Experimental modelling of nerve injury was carried out by its clamping and subsequent treatment with drug product Nucleo C. M. P. Forte. Ultrastructural study of femoral nerve after clamping detected significant dystrophic and destructive changes in axoplasm of axis cylinders of nerve fibers in rats not treated with the drug. Treatment with Nucleo C. M. P. Forte was found to be associated with significantly less pronounced ultrastructural changes as compared to the rats receiving no treatment after femoral nerve clamping. Taking into consideration all abovementioned ultrastructural morphological signs, it can be confidently stated that this drug product improves the recovery of nerve fiber, acting as a neuroprotective and restorative agent at tissue and cellular levels.

Key words: mandibular fracture, nerve injury, Nucleo C. M. P. Forte, ultrastructure.

Introduction. The search of ways and methods of therapeutic influence on the processes taking place in the nerves injured as a result of jaw fractures, in order to enhance their restoration, remains one of the urgent problems of modern operative dentistry. Treatment of peripheral nerve damage is not always successful and presents a great challenge for dentists. It is associated with the diversity of clinical manifestations, the presence of various complications (pain syndrome, motor and trophic disorders, contractures, etc.). From 60 to 80% of mandibular fractures occur at the site of mandibular canal, that is, in the area of jaw angle, molars and premolars. Usually the patients with mandibular fractures do not receive adequate drug therapy aimed at preservation and restoration of the damaged inferior alveolar nerve viability, or the treatment is ineffective because of its late administration. Therefore, the development of treatment modalities aimed at accelerated restoration of inferior alveolar nerve viability, can considerably reduce the time of treatment. One of the most common complications after mandibular fractures is the damage of inferior alveolar nerve in mandibular canal. In addition to anatomically determined causes, inferior alveolar nerve injury may occur as a result of errors in surgical treatment. Nerve dysfunction of varying degrees occurs as immediate injury of the nerve in the damage of mandibular canal during fragment displacement, as well as in compression of the nerve caused by postoperative edema or hematoma in canal lumen [1–3].

Whatever the type of nerve injury in the canal, compression and toxic injury of inferior alveolar nerve occurs [8–10]. This complication is manifested by the absence of tissue sensitivity in innervation zone or its long-term change in the form of anesthesia, hyperesthesia or paresthesia of the skin in the area of the chin, vermillion border and mucous membrane of lower lip. Nerve injury may be followed by prolonged face pain of varying intensity and paroxysmal character, leading to emotional and stress-induced disorders as well as to worsening of life quality of the patients [5]. The moment of damage to the nerve during the injury is the initial stage of its serious long-lasting changes. The process of axon alterations incomplete nerve transection have laborately been studied by histopathologists. But inferior alveolar nerve is rarely completely transected. Therefore, there is a need for deep study of possibilities to preserve and restore the structure of compressed nerve.

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The problem of restoration of inferior alveolar nerve function directly depends on the duration of nerve compression in the mandibular canal, because the process of rehabilitation is influenced essentially by the factors of toxic effect of decay products on the neurovascular bundle and impaired adequate blood supply to the nerve itself and the tissues it innervates [4, 7]. Early diagnosis of the damaged branches of trigeminal nerve in fractures is important as traumatic neuritis is one of the causes of disturbed reparative regeneration. The damage of trigeminal nerve was established to cause a number of functional and morphological alterations in the facial tissues and oral cavity organs [6]. The problem of preservation and restoration of the nerve after the injury is still of great significance. Therefore, further development of treatment modalities of this pathology is reasonable and well-grounded.

**Objective.** The aim of the study was to evaluate experimentally on laboratory rats the efficacy of the drug product Nucleo C. M. P. Forte in prevention and restoration of morphologic disturbances of the nerve fibers in modelling of nerve injury.

**Materials and Methods.** To achieve the target goal an experimental study was carried out. Because of morphologic similarity of inferior alveolar nerve and the femoral nerve of rats, ultrastructural changes in that particular nerve were studied in modelling nerve injury by the method of clamping. An experimental study was conducted on Wistar rats at the vivarium of Vinnitsa National Pirogov Memorial Medical University. Modelling of mechanical injury of the nerve by means of experimental femoral nerve clamping was performed. The surgical intervention consisted of mobilization of the nerve followed by its clamping with a soft Bilroth’s clamp for 5 minutes. The animals were followed-up, treated with Nucleo C. M. P. Forte for 10 days, and the samples were taken for histological examination at the end of the study.

The study lasted for 14 days. Three groups of experimental animals were selected: group 1 (7 animals) – intact rats; group 2 (7 animals) – those operated on by nerve compression with no use of the drug; group 3 (7 animals) – those operated on with compression of the nerve and treated with Nucleo C. M. P. Forte. The drug dose was calculated according to the protocol on preclinical drug studies. All the animals were males. Operative intervention and withdrawal from the experiment were carried out under anesthesia – intraperitoneal administration of Propofol Novo (Fresenius Kabi, Austria) at a dose of 60 mg/kg. For the histological examination, the nerve segments were taken at the point of clamping, 1 cm proximal and 1 cm distal to the site of compression. At the end of the study, electron microscopic study of ultra-thin sections was conducted.

**Results.** The femoral nerve structure of intact rats was studied first to select the comparison group. Electron microscopic study of the femoral nerve showed the presence of axis cylinders covered with myelin layer. In the axoplasm of axis cylinders, there were neurofilaments and neurotubules, large mitochondria with densely packed cristae and moderately osmiophilic matrix, as well as micro-vesicular vesicles. Meissaxon whorls had circular direction and were densely packed. In some places there were oblique-oriented myelincisures. In the axoplasm of the sites adjacent to nodes of Ranvier there were many multivesicular bodies. In the cytoplasm of lemmocytes there were tubules of endoplasmic reticulum, Golgi complex cisterns, mitochondria and free ribosomes. The nuclei of Schwann cells were oval-shaped and moderately osmiophilic. Nerve fibers were surrounded by basal membrane (Fig. 1).

In the group of rats with no use of the drug swelling, inflammatory infiltration and degeneration phenomena were noted in the proximal portion of the femoral nerve of the rats on the 14th day after clamping. Axis cylinders were swollen. Nervous fibers formed regeneration neuroma indicating the regeneration of destroyed fibers. Regenerating cylinders with no distinct orientation were seen between the nervous fibers. Ultrastructural study revealed dystrophic and destructive changes in axoplasm of axis cylinders after clamping of the proximal portion of the femoral nerve. In the axoplasm of axis cylinders there were regions of cytolysis, destructively altered mitochondria, dilated tubules of endoplasmic reticulum and cistern, as well as the are as with no ribosomes and Golgi complex. The cytoplasm of lemmocytes contained the regions of
cytolysis, destructively altered mitochondria, dilated tubules of endoplasmic reticulum and Golgi complex as well. Myelin was deformed, there were additional myelin whorls located both in the cytoplasm of neurolemmocytes and in the axoplasm of axis cylinders (Fig. 2).

**Fig. 1.** Myelin fiber ultrastructure of the femoral nerve in intact rats:
- 1 – axoplasmofaxis cylinder; 2 – mitochondria; 3 – vesicular bodies; 4 – myelin sheath; 5 – lemmocyte cytoplasm

**Fig. 2.** Ultrastructural changes in myelinated fibers of proximal portion of the femoral nerve, the 14th day after clamping, notreatment:
- 1 – narrowed axis cylinder; 2 – deep dystrophy of axoplasm; 3 – deformed myelin; 4 – additional myelin whorls

In the group of rats without use of the drug ultrastructural study of the site of femoral nerve clamping demonstrated homogenized and compacted axoplasmaxis cylinders, absence of longitudinal orientation of microfilaments and neurotubuleson the 14th day after clamping. Along with the disorganized axis cylinders, axon shaving even contours, cleared axoplasm and a large number of mitochondria were found. Detached from the myelin sheath fragments with a circular orientation of lamellae were seen. Such elements occurred both in the cytoplasm of the lemmocyte sand in the thickness of myelin. In some nerve fibers myelin sheath was homogeneous and structureless. In other nerve fibers, the myelin sheath appeared as randomly oriented filamentous for-
In the group of rats with no drug use swelling, inflammatory infiltration and degenerative changes were observed in the distal portion of femoral nerves on the 14th day after clamping, being more evident than those in the proximal portion. After femoral nerve clamping ultrastructural destructive alterations in axis cylinder axoplasm were more pronounced in the distal portion than in the proximal one. Fragmentation and swelling of axis cylinders, their indistinct orientation were detected. In the axis cylinder axoplasm there were the regions of cytolysis, destructed mitochondria, dilated tubules of endoplasmic reticulum containing no ribosomes, and Golgi complex cisternae. There were disorganized fibers containing constricted axis cylinders. Some nerve fibers contained no axis cylinders. Clearing of Schwann cell cytoplasm with the signs of its swelling as well as nuclei pyknosis were seen. Perinuclear spaces were enlarged. Myelin was disorganized. Myelin deformation and fragmentation, loss of organized alignment of myelin plates and their loosening; 5 – necrotic change of neurolemmocyte nucleus; 6 – vacuolar dystrophy of Schwann cell cytoplasm

The ultrastructure of proximal portion of the femoral nerve of rats receiving Nucleo C. M. P. Forte therapy had been subjected to changes as well, compared to intact animals. Increased myelin thickness, enlarged spaces between mesaxon whorls, curved layers of mesaxons, enlarged periaxonal spaces were observed. There were the signs of nerve fibers dystrophy manifested by irregular contours. Schwann cells were enlarged in sizes with increased number of entoblasts in their nuclei, indicating the beginning of regeneration neuroma formation. Degenerative alterations and disorganization of myelin sheath were detected (Fig 5).
In 14 days after clamping of the femoral nerve the signs of swelling were less evident in the site of compression of the rats treated by Nucleo C. M. P. Forte than in those undergoing clamping of the femoral nerve with no use of the drug. Axis cylinder swelling in the site of clamping was less evident as well. Isolated regenerating cylinders were located between the nerve fibers but unlike the rats undergoing clamping of the femoral nerve with no use of the drug, they were oriented along the nerve fibers. In the rats undergoing clamping of the femoral nerve with no use of the drug there were much more such cylinders with the signs of regeneration, and they were randomly located and formed regeneration neuroma (Fig. 6).

After the treatment ultrastructural changes both in proximal and distal portions of the femoral nerve were less evident than in the rats with no use of the drug. Nerve column swelling and nerve fibers degeneration were less pronounced as well. Organelle clusters in axon cytoplasm and Schwann cell swelling were found. However, along with isolated myelin fibers with destruction signs there were the fibers with insignificant changes as well as the fibers similar to those in intact rats. In the distal portion of the femoral nerve, granular endoplasmic reticulum profiles in axis cylinder axoplasm
were enlarged in diameter with distinct contoured membrane indicating increased intracellular regeneration. Shortened mitochondrial cristae and reduced matrix density were detected. There were multiple clusters of mitochondria and endoplasmic reticulum profiles. Schwann cell structure was altered. Ovoid bodies consisting of concentrically located plates were often determined both in Schwann cell cytoplasm and axis cylinder axonema. Hypertrophy of Schwann cell nuclei was observed. Well organized Golgi complex and endoplasmic reticulum tubules as well as numerous mitochondria were located in their cytoplasm. The structure of mitochondria was similar to that of intact animals (Fig. 7).

**Fig. 6.** Ultrastructure of the femoral nerve of rat sat the site of clamping, the 14th day after clamping, treatment with Nucleo C.M.P. Forte:
1 – hypertrophy of Schwann cell nucleus; 2 – alterations in axis cylinder with preserved myelin; 3 – swelling around the axis cylinder in the site of clamping; 4 – curved contours of axis cylinder axolemma; 5 – enlarged periaxonal space filled with hydropic fluid

**Fig. 7.** Ultrastructure of the distal portion of the femoral nerve of rats, the 14th day after clamping, treatment with Nucleo C.M.P. Forte:
1 – hypertrophy of Schwann cell nuclei; 2 – alterations in axis cylinder with preserved myelin; 3 – irregularly enlarged periaxonal space; 4 – Schwann cell cytoplasm

**Discussion.** Comparison of the studied groups of rats demonstrated the following data. Electron microscopic study of femoral nerve of rats after clamping without drug use revealed severe damage of myelin and axoplasm. Axoplasm was cleared having the signs of swelling. There was a decreased number of microtubules and neurofilaments. Endoplasmic reticulum cisternae were enlarged. Periaxonal space was enlarged and filled with hydropic fluid. Axons were corrugated. Axolemma structure was damaged, homog-
enized and thickened having curved contour. In periaxonal spaces multimembranous complexes, osmiophilic lysosomal bodies, multivesicular bodies, vacuoles, membrane fragments were often observed. Axons often had semilunar shape and were located in axolemma duplicate. In some axis cylinders the axoplasm was homogenized and dense. Axons with cleared axoplasm were located along with corrugated axis cylinders having the cytoplasm of high electronic density. Laminar structure of myelin sheath was damaged. Focal loss of regular alignment and degradation of myelin sheath with chaotic lamina orientation were noted. There was the swelling of in homogenous cytoplasm. The zones of decreased electronic density containing no organelles, bordered with the zones of focal organelle clusters. Enlarged elements of Golgi apparatus, swollen cisternae of endoplasmic reticulum were seen, multivesicular bodies were present. The number of vesicles bound to Schwann cell sheaths with medium electronic density contents was much larger than that in intact rats. There were isolated regions of indistinct plasmolemma with thickened basal membrane in those sites. Swollen mitochondria, decreased number of cristae, fine-grained matrix with cleared regions were detected.

After treatment with the drug product Nucleo C. M. P. Forte electron microscopy demonstrated much better histologic structure of nerve tissues. Electron microscopic study showed the presence of organelle clusters in axon cytoplasm, Schwann cell swelling. However, along with isolated myelin fibers with the signs of destruction there were the fibers with insignificant changes as well as the fibers similar to those found in intact rats. In the distal portion of the femoral nerve granular endoplasmic reticulum profiles in axis cylinder axoplasm were increased in diameter, their membranes were sharply contoured suggesting enhanced intracellular regeneration. Shortened cristae and decreased matrix density were noticed in mitochondria. Multiple clusters of mitochondria and endoplasmic reticulum profiles were often present. Schwann cell structure was altered. Ovoid bodies, consisting of concentrically located plates, were often found both in Schwann cell cytoplasm and axis cylinder axonema. There was hypertrophy of Schwann cell nuclei. In their cytoplasm well-organized Golgi complex and endoplasmic reticulum tubules as well as multiple mitochondria were located.

Conclusions. In view of all above mentioned ultrastructural morphologic signs it can be statedwith a high degree of confidence that treatment with the drug product Nucleo C. M. P. Forte has a positive impact on preservation and restoration of nerve structure, promoting its fast and complete recovery after the injury. This drug enhances restoration of nerve fibers in the proximal and distal portions, as well as in the site of the nerve fiber injury (clamping), actingas a neuroprotective and restorative agentat tissueand cellular levels.

**ЕФЕКТИВНІСТЬ ВИКОРИСТАННЯ НУКЛЄО Ц. М. Ф. ФОРТЕ У ВІДНОВНІЙ ТЕРАПІЇ ПІСЛЯ ТРАВМИ НЕРВА**

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Проведено експериментальне моделювання травми нерва шляхом перетискання та подальшого лікування препаратом Нуклео Ц. М. Ф. форте. Ультраструктурне дослідження дозволило виявити після перетискання значні дистрофічні та деструктивні зміни в аксоціліндричних зонах нервових волокон у щурів, яким не вводили препарат. При лікуванні препаратом Нуклео Ц. М. Ф. форте ультраструктурні зміни значно менше виражені, ніж у щурів, яким після перетискання стегнового нерва не проводили лікування. Враховуючи вищезазначені ультраструктурні ознаки, можемо стверджувати, що даний препарат покращує відновлення нервового волокна, діючи на тканинному та клітинному рівнях як нейропротективний та відновний засіб.

**Ключові слова:** перелом нижньої щелепи, травма нерва, Нуклео Ц. М. Ф. форте, ультраструктура.

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**ГИГІЕНА ТРУДА**

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**ОЦЕНКА РИСКА ФУНКЦИОНАЛЬНЫХ НАРУШЕНИЙ У РАБОТНИКОВ, ЗАНЯТЫХ НА ПОДЗЕМНЫХ ГОРНЫХ РАБОТАХ**

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Снижение функциональных возможностей организма наступает до первых признаков болезни и является прогностически неблагоприятным признаком. Группу наблюдения с