутрішньовенне введення озонованого фізіологічного розчину сприяє елімі-нації ранових макрофагів, в основному за рахунок генетично запрограмо-ваної загибелі кле-ток (апоптозу), яка відіграє важливу роль в механізмах регуляції запального процесу.
Ключові слова: рана, озон, цукровий діабет.

Pyoinflammatory lesions of soft tissues in patients with diabetes mellitus is of great significance, because it is diagnosed 20 times more often than in population without diabetes. The frequency of purulent infections are 10-25%.

Purulent-necrotic lesions of the extremities require amputation in 30-50% of cases [1, 2]. Among all cases of lower extremity amputations 50-70% are due to diabetes [3, 4]. Moreover, 5 out of 6 amputations, not related to the traumatic injury of the limb, are performed in patients with diabetes. The mortality rate among patients with diabetes, who undergo amputation varies from 28 to 40%, and 5-year surveillance is only 10-25% [5, 6, 7].

The average days of hospitalization due to amputation ranges from 86 to 91 that is 47% higher than hospitalization days due to other complications of diabetes [8, 9, 10]. The average duration of outpatient treatment is approximately 4 months that is 4.6 times longer than in patients without diabetes; in 10% of cases treatment exceeds the annual target period of treatment in the system of the polyclinic network of health facilities [11].

The aim: to determine the morphological substrate of purulent foci in patients with diabetes mellitus with the purpose of limiting and preventing the progression of secondary-alteration changes in this area.

Material and methods: *Twelve patients with diabetes mellitus accompanied by pyoinflammatory complications (6 patients in the main group and 6 patients in the control group) were enrolled in the study. The ultrastructural peculiarities of morpho-functional changes of macrophages have been studied with the purpose of determining the dynamics and thrust of destructive-necrotic processes in these cells when the ischemic-gangrenous form of diabetic foot syndrome develops. Sampling of biological material in the main and control groups was carried out at the time of bandaging on the 3rd, 7th and 16th days of the inpatient treatment.*

Discussion of the results. The study of ultrastructural changes of macrophages on the 3rd day of treatment revealed masses of chaotically located fibrillar structures in the cytoplasm of macrophages that occasionally had an increased electron density. This phenomenon was observed in the purulent-necrotic areas of soft tissues of patients from the main group, compared to the control group.

In all cases, mitochondria were enlarged in size, swollen, with a light matrix and contained a reduced amount of cristae. The cristae were deformed and shortened. Swollen matrix in mitochondria led to the formation of vacuoles on their place containing fine-grained contents (Fig. 1).

The nucleus had a usual form and size with the presence of single invaginations. Chromatin was predominantly concentrated in the form of solid electron-dense masses or evenly distributed throughout the nucleus. There were nuclei with partial chromatin dispersion. The contents of the nuclei included granular, fibrillar, and fine vacuolar material.

The nuclear membrane folding did not fluctuate significantly. The folds did not cover the whole surface of the nucleus. In some areas invaginates were represented by the continuation of perinuclear space only. The nuclear envelop pores, which connect the contents of the cytoplasm and nucleoplasm, have been observed.

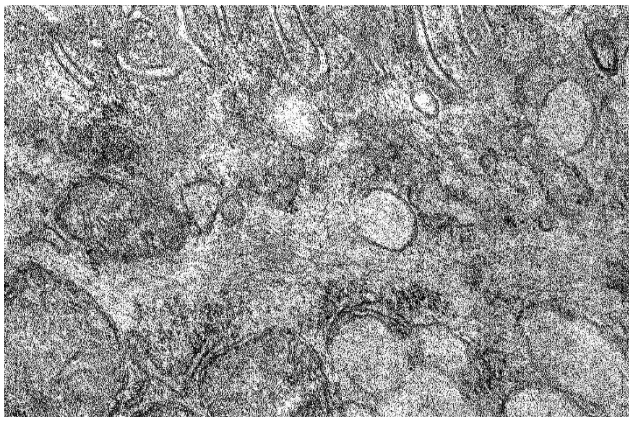

Fig. 1. Formation of vacuoles on the places of destructively altered mitochondria

The cytoplasm between the zones of the plate complex was occupied by small mitochondria, single polysomal rosettes, and cisternae of granular endoplasmic reticulum, which was represented by extended intracellular channels and vacuolar formations. The smooth endoplasmic reticulum was predominantly located in the central part.

The surface of macrophages in the process of their differentiation from monocytes was relatively plane. Occasionally there occurred small processes or pseudopodia. The number of pinocytic vesicles surrounded by a border was reduced in poorly differentiated cells.

Certain destruction of a large part of macrophages manifested in case of poorly organized plate-like complex.

The above-mentioned features of wound macrophages did not differ between the main and control groups of patients until the 7th day of treatment.

In the patients from the main group who underwent intraarterial administration of ozone (course of 5-7 sessions), the cytoplasm of many cells looked densified, microtubules and cytoplasmic fibers were preserved. The contact areas of the granular and smooth endoplasmic reticulum were clearly identified. There were masses of chaotically located and clearly visible fibrillar structures. The surface of macrophages, as a rule, was uneven, the finger-like processes or pseudopodia were observed.

The nucleus was usually round, oval or oblong with the presence of invaginations. The rate of the cytoplasmic membrane folding varied from small surface formations to profound invaginations, which gave the nucleus an irregular shape. Certain areas of invaginates were represented by a continuation of perinuclear space. Chromatin was relatively evenly distributed.

In addition to the changes described, the constant peculiarity was the emergence of large vacuoles, diverse in structure. The described changes in the state of the nuclear and perinuclear material were accompanied by compression and dehydration of the cell, which ended with its fragmentation and the formation of tightly contacting bodies of various forms (Fig. 2). The mitochondria of macrophages were often round or rarely oblong, and contained matrix of different densities. Occasionally there were round granules of high electron density between the cristae.

Therefore, the study of ultrastructural changes in on the 3rd day of treatment made it possible to reveal that in this category of patients some macrophages were characterized by the degenerative changes. Significant changes were often observed in macrophages

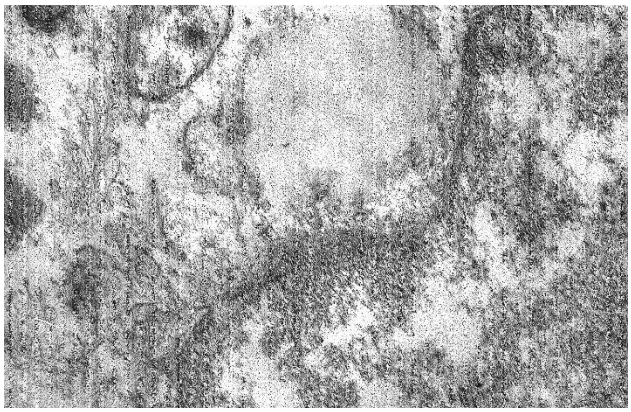

Fig. 2. Formation of a large electronically clarified vacuole near the nucleus

on the part of many organelles and, in the first place, mitochondria. Many of the mitochondria had pronounced signs of destruction. There was a disruption and fragmentation of the cristae that looked like short tubules. Many macrophages had signs of swelling and they contained destructively altered mitochondria, vacuoles, microtubules and microfilaments.

The study did not show any fundamental differences between the groups in wound macrophages until the 7th day of treatment.

In the control group of patients the same nature of changes was observed up to the 16th day of inpatient treatment.

The tendency to the wall location of chromatin in the main group in the form of solid electron-dense stripes was more determined in comparison with similar biological material in the control group.

There occurred uneven cell hypertrophy, formation of nuclei of queer form, chromatin clusters along the periphery of the nuclei, characteristic for the initial stage of apoptotic degeneration. Activation of these processes in the investigation of tissue macrophages from the biological material of patients in the control group was observed after the 17th day.

Despite the presence of destructive changes in the cell, the mitochondria of the macrophages in the main group of patients often retained their structure. Occasionally macrophages with signs of cellular degeneration were detected. Consequently, most of the macrophages of the wound surface of the patients in the main group did not have morphological characteristics peculiar for necrotic changes.

Under the influence of ozonotherapy, cells, which were at different stages of apoptosis, were often detected (compared with control). In addition to the initial signs, it was possible to observe the extended stage of apoptosis with pronounced condensation of the remnants of the nucleus and cell organelles at the beginning of the apoptotic bodies formation.

Furthermore, during the ozone therapy in destructively altered macrophages there were the signs of decrease in the synthesis of structural proteins – reduction of the nucleoli size and the absence of granular component in them, a small number of free ribosomes and polysomes, typical for programmed cell death.

Conclusions: Ozone therapy stimulates the functional activity of wound macrophages, as it causes destructive changes in these cells without necrotic lesions. Intravenous introduction of ozonized saline contributes to the elimination of wound macrophages, mainly through genetically programmed cell death (apoptosis), which plays a significant role in the regulatory mechanisms of the inflammatory process.

Perspectives of the further research. Study the morphological substrate of purulent foci in patients with diabetes mellitus.

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ИЗМЕНЕНИЯ РАНЕВЫХ МАКРОФАГОВ У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ

Резюме: Гнойно-некротические поражения конечностей в 30-50% случаев требуют ампутации. Среди всех случаев ампутации нижних конечностей 50-70% связаны с диабетом. Причем 5 из 6 ампутаций, не связанных с травматическим повреждением конечности, выполняются больным сахарным диабетом. Смертность среди пациентов с диабетом, перенесших ампутацию, колеблется от 28 до 40%, а 5-летнее наблюдение составляет всего 10-25%. При исследовании ультраструктурных изменений макрофагов на 3-й день лечения в цитоплазме макрофагов были обнаружены массы хаотично расположенных фибриллярных структур, изредка имеющих повышенную электронную плотность. Это явление наблюдалось на гнойно-некротических участках мягких тканей у пациентов основной группы по сравнению с контрольной группой. Во всех случаях митохондрии были увеличены в размерах, набухли, имели светлый матрикс и содержали уменьшенное количество крист. Кристы деформированы и укорочены. Набухший матрикс в митохондриях привел к образованию на их месте вакуолей с мелкозернистым содержимым. Ядро обычной формы и размеров с наличием единичных инвагинаций. Хроматин был преимущественно сконцентрирован в виде твердых электронно-плотных масс или равномерно распределен по ядру. Были ядра с частичной дисперсией хроматина. Содержимое ядер включало зернистый, фибриллярный и мелкодисперсный вакуолярный материал. Складывание ядерной мембраны существенно не колебалось. Складки не покрывали всю поверхность ядра. В некоторых областях инвагинаты были представлены продолжением только перинуклеарного пространства. Наблюдаются поры ядерной оболочки, которые соединяют содержимое цитоплазмы и нуклеоплазмы. Цитоплазма между зонами пластинчатого комплекса была занята мелкими митохондриями, одиночными полисомальными розетками и цистернами гранулярного эндоплазматического ретикулума, который был представлен протяженными внутриклеточными каналами и вакуолярными образованиями. Гладкая эндоплазматическая сеть преимущественно располагалась в центральной части. Озонотерапия стимулирует функциональную активность раневых макрофагов, так как вызывает деструктивные изменения в этих клетках без некротических повреждений. Внутривенное введение озонированного физиологического раствора способствует элиминации раневых макрофагов, в основном за счет генетически запрограммированной гибели клеток (апоптоза), которая играет важную роль в механизмах регуляции воспалительного процесса.
Ключевые слова: рана, озон, сахарный диабет.

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Abstract: Purulent-necrotic lesions of the extremities require amputation in 30-50% of cases. Among all cases of lower extremity amputations 50-70% are due to diabetes. Moreover, 5 out of 6 amputations, not related to the traumatic injury of the limb, are performed in patients with diabetes. The mortality rate among patients with diabetes, who undergo amputation varies from 28 to 40%, and 5-year surveillance is only 10-25%. The study of ultrastructural changes of macrophages on the 3rd day of treatment revealed masses of chaotically located fibrillar structures in the cytoplasm of macrophages that occasionally had an increased electron density. This phenomenon was observed in the purulent-necrotic areas of soft tissues of patients from the main group,

compared to the control group. In all cases, mitochondria were enlarged in size, swollen, with a light matrix and contained a reduced amount of cristae. The cristae were deformed and shortened. Swollen matrix in mitochondria led to the formation of vacuoles on their place containing fine-grained contents. The nucleus had a usual form and size with the presence of single invaginations. Chromatin was predominantly concentrated in the form of solid electron-dense masses or evenly distributed throughout the nucleus. There were nuclei with partial chromatin dispersion. The contents of the nuclei included granular, fibrillar, and fine vacuolar material. The nuclear membrane folding did not fluctuate significantly. The folds did not cover the whole surface of the nucleus. In some areas invaginates were represented by the continuation of perinuclear space only. The nuclear envelop pores, which connect the contents of the cytoplasm and nucleoplasm, have been observed. The cytoplasm between the zones of the plate complex was occupied by small mitochondria, single polysomal rosettes, and cisternae of granular endoplasmic reticulum, which was represented by extended intracellular channels and vacuolar formations. The smooth endoplasmic reticulum was predominantly located in the central part. The surface of macrophages in the process of their differentiation from monocytes was relatively plane. Occasionally there occurred small processes or pseudopodia. The number of pinocytic vesicles surrounded by a border was reduced in poorly differentiated cells. Ozone therapy stimulates the functional activity of wound macrophages, as it causes destructive changes in these cells without necrotic lesions. Intravenous introduction of ozonized saline con-tributes to the elimination of wound macrophag-es, mainly through genetically programmed cell death (apoptosis), which plays a significant role in the regulatory mechanisms of the inflammato-ry process.

Key words: diabetes, wounds. ozone.

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