REPORTS OF MORPHOLOGY

Official Journal of the Scientific Society of Anatomists,
Histologists, Embryologists and Topographic Anatomists
of Ukraine

journal homepage: <https://morphology-journal.com>

Histological changes of the arterial bed of the hind limbs of the rats under condition of the acute ischemia-reperfusion and correction with the carbacetam

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ARTICLE INFO

Received: 15 June, 2020

Accepted: 20 July, 2020

UDC: 616.137.83/93-091.8:616-005.4:615.214.3]-092.9

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The ischemic-reperfusion lesion is a complex multifactorial damage of the primary ischemic tissues as a result of restoration of the arterial blood circulation in them, which is accompanied by local morpho-functional reorganization of the vascular bed of the hind limbs of the rats. One of the promising means in the treatment and prevention of the reperfusion disorders is a carbacetam, which smooths the phenomena of hypo- and hyperperfusion in the post-ischemic period. The aim of the study was to establish the manifestations of the morpho-functional remodeling of the vascular bed of the hind limbs of the rats in ischemia-reperfusion and under conditions of correction with carbacetam. Histological examination of the vascular bed of the hind limbs of 30 rats under conditions of ischemia-reperfusion (group I) and 30 rats in the simulation of ischemia-reperfusion, which in the post-ischemic period administered carbacetam once a day (5 mg/kg) for 14 days (group II) were done. There were 6 intact animals in the control group. Simulation of ischemia was performed by applying SWAT rubber tourniquets on the hind limbs for 2 hours, and reperfusion - by removing of the tourniquet. The animals of the experimental groups were divided into 5 subgroups with reperfusion terms after 1, 2 hours and 1 day, as well as after 7 and 14 days. Histological examination was performed according to generally accepted methods. The vascular bed in the middle third of the thigh and the shin below the tourniquet was examined using a Bresser Trino Researcher 40x-1000x microscope. Analyzing of the obtained results, was established that after 1 hour of the reperfusion the histological changes became a systemic, and after 1 day it were more significant. It should be noted that the thickness of the vessel walls increased, and the elastic membranes were partially elined, thinned and torned. The stepwise clarity of the arterials walls structure was lost. The edema acquired a total nature. The histological examination of the vessels after 7 days revealed that the swelling of the walls decreased and the condition of the elastic frame was improved. There was a proliferation of collagen fibers in the adventitia, which was a response to ischemic effects. It is noted that after 14 days in all wall membranes the proliferative activity of fiboblasts was remained. Under the conditions of the correction with the carbacetam after 2 hours, the structural positive dynamics became more pronounced and increased to a maximum level after 7 days of the experiment. The number of the modified and exfoliated endothelial cells decreased, and the condition of smooth myocytes increased. Histologically, the gradual restoration of endothelial coverage of the intima was established. As follows, ischemia and reperfusion cause vascular remodeling after 1 hour with a peak of the manifestations after 1 day of the reperfusion, which includes edematous syndrome, dystrophic-degenerative changes with an inflammatory response to the damage, and in the late reperfusion period increased a fibroblasts activity. Gradual return of morphological changes occurs after 14 days of the experiment. Under the conditions of correction, the acceleration of the remodeling with stabilization of the process and the most possible structural restoration after 7 days of the study was noted.

Keywords: artery, remodeling, ischemia-reperfusion, elastic membranes, carbacetam.

Introduction

Ischemic-reperfusion lesion is a complex multifactorial process of damage and dysfunction of primary ischemic tissues as a result of restoration of arterial blood circulation in conditions of acute or chronic ischemia [9, 11, 13, 15, 19, 22]. Arterial ischemia is one of the main reasons for the deterioration of the quality of life of patients, their early disability and accounts for about 10% of all gunshot wounds in the structure of combat trauma [5, 15]. In 67% of cases of vascular damage at the prehospital stage, a tourniquet is applied, which can induce reperfusion damage [4, 9, 10, 15, 22].

Recently, more and more attention in the treatment and prevention of ischemic disorders is paid to nootropic drugs that have metabolic, neuroprotective, antiplatelet, antioxidant effects, as well as smoothing the phenomena of hypo- and hyperperfusion in the postischemic period, increase microcirculation in tissues. Among the new drugs, carbacetam, an endogenous modulator of the GABA-benzodiazepine receptor complex derived from β -carboline, is markedly secreted [7, 23]. The effectiveness of its use is shown in the work on the restoration of cognitive impairment, reduction of endogenous intoxication and oxidative stress in polytrauma [23].

Literature data indicate that the morphological manifestations of the syndrome of ischemia and reperfusion are impaired hemomicrocirculation, which is histologically manifested by endothelial edema, leukocyte-endothelial adhesion, hemodynamic disorders, reduction of microvascular and arteriovenous shunting, however, morpho-functional changes in arteries of large diameter, their dynamics, as well as the search for means of tissue protection remains open for further research [9, 13].

However, in reviewing the scientific and experimental literature, we did not find any publications on the use of this drug for the treatment and prevention of complications of ischemia-reperfusion, which prompted an attempt to use carbacetam to study the local correction of reperfusion.

The aim of the study was to establish the manifestations of morphofunctional reorganization of the vascular bed of the hind limbs of rats during ischemia-reperfusion and under conditions of correction with carbacetam.

Materials and methods

Histological examination of the vascular bed of 60 rats have been done.

Ischemia was modeled by applying SWAT (Stretch-Wrap-And-Tuck) rubber tourniquets to the right hind limb of the animal at the level of the inguinal fold for 2 hours under thiopental-sodium anesthesia. The reperfusion syndrome was modeled by removing the tourniquet and restoring blood circulation in the previously ischemic limb 2 hours after application. Observations of animals were carried out for 14 days.

During the experiment, the experimental animals were divided into three groups:

- first experimental group (30 rats) - animals with simulation of ischemic-reperfusion injury;
- second experimental group (30 rats) - animals with simulation of ischemic-reperfusion damage, in which 1-oxo-3,3,6-trimethyl-1,2,3,4-tetrahydroindolo [2,3-c] quinolines (carbacetam) was injected intraperitoneally in the reperfusion period for the purpose of correction at a dose of 5 mg per kilogram of body weight 1 time per day for 14 days of the reperfusion period;
- control group (6 rats) - intact animals.

Animals of the first and second experimental groups were divided into 5 subgroups (6 animals each). The model of the early post-ischemic period in both groups was presented by subgroups of animals with reperfusion changes after 1, 2 hours and 1 day, and the model of the late reperfusion period - by subgroups of animals 7 and 14 days after removal of the tourniquet.

All studies were conducted in compliance with the main provisions of the Law of Ukraine № 3447-IV of 21.02.06 "On the protection of animals from cruel treatment" (2006) and Council of Europe Directive 2010/63 EU on animal experiments.

Euthanasia of animals was performed by administration of thiopental-sodium anesthesia (500 mg/kg body weight intraperitoneally), followed by decapitation and collection of biological material. Soft tissue samples, together with the vascular-nervous bundle of the hind limb of the animals, were taken below the level of the tourniquet at the level of the middle third of the thigh, followed by fixation, dehydration and paraffin removal, which were performed according to conventional methods. Prepared a series of sections with a thickness of 4-5 μ m, made on a microtome MS-2. The latter, after dewaxing, were stained with hematoxylin and eosin, picrofuchsin by Van Gieson's method, resorcin fuchsin by Weigert's elastic stain method, and Heidenhain's AZAN trichrome stain method [17]. This made it possible to obtain differentiated tissue staining due to the heterogeneity of the perception of different dyes by biological tissues.

Examination of the preparations was performed using a Bresser Trino Researcher 40x-1000x microscope (serial number 0913137). The most demonstrative histological specimens were photographed using a Digital Camera for Microscope Science Lab DCM 820 Resolution 8.0 Mp.

Results

Histological examination of the main arteries and veins of the thigh and lower leg showed that the structural organization of their walls corresponds to the generally accepted criteria. Elastic membranes are clearly contoured in the form of a homogeneous eosinophilic strip. Arteria of the tibia and femur segments differ only in diameter (Fig. 1).

After 1 hour of reperfusion changes became systemic and appeared at all levels of structural organization of blood vessels. Rounded, due to the swelling of the cytoplasm, endothelial cells protruded into the lumen of blood vessels,

Fig. 1. Clearly contoured inner and outer elastic membranes of the femoral artery of the rat of the control group. 1 - inner elastic membrane; 2 - outer elastic membrane. Weigert's elastic stain, x200.

Fig. 2. Endothelial cell exfoliation, aggregation and adhesion of erythrocytes. The edema of the media. Section of the femoral artery of the rat after 1 hour of reperfusion. 1 - exfoliation of endothelial cells; 2 - aggregation and adhesion of erythrocytes; 3 - edema of the artery wall. Hematoxylin and eosin stain, x200.

and some of them peeled off. The tortuosity of the inner elastic membrane became irregular, split. There have been sticking and adhesion erythrocytes. As a result of the swelling, the subendothelial prosthesis expanded, and smooth myocytes sometimes lost their compact position. Dystrophic changes of myocytes were determined in parallel. Lymphocytes were occasionally detected. Adventitia was disturbed (Fig. 2).

Histologically, after 2 hours of reperfusion, structural changes intensified. Often endothelial cells were exfoliated in layers. The intima thickened, lost its integrity, and sometimes exfoliated along with endothelial cells. Swelling of the subendothelial layer increases. Elastic membranes at this time are split, straightened and often fragmented,

which we regarded as a sign of loss of vascular wall tone. Smooth myocytes with signs of vacuolar dystrophy were distinguished by intercellular substance. The swelling of the outer membrane, enriched at this time by cells of hematogenous origin, including lymphocytes, visually thickened the vascular wall (Fig. 3).

After 1 day, structural changes became the most significant. The wall thickness increased. Elastic membranes were partially leveled, thinned and developed, sometimes transforming into chains of fragments of various sizes. Endotheliocytes did not differ structurally from those we had already found in the previous terms of the experiment. The difference was that after 1 day these manifestations became systemic and almost uniformly expressed in all segments of the vascular bed. The clarity of the layered structure of the arteria walls was lost. Dystrophic and degenerative changes of smooth myocytes in the form of their vacuolation, focal and total cytolysis were manifested. Round-cell infiltrates are present in all layers. Defects of the endothelial lining and the appearance of vasculitis created the preconditions for parietal aggregation of erythrocytes and thrombosis. Edema acquired a total character and tended to spread to the paravascular prostheses and skeletal muscles (Fig. 4).

Changes in the main vessels after 7 days were generally identical to the previous reperfusion period. The swelling of the arteria walls decreased due to the restoration of the endothelial covering of the intima. Among myocytes, dystrophically altered cells and low-intensity cellular infiltrates, predominantly lymphocytes with an admixture of proliferating fibroblasts and single histiocytes, were not uncommon. In general, the condition of the elastic membranes improved. In most cases, the integrity of the elastic membranes was preserved. However, straightening and fragmentation of elastic fibrils were also not uncommon (Fig. 5).

In adventitia and perivascular stroma there was a

Fig. 3. Thinned, split and partially lysed elastic fibers. Cranial tibial artery of the rat after 2 hours of reperfusion. 1 - thinned and split inner elastic membrane; 2 - partially lysed outer elastic membrane. Weigert's elastic stain, x100.

Fig. 4. Destructive changes in the wall of the arteria, combined with an intense inflammatory reaction, expressed in swelling around the vessel with spread to the muscle. Section of the femoral artery of the rat after 1 day of perfusion. 1 - paravasal edema; 2 - wall infiltration. Hematoxylin and eosin stain, x100.

Fig. 5. Multiple foci of desquamation of damaged endothelial cells of the arteria and veins of the tibial segment after 7 days of reperfusion. Low-intensity cellular infiltration of adventitia and media. 1 - desquamation of endothelial cells of the cranial artery of the tibia; 2 - desquamation of endothelial cells of the tibial vein intima; 3 - moderate lymphocytic infiltration of the adventitia. Hematoxylin and eosin stain, x100.

proliferation of collagen fibers, which was an adequate response to long-term ischemic effects.

After 14 days in the arteria of all segments revealed an increase in the number of collagen fibers in the subendothelial space and the outer membrane of the arteria. Leiomyocyte hyperopia and an increased proportion of connective tissue component were detected in the thickened media. The elastic fibers of the inner elastic membrane were unevenly polished, restoring integrity. However, in the media and at the sites of localization of the outer membrane, the elasticity was blurred or absent. Along with this, swelling, desquamation of individual and dystrophic changes of smooth myocytes were preserved. In all wall membranes,

the intensity of lymphohistiocytic infiltration was significantly reduced, but the proliferative activity of fibroblasts was preserved (Fig. 6).

The morphological studies of the vascular bed under the conditions of carbacetam correction showed that after 1 hour the systemic hemodynamic disturbances in all segments of the vascular bed did not differ much from the ischemia-reperfusion already detected by us at the same time, without correction and increased in the direction of shin.

After 2 hours, the positive dynamics of structural changes in the arteria became more pronounced. The number of modified and exfoliated endothelial cells decreased. Most of the cells were located on the basement membrane, which reduced the intensity of plasma penetration of arteria walls.

Fig. 6. Proliferation of connective tissue fibers in all membranes of the wall of the femoral arteria of rat after 14 days of reperfusion. Heidenhain's AZAN trichrome stain, x100.

Fig. 7. The inner elastic membrane of the arteria is preserved, the elastic of the middle membrane is absent, in the vein wall it is partially fragmented. Knee arteria and vein of the rat after 2 hours of reperfusion with correction. 1 - clear inner elastic membrane; 2 - lack of elastic fibers in the media; 3 - outer elastic membrane. Weigert's elastic stain, x100.

Fig. 8. Clear inner and outer elastic membranes of arteria and fragmentation of elastic fibers of media. Femoral arteria of the rat after 14 days of reperfusion with correction. 1 - clear inner elastic membrane; 2 - outer elastic membrane; 3 - fragmentation of elastic fibers of the media. Weigert's elastic stain, x200.

The reduction of ischemic effects contributed to the deterioration of smooth myocytes - the number of cells with vacuolated sarcoplasm decreased. The intercellular amorphous substance was poorly differentiated. Single lymphocytes, macrophages, and fibroblasts were present in all wall membranes (Fig. 7).

Destructive changes in elastic fibers lost their systemicity and were rather sporadic. In the large and middle veins, plethora prevailed. The endothelial lining largely preserved integrity.

After 1 day, the positive dynamics increased and reached its maximum after 7 days of the study, slowing down and stabilizing after 14 days of the experiment.

During these periods, we found a gradual restoration of endothelial coverage of the intima. Most cells had normal outlines, but among them there were cells with cytoplasmic swelling. Elastic membranes had a characteristic configuration. Closer to the end of the experiment, we did not notice their splitting or fragmentation (Fig. 8).

Despite the fact that under the condition of correction a significant share of structural disturbances has consistently returned to its initial state, starting from the early perfusion period, at the final stage we detected residual phenomena. Thus, hypertrophy of a part of smooth myocytes of arteria (together with atrophic ones) and an increase in the number of thin collagen fibers in the extracellular matrix, mainly on the border with the outer shell, were detected. In adventitia, the proportion of collagen fibers also increased.

Discussion

It is known that ischemia and reperfusion is a complex multifactorial process with changes in cellular metabolism, disruption of ion transport and activation of proteinases, processes of lipid peroxidation, which leads to cell death in cells and release of cytotoxic intracellular components

and is a consequence not only of ischemia but also of reoxygenation, which often significantly activates pathological processes and causes local and systemic disorders [2]. Their morphological manifestations are: remodeling of vessels of both large caliber and microvessels of the hemomicrocirculatory tract, soft tissues with the development of edema, necrotic-destructive changes and violation of the rheological properties of blood [3].

The mechanism of perfusion damage is associated with impaired microcirculation, with pronounced endothelial swelling, leukocyte-endothelial adhesion, albumin extravasation and impaired arteriole relaxation [16]. Thus, the processes that occur during ischemia are the result not only of ischemia, but also of reoxygenation, which not only does not stop their development, but also often significantly activates pathological processes [21].

At long ischemia of extremity tissue there is a desolation of a microcirculatory channel with the subsequent reduction of microvessels that is shown by decrease in their quantity and decrease in a tissue blood flow. Against the background of restoration of arterial hemodynamics in the limb, the volume of blood entering the microcirculatory tract approaches normal, and the volume of the microcirculatory tract does not increase, which leads to a simultaneous increase in blood flow velocity in all microvessels of basal blood flow [8, 14, 18]. At the same time, these capillaries are not enough to provide adequate blood volume through the arterial bed, which leads to increased arteriovenous shunting [12]. Direct revascularization is accompanied by progression of regional venous hypertension and profound disruption of tissue circulation.

In studies of vascular reorganization during 6-hour ischemia and 6-hour perfusion, it is noted that in morphological examination - there are signs of moderately expressed perivascular edema, as well as a significant number of petechial hemorrhages [2, 6]. In certain fields, hemorrhages merge and resemble hemorrhagic infiltration [20]. Signs of sludge, as well as a significant number of fibrin clots are expressed in the lumen of some vessels, and inflammatory cells are located along the vessels [1].

As a result of our research, it was found that ischemia and perfusion cause remodeling of vessels of both large and small diameters. In the early perfusion period, structural changes included edematous syndrome, dystrophic-degenerative, and destructive changes in vessels with an inflammatory response to injury; the initiating factor can be considered ischemic endothelial damage.

In the late perfusion period, the remodeling of structural components was characterized by a slowing down of acute manifestations with a partial return to the initial state and the onset of fibroblastic diferon activity and the synthesis of connective tissue matrix in the walls of vessels and near them.

Changes in the histoarchitectonics of the vascular walls intensified in the distal direction, and after 14 days of reperfusion most of the signs returned to the control group.

In the stages of post-ischemic reperfusion under the conditions of carbacetam correction, soft tissue remodeling is sequential with stabilization of the process and structural recovery after 7 days of the experiment, while in animals without correction morphological signs of recovery were detected after 14 days.

Conclusions

Ischemia and reperfusion cause vascular remodeling after 1 hour with a peak of manifestations after 1 day of

reperfusion, which includes edematous syndrome, dystrophic-degenerative changes with an inflammatory response to damage, and in the late reperfusion period increase in the activity of fibroblastic diferon. Gradual return of morphological changes occurs after 14 days of the experiment. Under the conditions of correction, there was an increase in remodeling with stabilization of the process and the most possible structural restoration after 7 days of the study.

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ГІСТОЛОГІЧНІ ЗМІНИ СУДИННОГО РУСЛА ЗАДНІХ КІНЦІВОК ЩУРІВ ЗА УМОВ ГОСТРОЇ ІШЕМІЇ-РЕПЕРФУЗІЇ ТА ПРИ КОРЕКЦІЇ КАРБАЦЕТАМОМ

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Ішемічно-реперфузійне ураження - це складне мультифакторне пошкодження первинно ішемізованих тканин у результаті

відновлення в них артеріального кровообігу, яке супроводжується локальною морфофункціональною реорганізацією судинного русла задніх кінцівок щурів. Одним із перспективних засобів у лікуванні і профілактиці реперфузійних порушень є карбацетам, котрий згладжує феномени гіпо- та гіперперфузії в постішемичному періоді. Мета дослідження - встановити прояви морфофункціонального ремоделювання судинного русла задніх кінцівок щурів при ішемії-реперфузії та за умов корекції карбацетамом. Проведено гістологічне дослідження судинного русла задніх кінцівок 30 щурів за умов ішемії-реперфузії (I група) та 30 щурів при моделюванні ішемії-реперфузії, котрим у постішемичному періоді вводили карбацетам 1 раз на добу (5 мг/кг) протягом 14 діб (II група). У контрольній групі було 6 інтактних тварин. Моделювання ішемії проводили шляхом накладення гумових джгутів SWAT на задні кінцівки впродовж 2 годин, а реперфузії - шляхом зняття турнікету. Тварини експериментальних груп були розподілені на 5 підгруп з реперфузійними термінами через 1, 2 години, 1 добу, а також через 7 та 14 діб. Гістологічне дослідження проводили за загальноприйнятими методиками. Досліджували судинне русло в середній третині стегна та гомілки нижче ділянки накладання турнікету за допомогою мікроскопа Bresser Trino Researcher 40x-1000x. Аналізуючи отримані результати встановили, що через 1 годину реперфузії гістологічні зміни набували системності, а через 1 добу були найбільш вираженими. Необхідно відмітити, що товщина стінок судин збільшувалася, а еластичні мембрани частково вирівнювались, витончувались та розривались. Нерідко втрачалась чіткість пошарової структури стінок артерій. Набряк набував тотального характеру. При гістологічному дослідженні судин через 7 діб виявили, що набряк стінок зменшувався, а стан еластичного каркасу покращувався. В адвентиції мала місце проліферація колагенових волокон, що було відповіддю на ішемічні впливи. Відмічено, що через 14 діб у всіх оболонках стінки зберігалася проліферативна активність фібробластів. За умов корекції карбацетамом через 2 години структурна позитивна динаміка ставала більш виразною та сягала максимуму через 7 діб експерименту. Зменшувалася кількість видозмінених та злуценних ендотеліоцитів, а стан гладких м'якоцитів покращувався. Гістологічно встановлено поступове відновлення ендотеліального покриття інтими. Таким чином, ішемія та реперфузія зумовлює ремоделювання судин вже через 1 годину із піком проявів через 1 добу реперфузії, що включає в себе набряковий синдром, дистрофічно-дегенеративні зміни із запальною відповіддю на пошкодження, а у пізньому реперфузійному періоді наростання активності фібробластичного дифферону. Поступове повернення морфологічних змін відбувається через 14 діб експерименту. За умов корекції відмічено прискорення ремоделювання зі стабілізацією процесу та найбільш можливим структурним відновленням уже через 7 діб дослідження.

Ключові слова: артерія, ремоделювання, ішемія-реперфузія, еластичні мембрани, карбацетам.

ГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ СОСУДИСТОГО РУСЛА ЗАДНИХ КОНЕЧНОСТЕЙ КРЫС ПРИ ОСТРОЙ ИШЕМИИ-РЕПЕРФУЗИИ И ПРИ КОРРЕКЦИИ КАРБАЦЕТАМОМ

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Ишемически-реперфузионное повреждение - это сложное мультифакторное повреждение первично ишемизированных тканей в результате восстановления в них артериального кровообращения, которое сопровождается локальной морфофункциональной реорганизацией сосудистого русла задних конечностей крыс. Одним из перспективных средств в лечении и профилактике реперфузионных повреждений является карбацетам, который сглаживает феномены гипо- и гиперперфузии в постишемическом периоде. Цель исследования - установить проявления морфофункционального ремоделирования сосудистого русла задних конечностей крыс при ишемии-реперфузии и в условиях коррекции карбацетамом. Проведено гистологическое исследование сосудистого русла задних конечностей 30 крыс в условиях ишемии-реперфузии (I группа) и 30 крыс при моделировании ишемии-реперфузии, которым в постишемическом периоде вводили карбацетам 1 раз в сутки (5 мг/кг) в течение 14 суток (II группа). В контрольной группе было 6 интактных животных. Моделирование ишемии проводили путем наложения резиновых жгутов SWAT на задние конечности в течение 2 часов, а реперфузии - путем снятия турникета. Животные экспериментальных групп были разделены на 5 подгрупп с реперфузионными сроками через 1, 2 часа, 1 сутки, а также через 7 и 14 суток. Гистологическое исследование проводили по общепринятым методикам. Исследовали сосудистое русло в средней трети бедра и голени ниже участка наложения турникета с помощью микроскопа Bresser Trino Researcher 40x-1000x. Анализируя полученные результаты установили, что через 1 час реперфузии гистологические изменения становились системными, а через 1 сутки были наиболее выраженными. Необходимо отметить, что толщина стенок сосудов увеличивалась, а эластичные мембраны частично выравнивались, истончались и разрывались. Терялась четкость послойной структуры стенок артерий. Отек приобретал тотальный характер. При гистологическом исследовании сосудов через 7 дней обнаружили, что отек стенок уменьшался, а состояние эластичного каркаса улучшалось. В адвентиции имела место пролиферация коллагеновых волокон, что было ответом на ишемические воздействия. Отмечено, что через 14 дней во всех оболочках стенки сохранялась пролиферативная активность фибробластов. В условиях коррекции карбацетамом через 2 часа структурная положительная динамика становилась более выразительной, достигая максимума через 7 дней эксперимента. Уменьшалось количество видоизмененных и слущенных эндотелиоцитов, а состояние гладких миоцитов улучшилось. Гистологически установлено постепенное восстановление эндотелиального покрытия интимы. Таким образом, ишемия и реперфузия предопределяет ремоделирование сосудов уже через 1 час с пиком проявлений через 1 сутки реперфузии, что включает в себя отечный синдром, дистрофически-дегенеративные изменения с воспалительным ответом на повреждение, а в позднем реперфузионном периоде - нарастание активности фибробластического дифферона. Постепенное возвращение морфологических изменений происходит через 14 дней эксперимента. В условиях коррекции отмечено ускоренное ремоделирование со стабилизацией процесса и наиболее возможным структурным восстановлением уже через 7 суток исследования.

Ключевые слова: артерия, ремоделирование, ишемия-реперфузия, эластические мембраны, карбацетам.