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STUDY OF THE ANESTHETIC SOLUTIONS` EFFECT ON THE ULTRASTRUCTURE OF THE MUCOUS MEMBRANE OF THE GUMS AND MUSCLES OF RATS

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Improving the safety and efficiency of anesthesia in the treatment of oral diseases is one of the pressing problems of modern dentistry, where the choice of anesthetic solution is important, taking into account its physicochemical properties. The presence of vasoconstrictors in the local anesthetic solution improves the quality of anesthesia, but at the same time creates a number of relative and absolute contraindications for the use of such drugs. A reliable resolution to this problem can be the development of a new composition of anesthetic solution based on sodium hyaluronate and without the use of vasoconstrictors. To study the direct effect of the proposed solution on soft tissues in the area of its administration, their ultrastructure was researched. Analysis of the results of the study showed that changes in the ultrastructure of tissues in the area of administration of solutions occurred in all experimental groups of animals in comparison with the intact group and had both common features and certain differences. Ultrastructural changes in the mucous membrane and muscles in animals after the introduction of a new composition of the anesthetic were characterized by the presence of mitochondrial and ribosomal reactions, which may be due to the activation of hyaluronidase and protein synthesis processes. 7 days after administration of the solutions, normalization of the ultrastructure of the mucous and masticatory muscles was observed in all experimental animals, which indicates their reverse and functional nature.

Key words: sodium hyaluronate, electron microscopy, tissue ultrastructure, dental anesthetic, experimental animals.

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ВИВЧЕННЯ ВПЛИВУ АНЕСТЕЗУЮЧИХ РОЗЧИНІВ НА УЛЬТРАСТРУКТУРУ СЛИЗОВОЇ ОБОЛОНКИ ЯСЕН ТА М'ЯЗІВ ЩУРІВ

Підвищення безпеки та ефективності анестезії при лікуванні захворювань порожнини рота є однією з актуальних проблем сучасної стоматології, де важливим є вибір розчину анестетика з урахуванням його фізико-хімічних властивостей. Наявність судинозвужувальних засобів у розчині місцевого анестетика покращує якість анестезії, але в той же час створює ряд відносних і абсолютних протипоказань для застосування таких препаратів. Надійним вирішенням цієї проблеми може стати розробка нового складу анестезуючого розчину на основі гіалуронату натрію без використання судинозвужувальних засобів. Для вивчення безпосереднього впливу запропонованого розчину на м'які тканини в зоні його введення досліджено їх ультраструктуру. Аналіз результатів дослідження показав, що зміни ультраструктури тканин у зоні введення розчинів відбувалися в усіх дослідних групах тварин у порівнянні з інтактною групою і мали як спільні риси, так і певні відмінності. Ультраструктурні зміни слизової оболонки та м'язів у тварин після введення нового складу анестетика характеризувались наявністю мітохондріальних та рибосомальних реакцій, що може бути пов'язано з активацією процесів гіалуронідази та синтезу білка. Через 7 діб після введення розчинів у всіх піддослідних тварин спостерігалася нормалізація ультраструктури слизових і жувальних м'язів, що свідчить про їх зворотний і функціональний характер.

Ключові слова: гіалуронат натрію, електронна мікроскопія, ультраструктура тканин, зубний анестетик, експериментальні тварини.

The work is a fragment of the research project "Correction of pathogenetic mechanisms of metabolic disorders in oral tissues in patients depending on environmental and nutritional factors that affect carbohydrate and lipid metabolism", state registration No. 0118U0006966.

Improving the safety and effectiveness of anesthesia in the treatment of oral diseases is one of the urgent problems of modern dentistry. 98 % of all dental interventions are performed using local anesthesia, where the choice of anesthetic solution is important, taking into account its physicochemical properties. The most common anesthetic used for infiltration and block anesthesia are articaine-based anesthetics. Articaine is the first thiophene derivative, and a weak base, slightly soluble in water, therefore it is used in the form of a water-soluble hydrochloric acid salt. For a local anesthetic effect in the tissues, hydrolysis must occur with the formation of a fat-soluble base, which enters the phospholipid membrane of the nerve endings or fibers everywhere. Elimination of articaine is exponential with an elimination half-life of 20 minutes. Since articaine is very rapidly hydrolyzed in the blood, the risk of systemic intoxication is lower [1].

The presence of vasoconstrictors in a local anesthetic solution improves the quality of anesthesia, but creates a number of relative and absolute contraindications associated with hypersensitivity reactions and diseases of the cardiovascular system, but in most cases we observe local complications from anesthesia associated with microtrauma, vasospasm of the microvasculature, a decrease in pH of tissues adjacent to the target site of anesthesia [11].

We believe that the most probable way of solving the set tasks is the development of a fundamentally new composition of a local anesthetic solution without the use of vasoconstrictors and preservatives by introducing a gel-like agent into its composition, due to which the consistency of the solution will change, absorption in tissues will slow down, the pH of the solution will increase, which will increase the efficiency and safety of local anesthesia [3].

As such an agent, hyaluronic acid can be chosen, which is a natural substance of the body, synthesized by a class of built-in membrane proteins by hyaluronate synthetases and biodegradable using a family of hyaluronidase enzymes [5]. Due to its physicochemical properties, hyaluronic acid has been widely used in medicine in recent decades, in particular in ophthalmology, cosmetology, traumatology, arthrology, dentistry and other branches of medicine [6, 7]. In the medical scientific literature, the results of many experimental studies have been published, where, using morphology, histochemistry, and electron microscopy, it has been established that: hyaluronic acid can cause a hydrating effect in tissues. serve as a carrier of bioactive components; temporarily embedded in a matrix of glucosaminoglycans and intercellular proteins forming a protective barrier of cells against toxic compounds; has a specific ability to retain water and form proteoglycan aggregates, thereby strengthening the functions of connective tissue (trophic, barrier, plastic), providing elasticity and resistance to external stimuli [8].

Hyaluronic acid is distinguished by its origin: synthetic and extracted from biological material, stabilized or unstabilized, as well as by molecular weight, which significantly affects its properties. The most optimal for our study was the choice of synthetic unstabilized hyaluronic acid with a molecular weight of about 2600 kDa [9]. This type of sodium hyaluronate is already used in medicine, in particular, in ophthalmology for the introduction into the eye, as well as in other surgical operations [10]. There is also already extensive experience in the use of injectable derivatives of hyaluronic acid with a local anesthetic to reduce pain during invasive procedures [2].

Electron microscopy is one of the main methods for studying the state of tissues at the cellular level, widely used in fundamental and applied research for the structural and functional analysis of its vital activity in normal conditions and with various types of intervention [12].

Therefore, we consider it expedient to study the biological effect of the proposed composition for local anesthesia, containing sodium hyaluronate, anesthetic and water as a solution, on the ultrastructure of tissues in the area of injection.

The purpose of the work was to study the ultrastructure and evaluate the nature of changes in the soft tissues adjacent to the lower jaw of experimental animals during infiltration with their traditional dental anesthetic and the proposed gel-like anesthetic composition based on sodium hyaluronate.

Materials and methods. The proposed injectable gel-like aqueous composition for local anesthesia contains: sodium hyaluronate (HYARAL PLUS, state registration certificate 711/12-300200000) – 0.0018 g; anesthetic Lidocaine 2 % – 0.04 g; water for injections up to 1 ml; the viscosity of the solution at 20°C – 5 mPa·s, pH=5 [2].

A patented form of articaine-based dental anesthetic was used as a local anesthetic ("ARTIFRIN-ZDOROVYA" Manufacturer: Limited Liability Company "Pharmaceutical Company "Zdorovya", Ukraine). Experimental studies were conducted on the basis of the laboratory of biochemistry and vivarium SE "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine".

The study included male Wistar rats of herd breeding weighing 217–281 grams, divided into four groups. A total of 21 animals were used in the experiment. The intact group (group 1) consisted of three animals, the other groups – six animals. In the control group (group 2), 0.02 ml of sterile water for injection was injected into the soft tissues of the lower jaw in the area of attachment of the masticatory muscle and the attached gum of the lower jaw from the opposite side with an insulin syringe. Animals of the first experimental group (group 3) were injected with 0.02 ml of anesthetic ARTIFRIN-ZDOROVYA by the same method. Animals of the second experimental group (group 4) were injected with 0.02 ml of the proposed injectable gel composition for local anesthesia.

The animals were removed from the experiment in two stages (three animals from the control and each experimental group) two hours after the administration of the drugs and after 7 days. Euthanasia of experimental animals was carried out in a state of deep anesthesia in accordance with the "European

Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" using sodium thiopental. (40 mg/kg rat weight) [4].

The objects of study were fragments of soft tissues adjacent to the lower jaw of experimental animals: the mucous membrane of the gums and masticatory muscles.

For electron microscopic examination, areas of the gingival mucosa and muscles of rats were fixed in a 2.5 % glutaraldehyde solution in phosphate buffer at a pH of 7.4 with additional fixation with a 1 % osmic acid solution at the same buffer pH. The samples were then dehydrated in alcohols of increasing concentration. Impregnation of tissues and their polymerization took place in a mixture of epon-araldite resins. The contrast of ultrathin sections was performed according to the Reynolds method. Objects were studied and photographed in an electron microscope PEM-100. The work was carried out in the group of electron microscopy of the laboratory of pathological anatomical and electron microscopic studies SE "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine".

Results of the study and their discussion. The mucous membrane of the gums of intact animals (group 1) was characterized as follows: the epithelial layer is preserved, the cells of the basal layer are located in a wave-like manner in relation to the lamina propria and had a round, oval or cylindrical shape, containing a typical set of organelles and large nuclei, mainly round and oval in shape. Some large nuclei contained two nucleoli and deep folds of the karyolem, or its invagination was observed. Intercellular contacts were preserved, and numerous desmosomes were well defined. The cells of the granular layer contained a small number of keratohyalin granules. The surface layers of the epithelium were without visible structural changes. Muscle tissue had a predominantly normal ultrastructure, well-defined myofibrils and contractile structures.

In the first part of the experiment, 2 hours after the injections in the control group (group 2), free ribosomes were determined on the background of the preserved epithelial layer of the gingival mucosa in the cytoplasm of the cells of the basal layer. In some cells, mitochondrial vacuolization took place. In the papillary layer of the lamina propria, loosely located collagen fibrils and their individual bundles, single cells of connective tissue and microvessels with a normal ultrastructure were observed. In some areas, homogeneous material was observed. In the reticular layer, bundles of collagen fibrils were located somewhat sparsely (fig. 1a).

Fragments of the cytoplasm of muscle cells with slightly cleared sarcoplasm were located between the myofibrils in the muscle tissue; the sarcosomes were large and had an increased electron density of the matrix. Areas where muscle cells with signs of hydropic dystrophy were centrally identified. Swelling of hyaloplasm and membrane organelles was observed in them. Myosatellitocytes that were located at the edge of the fiber had a normal ultrastructure (fig. 1b).

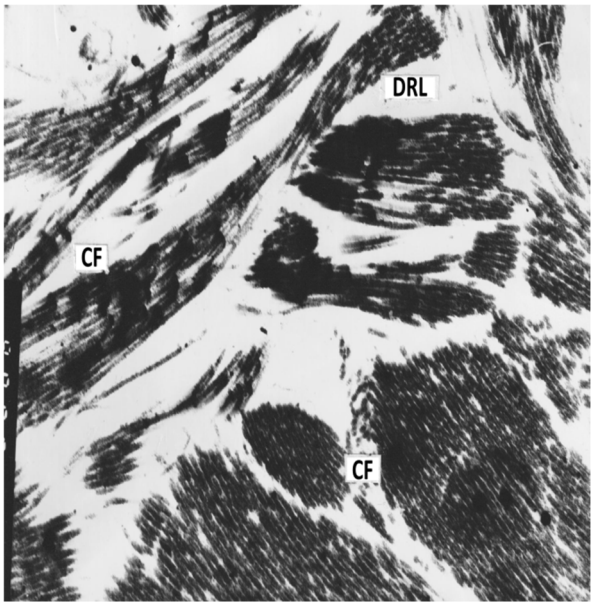

Fig. 1a Ultrastructure of the gingival mucosa of rats in the control group. Group 2. Sparse arrangement of collagen fibril bundles in the reticular layer.
Electronic microphotography: X 10 000.

Note: DRL – deep reticular layer of the lamina propria, CF – collagen fibrils.

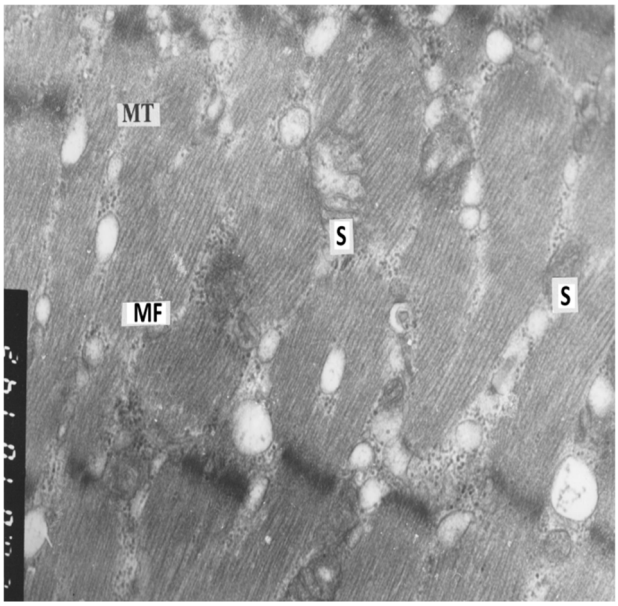

Fig. 1b The ultrastructure of the muscular tissue of the gums of the rats of the control group. Group 2. Light hydropic changes in the cytoplasm of individual myosatelytocytes of muscle tissue.

Electronic microphotography: X 15 000.

Note: MT – muscle tissue, MF – myofibrils, S – sarcosomes

In the epithelium of the gingival mucosa of group 3 rats, 2 hours after anesthesia with an anesthetic based on articaine, reactive changes in mitochondria in the ultrastructure of individual epithelial cells were found, which were more significant than in other groups of animals (fig. 2a).

The ultrastructure of the lustrous and stratum corneum was unchanged.

In the lamina propria, the structures of the papillary layer of connective tissue were within normal limits. We also did not find any special changes in the reticular layer. In the cells of the granular layer of the epithelium, there were signs of edema of the hyaloplasm and membrane organelles.

Signs of edema of sarcoplasma and organelles were observed in muscle tissue under standard anesthesia, as well as in animals of the control group.

At the same time, free ribosomes are found in it. The muscle tissue had a predominantly normal ultrastructure with the exception of minor areas with elements of hydropic dystrophy in its cells and revealed changes in mitochondria with almost complete destruction (fig. 2b). In addition, in some cells, hyaloplasmic edema was observed and the number of organelles was reduced, that is, the existing phenomena of hydropic dystrophy.

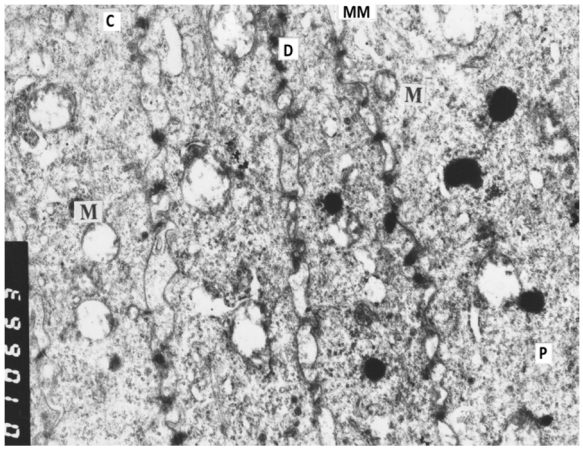

Fig. 2a Ultrastructure of rat gingival mucosa after standard anesthesia. Group 3. Epithelial cells with signs of edema of the hyaloplasm and membrane organelles.

Electron micrograph: X 10 000.

Note: MM - mucous membrane, M - mitochondria, C – cytoplasm, D – desmosomes, P – polysomes.

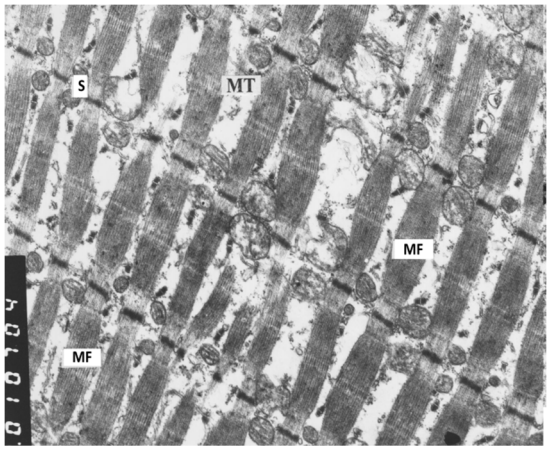

Fig. 2b Ultrastructure of rat muscle tissue after standard anesthesia. Group 3.

Hydropic dystrophy of symplasts.

Electron micrograph: X 10 000.

Note: MT – muscle tissue, S – sarcosome, MF – myofibrils.

Thus, in the epithelial cells of the mucosa and muscles, more significant changes in mitochondria were observed.

In the epithelium of the mucous membrane of group 4 rats after anesthesia of the proposed injection gel composition in the basal and spinous layers of the epithelium, cells in different functional states were observed.

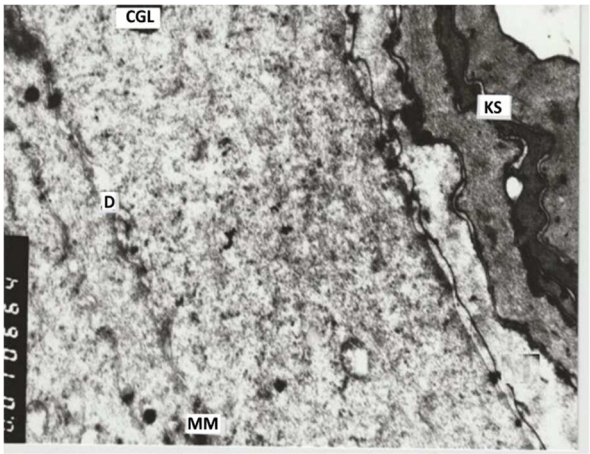

Fig. 3a Ultrastructure of rat gingival mucosa after anesthesia with a new drug. Group 4. Cells of the granular layer of the epithelium with signs of edema of the hyaloplasm and membrane organelles, cells of the stratum corneum of increased electron density.

Electron micrograph: X 10 000.

Note: MM – mucous membrane, CGL – cells of the granular layer, D – desmosomes, KS – keratinized scales.

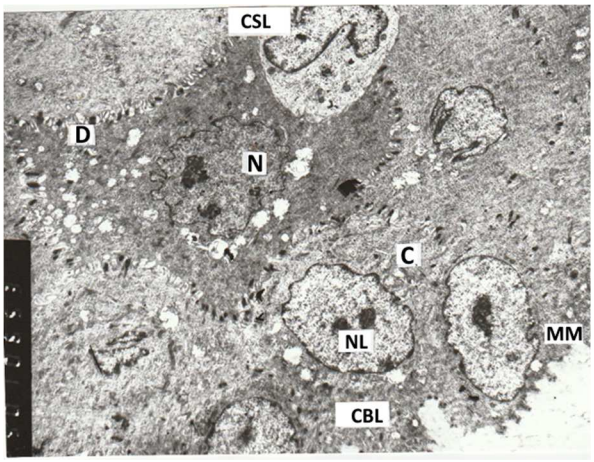

Fig. 3b Ultrastructure of rat gingival mucosa after anesthesia with a new drug. Group 4. In the cells of the basal and spinous layers of the epithelium, a large number of free ribosomes and vacuolization of mitochondria in their individual cells.

Electron micrograph: X 10 000.

Note: MM – mucous membrane, CBL – cells of the basal layer, CSL – cells of the spinous layer, N – nucleus, NL – nucleolus, C – cytoplasm, D – desmosome.

Some of the cells showed signs of edema of both cytoplasm and karyoplasm, and mitochondrial vacuolization was also determined (fig. 3 a).

At the same time, signs of active protein-synthesizing activity were observed in them, as evidenced by the increased content of free ribosomes in the cells, and the nuclei contained two nucleoli, one of which was densely located near the plasmolemma. Other cells were found with an increased electron density of the cytoplasm and with a large number of polisome and ribosomes, as well as with nuclei that had deep karyolem folds (fig. 3b, 3c).

Superficial cells were without structural changes. The lamina propria structures were in a normal state.

In the muscle tissue of this group of animals in relation to intact animals and after standard anesthesia, hyaloplasmic edema was observed to a somewhat greater extent in its cells. Vacuolization of part of mitochondria or partial destruction of their crista was also observed, but in smaller quantities than in the previous group. At the same time, the number of free ribosomes was increased, ie, protein-synthetic processes were more active (fig. 3d).

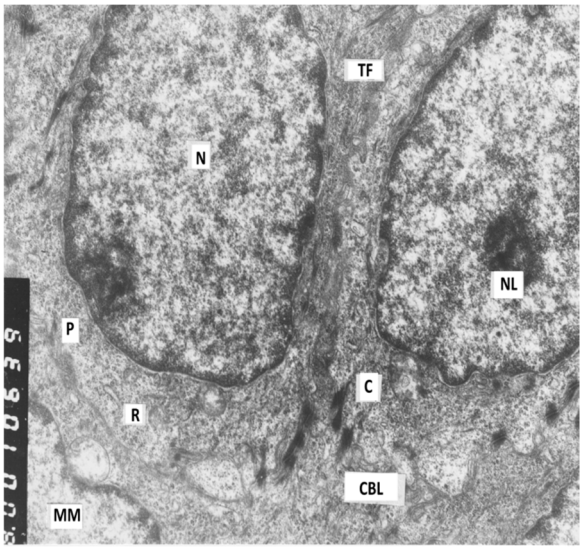

Fig. 3c Ultrastructure of rat gingival mucosa after anesthesia with a new drug. Group 4. Cells of the basal layer of the epithelium with an increased content of polysome and free ribosomes.

Electron micrograph: X 10 000.

Note: MM – mucous membrane, CBL – cells of the basal layer, N – nucleus, NL – nucleolus, C – cytoplasm, R – free ribosomes, P – polysomes, TF – tonofibrils.

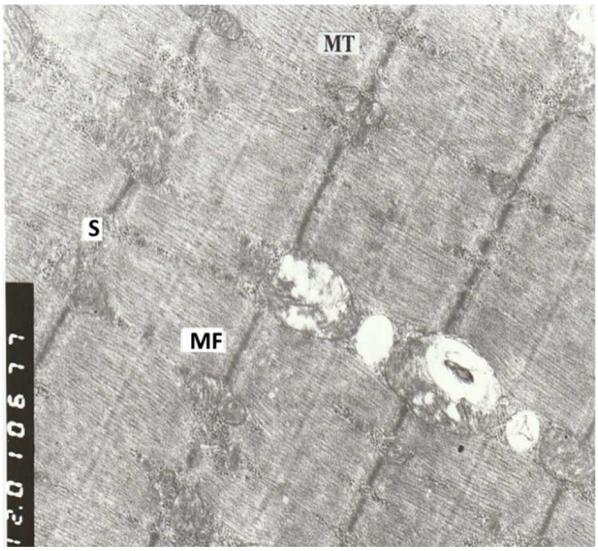

Fig. 3d Ultrastructure of rat muscle tissue after anesthesia with a new drug. Group 4. Vacuolization of individual mitochondria of muscle cells.

Electron micrograph: X 12 000.

Note: MT – muscle tissue, S – sarcosome, MF – myofibrils.

In the second part of the study, the animals were removed from the experiment after 7 days to analyze the long-term results of exposure to anesthetic solutions directly on the “target tissue”.

In all studied groups, after a week, the electron microscopic picture of tissue sites did not differ from intact animals and had a typical structure. (fig. 4a, 4b, 4c).

The epithelial layer of the gingival mucosa was preserved, the cells of the basal membrane were predominantly oval-cylindrical in shape and contained a typical set of organelles. Cell ultrastructures and intercellular contacts were preserved. Superficial layers of the epithelium without visible structural changes. The muscle tissue had a characteristic ultrastructure, myofibrils were clearly visualized, the structures of the contractile apparatus were present.

Analysis of the results of the study showed that changes in the ultrastructure of tissues in the area of administration of solutions occurred in all experimental groups of animals in comparison with the intact group and had both common features and certain differences. Thus, the common features in the ultrastructural picture were probably due to the factors of tissue injury during injections - the introduction of a needle and an increase in pressure in the tissues during the administration of solutions, as well as injury during material collection.

In our opinion, the changes in the tissues of the animals of the control group were due to tissue hydration, which is a common occurrence when sterile water with a neutral pH value and the opposite is introduced.

After the introduction of an anesthetic based on articaine in animals of the third group, changes in the ultrastructural picture of the mucous membrane of the gums and masticatory muscle had more

pronounced excellent signs compared to the control. Most modern anesthetics have a pH of 3–3.5, containing epinephrine, sulfites and parabens as preservatives, which not only increase the risk of developing general hypersensitivity reactions to these drugs and complications in the work of the cardiovascular system, but can negatively affect tissues right at the site of their introduction. Therefore, isolated epithelial and muscle cells with hydropic changes in the karyoplasm and hyaloplasm and vacuolization of individual mitochondria with destruction of part of the cristae were identified, and the expansion of the cisterns of the granular endoplasmic reticulum, in our opinion, may be due to the action of a vasoconstrictor. It should be noted that the changes are of a functional nature and are reversed.

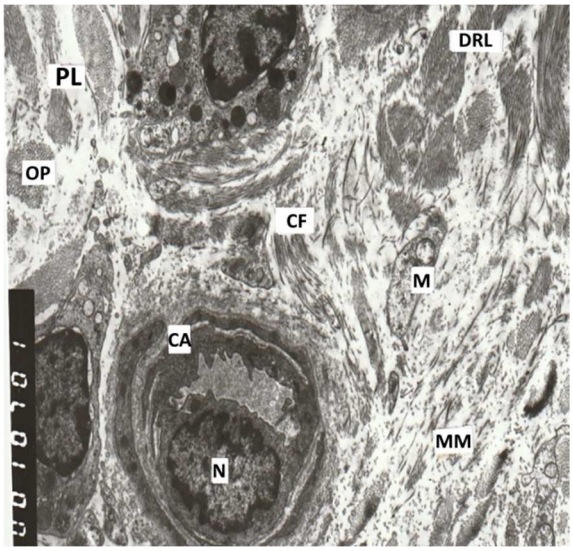

Fig. 4a Ultrastructure of the gingival mucosa of the rat of the control group after 7 days. Group 2. A fragment of the lamina propria of the papillary layer with connective tissue structures in a normal state.

Electron micrograph: X 10000.

Note: MM – mucous membrane, PL – papillary layer, OP – own plate, N – nucleus, CA – capillary, CF – collagen fibrils, DRL – deep reticular layer of the lamina propria.

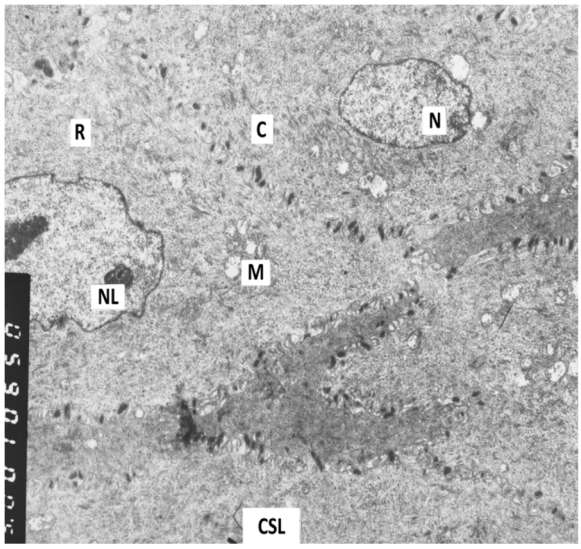

Fig. 4b Ultrastructure of the rat gingival mucosa after standard anesthesia after 7 days. Group 3. The ultrastructure of the spinous layer of the epithelium is close to normal.

Electron micrograph: X 10 000.

Note: CSL – cells of the spinous layer, N - nucleus, NL – nucleolus, C – cytoplasm, M – mitochondria, R – free ribosomes.

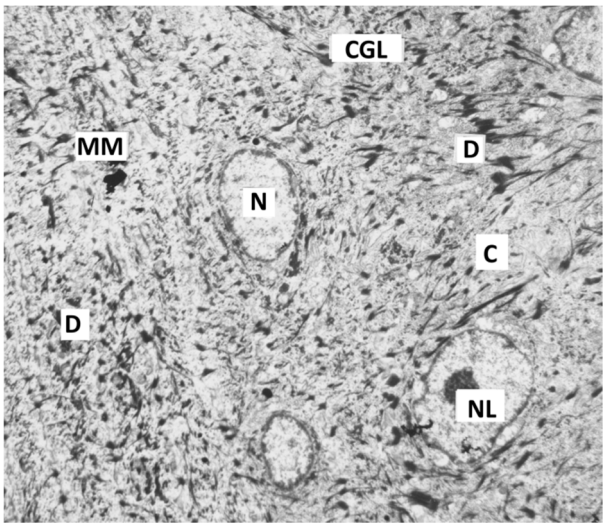

Fig. 4c. Ultrastructure of rat gingival mucosa after anesthesia with a new drug after 7 days. Group 4. The structure of cells of the granular layer of the epithelium.

Electron micrograph: X 10 000.

Note: MM – mucous membrane, CGL – cells of the granular layer, N – nucleus, NL – nucleolus, D – desmosome, C – cytoplasm.

Differences in ultrastructural changes in the mucous and masticatory muscles in animals of the fourth group after the introduction of a new composition of the anesthetic with hyaluronic acid were also more pronounced in comparison with the control group and had a number of characteristic features. In our opinion, this may be signs of an aseptic inflammatory reaction: tissue edema, loosening of the structures of the external cellular matter, slight diffuse infiltration by macrophages and, to a lesser extent, lymphocytes. In the cells of the gingival mucosa, hyaloplasmic edema, mitochondrial and ribosomal reactions were more often observed. It should be noted that, taking into account the biochemical mechanism of action of hyaluronic acid, these changes were expected and characteristic, as evidenced by the already known studies [5, 6]. Gradually, under the influence of tissue hyaluronidases and free radicals, the injected hyaluronic acid was biodegraded into low molecular weight fragments with bioactive properties and affecting cell proliferation, differentiation, migration and angiogenesis [9].

Thus, in our opinion, the leading factors of such an ultrastructural picture were tissue hydration, active work of hyaluronidase, and activation of protein-synthesizing processes in cells, which can enhance regenerative processes with the proliferation of gingival cells, as well as the biosynthetic activity of muscle tissue cells [4, 12]. These changes were also functional and reversed.

In the second part of the study, 7 days after the administration of the drugs, all animals of the control and both research groups showed a normalization of the ultrastructural picture of the mucosa and muscles and practically did not differ from intact animals. The absence of irreversible changes in the areas of administration of solutions in all animals of the second part of the experiment gives the authors of the study reason to consider the researched solutions relatively safe.

Conclusions

1. Changes in the ultrastructure of tissues in the area of administration of solutions occur in all experimental groups of animals in comparison with the intact group and have both common features and certain differences.

2. After the administration of an anesthetic based on articaine, changes in the mucous membrane and muscles in animals show signs of ischemia, which may be due to the action of a vasoconstrictor.

3. Ultrastructural changes in the mucous membrane and muscles in animals after the introduction of a new composition of the anesthetic were characterized by the presence of mitochondrial and ribosomal reactions, which may be due to the activation of hyaluronidase and protein synthesis processes.

4. In all experimental animals, 7 days after administration of the solutions, normalization of the ultrastructure of the mucous and masticatory muscles was observed, which indicates their reverse and functional nature.

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