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O.V. Malikov
Bogomolets National Medical University, Kyiv

MORPHOLOGICAL STATE OF THE RENAL MEDULLA IN RATS IN CASE OF AN EXPERIMENTAL BURN INJURY UNDER CONDITIONS OF GEKOTON

E-mail: alexandrvm@ukr.net

The article presents structural changes of the renal medulla in experimental traumatic skin injury in rats under conditions of intravenous infusion of a colloid-hyperosmolar solution of gekoton. It was found that gekoton acts on the structure of the body as a cytoprotector. The cytoprotective effect is caused by induction of hypertrophy and hyperplasia of mitochondria. It also acts as a stimulator of mitophagia and an inhibitor of mitochondrial transduction of apoptotic signal. The obtained results are a kind of control and necessary for interpretation in comparison with the data to be obtained in the study of changes in the structural components of the renal medulla under conditions of infusion of other combined hyperosmolar solutions.

Key words: burn, renal medulla, light and electron microscopy.

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Pathogenesis and treatment of burns is a topical and not enough elaborated issue [3]. However, it has been established that the administration of infusions of hyperosmolar solutions in case of experimental skin burns of II-III grades of 21-23 % body surface area has an effective influence over endogenic intoxication [1], which causes an increase of catabolism level, destruction of cellular and non-cellular components of the organs [2, 5, 6, 7] and which is the cause of numerous characteristic burn disease manifestations of multiple organ failure.

The purpose of the work was to study structural changes of the renal medulla in case of an experimental skin burn injury in rats under conditions of an intravenous infusion of gekoton.

Materials and methods. The experimental study of morphological changes of the renal medulla in case of burn disease (within 1, 3, 7, 14, 21, 30 days) under condition of exposure to 0,9 % NaCl solution and an infusion of colloidal hyperosmolar drugs with detoxicating, rheological, energetic, antishock effect gekoton and lactoproteinum with sorbitol has been executed in 90 male Wistar rats of 155-160 g.

Results of the study and their discussion. The destruction of microvilli of limbus penicillatus and the breakage of apical region epithelial cells cytoplasm of proximal convoluted tubules of the nephron are typical for the renal medulla in rats with skin burns which were given the gekoton solution during the first three days, one and three days after the experiment began.

These modifications were observed on the background of destruction of mitochondria, formation of lysosomes and autophagosomes (mitophagosomes). Most often the mitochondria pathology is expressed in matrix focal clearing, partial or total disappearance of cristae, swelling, damage of internal membrane integrity. The internal membrane of some mitochondria is not detected and the organelle is converted into a vacuole, but such vacuoles are bigger than primary vacuoles (which are detected in cytoplasm of epithelial cells in a norm) and the residues of the cristae or the internal membrane allow their origin to be specified from mitochondria.

While assessing the state of mitochondria it is important to emphasize morphological signs of irreversibility of their changes. First of all they are: destruction of the membrane limiting the mitochondrion that results in decomposition of the organelle. A sign of irreversible changes of a damaged mitochondrion is also its fusion with a lysosome what leads to cristae homogenization and to the creation of an autophagosome (a mitophagosome) which looks like a vacuole densely filled with quite a homogenous material consisting of osmiophilic and osmiophobic areas. In some mitophagosomes the residues of membrane and/or globular inclusions of high electron density remarkably seen on the background of electronically-transparent matrix surrounded with the conserved organelle's membrane are detected.

The described phenomenon completely meets the picture of selective form of mitochondria autophagy or mitophagy [10], which final stage is a complete (or partial) digestion of phagosome's content and the release of the latter out of cytoplasm. Within 3 days after the skin burn mitochondria of different form and size (giant mitochondria, mitochondria with constrictions and buds of different form) are detected in cytoplasm of epithelial cells of renal tubules (fig. 1).

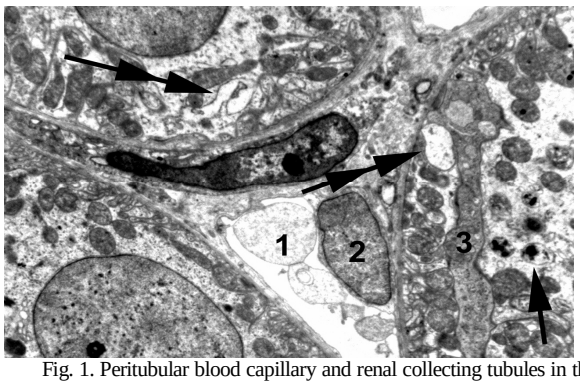

Fig. 1. Peritubular blood capillary and renal collecting tubules in the renal medulla of a rat with skin burn injury to which was given gekoton during 3 days, 3 days after the experiment began. 1 − capillary lumen; the nucleus of an epithelial cell; 2 – giant mitochondrion in cytoplasm of an epithelial cell of renal collecting tubule. Image magnification x 5000.

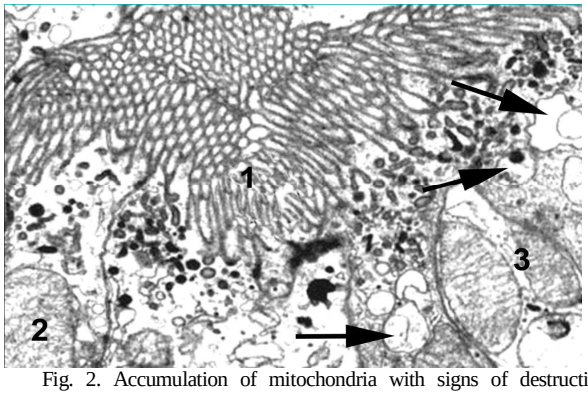

Fig. 2. Accumulation of mitochondria with signs of destruction, mitophagosomes, autophagic vacuoles and autophagic lysis areas on the apical pole of an epithelial cell of the proximal convoluted tubule of the nephron in the renal medulla in a rat with skin burn injury which were given gekoton solution during 7 days after the beginning of the experiment, 7 days after the experiment had begun. 1–structurally conserved microvilli of limbus penicillatus; 2−mitochondrion with cleared matrix and destructed cristae; 3–area of autophagic lysis. Image magnification x 24000.

This phenomenon meets the established view [9] about the new growth of mitochondria in the way of budding. The process of mitochondria hypertrophy and hyperplasia is performed on the background of selective autophagy of a part of them. On the final phase of their development the autophagosomes look like autophagic vacuoles filled with electronically transparent contents. These vacuoles' form and size are approximately correspondent to the form and size of mitochondria, that is why there is a reason to consider that not only «old», but also "young" mitochondria in the phase of «budding» can be injured (and lysed as a consequence of the autophagy). In this follow-up period the wall of peritubular blood capillaries conserves its typical structure. In the lumen of blood microcirculation vessel sometimes the cell debris is detected (most often the residues of lysed red blood cells).The processes of mitophagy, destruction of certain groups of mitochondria, as well as mitochondria hypertrophy and its new growth (budding of large mitochondria and small mitochondria formation) are typical for the cells of the renal medulla in rats with burns 7 days after daily injection of gekoton. The fact that the above mentioned processes are moderate and they are not associated with either apoptotic or necrotic cell disruption is the evidence of its effectiveness. Under these conditions, particularly, the microvilli of limbus penicillatus of epithelial cells of proximal convoluted tubule of the nephrons conserve their structure undamaged. There is a gradient of motion (transport) of autophagic vacuoles directed to apical area of epithelial cells cytoplasm and a similar gradient of mitochondria damage and forming of mitophagosomes.This is what can allow us to explain the resistance of cytoplasm basal areas (and then, basement membrane) and the tendency of apical areas of cytoplasm (and microvilli of limbus penicillatus related to them) to destruction. Even if the microvilli of limbus penicillatus conserve their structure there is a tendency to accumulate mitochondria with cleared matrix and with destroyed cristae, mitophagosomes, autophagic vacuoles and areas of autophagic lysis on the apical Removal of the single vacuoles' contents is pole of cytoplasm of epithelial cells (fig. 2). We have never registered such a phenomenon in the norm.

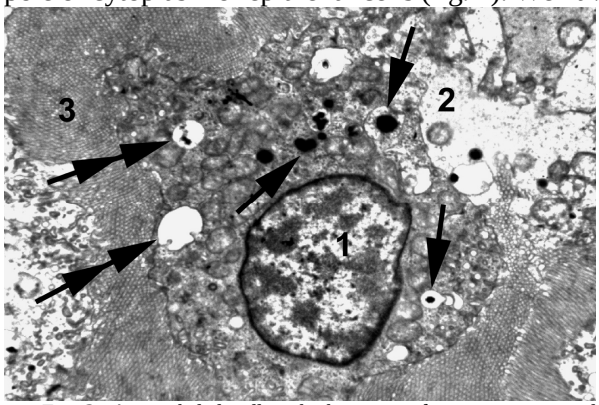

Fig. 3. An epithelial cell with the signs of an excessive areal autophagic vacuolization in the lumen of proximal convoluted tubule of the nephron in the renal medulla of a rat with skin burn injury which was given gekoton solution during 7 days after the beginning of the experiment, 14 days after the beginning of the experiment. 1 – nucleus of an epithelial cell; 2 – fusion area of vacuoles and mitophagosomes with formation of a locus of partial necrosis; 3 –microvilli of limbus penicillatus. Image magnification x 8000.

performed by due to an autophagic vacuole's membranes fusion with plasmatic membrane of a cell and, in this case, the separating (with an outside medium) function of the plasmalemma is conserved. However, in case of massive autophagic vacuolization the vacuoles and mitophagolysosomes fusion (in different phases of maturation) results in «vacuolar explosion» when the membrane is fragmented into "pieces" and its separating function levels out. The areas of autophagic lysis are burst out through the cytomembrane, initiating in this way the progress of necrotic destruction of a cell. (fig. 3). We have not detected the processes of mitophagy and hypertrophy and new growth of mitochondria in the cells of the renal medulla in rats with skin burn injury to which gekoton solution was administered during 7 days after the beginning of the experiment, 21 and 30 days after the beginning of the experiment.

During this period of follow-up a segmental, irregular reduction of areas of hyperosmotic cytoplasm, their clasmatosis, subtotal (and sometimes, total) peeling the deformed and fragmented hyperosmotic cells off the basal membrane has been detected (fig. 4). This phenomenon reminds the phenomenon of anoikis which The Nomenclature Committee on the Cell Death proposes to consider a kind of classical apoptosis [8].

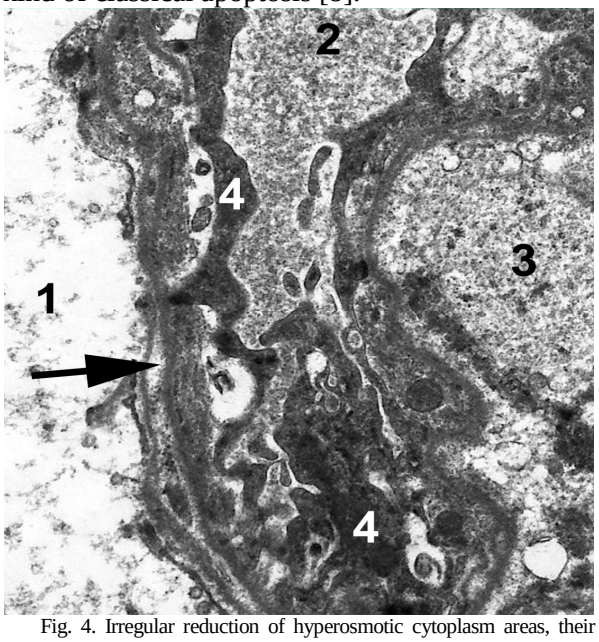

Fig. 4. Irregular reduction of hyperosmotic cytoplasm areas, their clasmatosis, subtotal peeling of deformed and fragmented hyperosmic cells off the basement membrane of the nephron thin tubule in the renal medulla of a rat with skin burn injury which was given gekoton solution during 7 days after the experiment had begun, 21 days after the beginning of the experiment. 1 – paravasal edema; 2 – tubular lumen; 3 – paravasal cell debris; 4 – cytoplasm of an epithelial cell. Image magnification x 10000.

Nowadays in scientific literature [7, 8, 10] the following types of cell death are mentioned: 1) macroautophagy (or the autophagy itself); 2) microautophagy or selective autophagy to which particularly belongs the mitophagy. The paradox of autophagy phenomenon consists in the fact that it can be not only a variation of realization of thanatogenic signal, but, on the contrary, the cell's survival programme. After skin burn injury under conditions of an infusion of gekoton we have observed two kinds of mitochondria death in the cells of the renal medulla: 1) breakdown of the cristae and limiting membranes with the following organelle disintegration (that can be assessed as a component of necrotic modifications in the cell); 2) mitophagy. If the first kind of mitochondria death is certainly pathological, mitophagy has an adaptive nature as a factor of mitochondria renovation and a factor of suppression of mitochondrial transduction of apoptotic signal [9]. There are certain dynamics in progress of mitophagy which achieve their peak level 14 days after the skin burn and becomes redundant or even excessive.

Increase of mitophagolysosomes number and their intensive transformation into autophagic vacuoles which ends in a massive autophagic vacuolization of the cytoplasm of some epithelial cells is considered a sign of an excessive mitophagy. Such vacuolization (the vacuole's contents are removed out of the cytoplasm) results in formation of numerous defects of cytomembrane (the consequence of «vacuolar explosion»), cell disruption by type of necrosis and desquamation of the latter into the tubular lumen.

Conclusions

1. In case of an experimental skin burn of II-III grade of 21-23 % of body surface area in rats a remarkable cytoprotective effect of gekoton on the structure of the renal medulla is a morphologic equivalent of positive effect of an intravenous infusion of gekoton. The above-mentioned cytoprotective effect is caused by the induction of mitochondria hypertrophy and hyperplasia, as well as the stimulation of mitophagy which provides the removal of damaged mitochondria and suppresses the progress of cell apoptosis.

2. During 30 days of structural modifications progress in the renal medulla in rats which were given the solution gekoton, the mitophagy in the cytoplasm of epithelial cells of renal tubules had been characterized with full timely dynamics. The mitophagy reaches its peak within 14 days after skin burn and it becomes redundant or even excessive. A sign of mitophagy excessiveness is an increase of number of mitophagolysosomes and their intensive transformation into autophagic vacuoles, which finishes with a massive autophagic vacuolization of some epithelial cells the cytoplasm. Such vacuolization (the vacuole's contents are removed out of the cytoplasm) results in formation of numerous defects of cytomembrane (a consequence of "vacuole explosion"), cell disruption by type of necrosis and its desquamation into the tubular lumen.

3. Within a long period of time after the last administration of gekoton (21 and 30 days after skin burn, that means, 14 and 23 days after the last infusion) we have not noted the mitophagy in the cells of the renal medulla in rats with burns. Instead of it, the peeling of the deformed hyperosmotic cells off the basement membrane is observed, what morphologically corresponds to a kind of classical apoptosis, that means, to anoikis.

4. The comparison of terms of mytophagy progress, as well as the induction of mitochondria hypertrophy and hyperplasia in the cells of the renal medulla in rats given gekoton, with the terms of progress of anoikis allows us to suppose that during a certain period of time (21 and 30 days after the skin burn) an unblocking of mitochondrial transduction of apoptotic signal is performed. These data are not only a morphologic sign of cytoprotective effect of gekoton, but also an evidence of the existence of a period of time for an optimal cytoprotective effect of the drug in case of its short – time (a daily injection during the first 7 days) administration.

Prospects of further research: we consider that the obtained results are a peculiar control and they are necessary for an interpretation in comparison with the data which are to be obtained during the investigation of the modifications of structural components of the renal medulla under conditions of an infusion of other combined hyperosmolar solutions.

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Реферати

МОРФОЛОГІЧНИЙ СТАН МОЗКОВОЇ РЕЧОВИНИ НИРКИ ЩУРІВ ПРИ ЕКСПЕРИМЕНТАЛЬНІЙ ОПІКОВІЙ ТРАВМІ ШКІРИ ЗА УМОВ ЗАСТОСУВАННЯ ІНФУЗІЇ ГЕКОТОНУ

Маліков О. В.

В статті представлені структурні зміни мозкової речовини нирки при експериментальній опіковій травмі шкіри у щурів за умов застосування внутрішньовенної інфузії колоїдно-гіперосмолярного розчину гекотону. З'ясовано, що гекотон діє на структуру органа як цитопротектор. Цитопротекторний ефект обумовлений індукцією гіпертрофії та гіперплазії мітохондрій. Також він діє як стимулятор мітофагії та інгібітор мітохондріальної трансдукції апоптозного сигналу. Одержані результати є своєрідним контролем і необхідні для інтерпретації у співставленні з даними, які мають бути отримані при дослідженні змін структурних компонентів мозкової речовини нирки за умов застосування інфузії інших комбінованих гіперосмолярних розчинів.

Ключові слова: опік, мозкова речовина нирки, світлова та електронна мікроскопія.

Стаття надійшла 16.03.18 р.

МОРФОЛОГИЧЕСКОЕ СОСТОЯНИЕ МОЗГОВОГО ВЕЩЕСТВА ПОЧЕК КРЫС ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ОЖОГОВОЙ ТРАВМЕ КОЖИ ПРИ УСЛОВИИ ПРИМЕНЕНИЯ ИНФУЗИИ ГЕКОТОНА

Маликов А. В.

В статье представлены структурные изменения мозгового вещества почек при экспериментальной ожоговой травме кожи у крыс при условии применения инфузии коллоидно-гиперосмолярного раствора гекотона. Выяснено, что гекотон действует на структуру органа как цитопротектор. Цитопротекторный эффект обусловлен индукцией гипертрофии и гиперплазии митохондрий. Также он действует как стимулятор митофагии и ингибитор митохондриальной трансдукции апоптозного сигнала. Полученные результаты являются своеобразным контролем и необходимы для интерпретации в сопоставлении с данными, которые должны быть получены при исследовании изменений структурных компонентов мозгового вещества почек в условиях применения инфузии иных комбинированных гиперосмолярных растворов.

Ключевые слова: ожог, мозговое вещество почки, световая и электронная микроскопия.

Рецензент Шепітько В.І.