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**MORPHOLOGY OF THE RAT KNEE JOINT
AFTER INTRAARTICULAR INJECTION OF
HYDROXYMETHYL-QUINOXALINE DIOXIDE**

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Abstract. Morphology of the rat knee joint after intraarticular injection of hydroxymethyl-quinoxaline dioxide.

Degtiar A.V. Topical antiseptics are widely used in orthopedics and traumatology. However, studies highlighting the effects of antiseptics during long-term use on joint tissues are limited. In the performed work, the peculiarities of the structure of intra-articular tissues of the knee joint and muscles in rats in two series of experiments were noted: control group (saline injection into the joint) and experimental group (hydroxymethyl-quinoxaline dioxide injection into the joint) daily for 5 days. Histological methods with semi-quantitative assessment of articular cartilage and synovial membrane were used. During the first day after the drug administration, similar reactive changes to the injection of saline and hydroxymethyl-quinoxaline dioxide were observed in the joint tissues of the two groups of rats. The synovial membrane of the capsule was thickened, and synoviocytes were hypertrophied. The articular cartilage contains single cells with dense nuclei only in the superficial parts, but in general, the cytoarchitectonics of articular cartilage was preserved. In 5 days after injection, no statistically significant differences between saline and hydroxymethyl-quinoxaline dioxide were observed for the study periods. According to the studies the safety of long-term use of the drug as an antiseptic for articular tissues was confirmed.

Реферат. Морфологія колінного суглоба щурів після внутрішньосуглобового введення гідроксиметилхіноксаліндіоксиду. Дегтяр А.В. Антисептики місцевої дії широко використовують в ортопедії та травматології. Однак дослідження, які б висвітлювали вплив антисептиків при довготривалому їх використанні на тканини суглобів, нечисленні. У цій роботі відзначені особливості структури внутрішньосуглобових тканин колінного суглоба та м'язів у щурів у двох серіях експерименту: контрольна група (введення в суглоб фізіологічного розчину) та дослідна група (введення в суглоб гідроксиметилхіноксалін діоксиду) щоденно протягом 5 днів. Використано гістологічні методи з напівкількісною оцінкою суглобового хряща та синовіальної оболонки. На першу добу після введення препарату та фізіологічного розчину в тканинах суглобів щурів відзначені аналогічні реактивні зміни: синовіальна оболонка потовщена, синовіоцити на ділянках гіпертрофовані. У суглобовому хрящі тільки в поверхневих відділах наявні одиничні пікнотичні клітини, але в цілому цитоархітекtonіка суглобового хряща збережена. Через 5 днів після введення статистично значущих відмінностей між фізіологічним розчином та гідроксиметилхіноксалін діоксидом за терміни дослідження не відзначено. За проведеними дослідженнями підтверджено безпечність для тканин суглобів довготривалого використання цього препарату в якості антисептика.

Surgical risk factors for periprosthetic joint infection after total arthroplasty are the most devastating complications that require extensive research [1]. Despite improvements in surgical techniques, the use of new generation antibiotics, the incidence of infection in primary arthroplasty is 0.3-1% of cases, but reaches high rates of 3 to 38% in revision arthroplasty [2]. The solution to the problem of surgical infections is under revision because of the constantly changing relationship between the microbial pathogen and the human body [3].

Microorganisms resistant to most antibiotics and antiseptics have emerged, the ways of transmission and their persistence in the body are transforming. Antiseptics with different properties have been developed and used [4, 5, 6, 7]. However, microbial strains with the ability to survive despite the effects of antibiotics and antiseptics are spreading. At the same time, there are rapid changes in both the spectrum of drugs and the microbial background of hospitals, which requires new approaches to the problem have shown that irrigation of the surgical

wound with antiseptic solutions leads to a decrease in the number of infectious complications after primary total and revision endoprosthesis [4, 8, 9]. In surgery an antiseptic agent of local action – hydroxymethyl-chinoxaline dioxide (HMCD, trade name Dioxydin) is used, the distinctive feature of which is the marked antibacterial action. However, the effect of long-term use of HMCD during revision endoprosthesis on the tissues of the joint and the muscles in patients with periprosthetic infection is unknown.

Thus, taking into account all of the above, the need to carry out experimental studies aimed at developing a scheme of optimal use of local antiseptics when performing revision endoprosthetics, especially in patients with periprosthetic infection, does not cause doubts.

The purpose: to study the effect of intraarticular injection of HMCD antiseptic on the morphology of rat knee joint structures.

MATERIALS AND METHODS OF RESEARCH

Experimental studies were performed on 20 white laboratory rats (age 3-3.5 months, mean weight 170-200 g) of the population of the Experimental-Biological Clinic of the State Institution "Sytenko Institute of Spine and Joint Pathology National Academy of Medical Sciences of Ukraine" The plan of the experiment was approved by the Bioethics Committee at the Sytenko Institute (protocol No. 163 dated March 20, 2017).

The skin of rats in the area of the left knee joint was treated with 70°ethyl alcohol. Then 0.1 ml (0.001 g) of HMCD (trade name Dioxidine, Farmak, Ukraine) or saline solution (0.9 % sodium chloride) was injected intraarticularly. The injections were performed daily for 5 days. Rats were withdrawn from the experiment after 1 day and 5 days after the injections.

Nutritional and motor activity of white laboratory rats, resistance of the lower limbs, and the condition of the knee joint were evaluated by the presence or absence of edema and hyperemia. The animals were withdrawn from the experiment by a lethal dose of anesthetic (sodium thiopental, 90 mg/kg intramuscularly) on the 1st day and after 5 days of daily administration of the drug. During the experiment, we were guided by the following recommendations: the "European Convention for the protection of vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986) [10] the "General Principles of Animal Experiments" approved by the First National Congress of Bioethics, and the requirements of the "Procedure for conducting scientific experiments, experiments on animals" (2012) were followed [11].

Macroscopic analysis was performed after opening the rat knee joint. The condition of articular

cartilage of the femoral and tibial condyles, menisci, and joint capsule was studied. When assessing articular cartilage, we paid attention to the congruence of the coating, its integrity, luster and color, recorded the presence or absence of hemorrhages and erosions. Synovial membrane was analyzed to determine the presence or absence of edema, hemorrhages and inflammatory infiltrates.

Histological studies. Rat knee joints were isolated, fixed in 10% neutral formalin solution, decalcified in 5% nitric acid solution, dehydrated in alcohol with increasing concentration (60° to 96°), in a mixture of ethyl alcohol and diethyl ether (ratio 1:1) and placed in a celloidin [12]. Slices 7-10 µm thick were made and stained with hematoxylin and eosin and van Gieson picrofuchsin. Material was analyzed and photographed under an Olympus BX-6 microscope. Morphometric analysis was performed using the recommendations of the Osteoarthritis Research Society International (OARSI) [13], developed for rats, based on the condition of articular cartilage and synovial membrane, as preparations were injected into the joint cavity. Given the short duration of the experiment, other indicators recommended by OARSI (osteophytes, subchondral bone sclerosis, etc.) were not included. The condition of articular cartilage was noted on each slice, taking into account changes at three sites (standardly designated by OARSI), (marginal and central) from each of the 5 animals (15 measurements) during the experiment. The synovial membrane was evaluated in the lateral and medial areas of the capsule.

The following morphometric indicators of articular cartilage were studied: 0 – no degeneration; 1 point – minimal degeneration of matrix or chondrocytes, 5-10% of the total cartilage area; 2 points – mild disturbance, 11-25% of the area; 3 points – moderate disturbance, 26-50% of the area affected; 4 points – noticeable degeneration, 51-75% affected; 5 points – severe degeneration. over 75% affected [13]. In the synovial membrane: 0 – no changes (1-2 layers of synovial cells); 1 point – increase in the number of layers of cells lining the synovial membrane ($\leq 3-4$ layers) or slight overgrowth of subsynovial tissue; 2 points – increase in the number of layers of cells lining the synovial membrane ($\geq 3-4$ layers) and/or proliferation of subsynovial tissue; 3 points – increase in the number of layers of covering cells (>4 layers) and/or subsynovial tissue proliferation and infiltration with inflammatory cells; 4 points – increase in the number of layers of lining cells (>4 layers) and/or subsynovial tissue proliferation, infiltration with a big number of inflammatory cells. Slices were analyzed under an Olympus BX63 light microscope (Germany) with CellSens Dimention 1.8.1 software.

Statistical analysis. After determining the non-parametric distribution of numerical indices by the Kolmogorov-Smirnov method, analysis was performed using the Mann-Whitney test [14] (the package program "Statistics 6.0, serial number 31415926535897). The decision about the reliability of differences observed between the levels of the trait in the samples was made on the basis of a comparison of the statistic (U) and critical (Ucr) values of the Mann-Whitney criterion. If the calculated $U_{st} \geq U_{cr}$ (U_{cr} from Mann-Whitney table), we accepted the null hypothesis (H_0).

RESULTS AND DISCUSSION

Observation of the animals. During administration of HMCD and physiological solution, the animals remained active, fully stepped on the limb, no increase in limb volume or edema was detected. The knee joint did not differ in size from the contralateral one. The animals were normally fed and active. Macroscopic examinations revealed no abnormalities in the components of the knee joint and surrounding tissues after the action of HMCD (Fig. 1 A, B). The articular cartilage was lucid without signs of degeneration.



Fig. 1. A. Introduction of HMCD into the rat knee joint. Articular cartilage. B. Micropreparation of articular cartilage

Microscopic studies. In the tissues of the joint on the first day after the introduction of the drug, there were reactive changes to the introduction of

physiological solution and HMCD. The synovial membrane of the capsule was thickened, the synoviocytes were hypertrophied (Fig. 2).

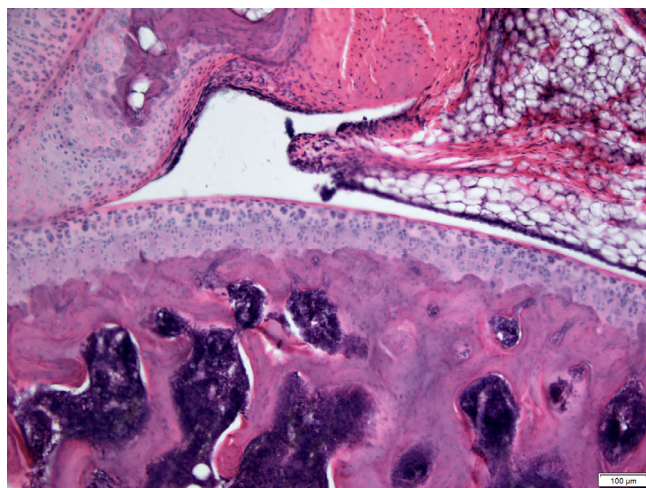


Fig. 2. Articular surfaces of the rat knee joint. Thickening of the synovial membrane. Introduction of HMCD into the joint. Hematoxylin and eosin staining. x100

The articular cartilage contains single cells with pycnosis nuclei only in the superficial parts, but on the whole, the cytoarchitectonics of articular cartilage is preserved (Fig. 3).

In the intermediate and deep zones, chondrocytes were located in capsules and had large, weakly

basophilic stained nuclei. The matrix was uniformly eosinophilic stained. The Tide mark separating uncalcified cartilage from calcified cartilage has the same contours. The osteochondral border had even contours. No areas of degeneration were detected.

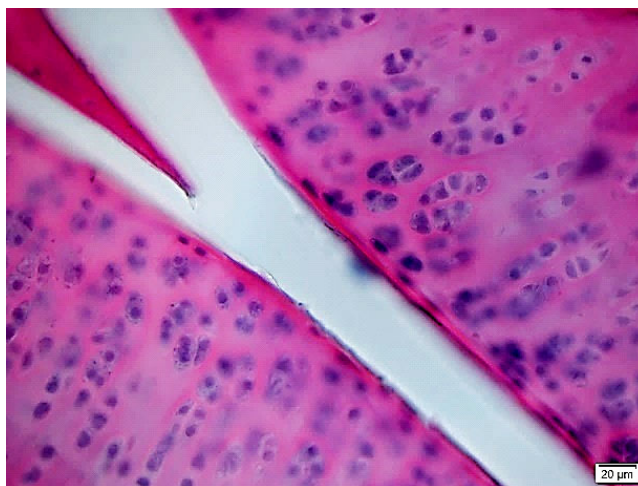


Fig. 3. Knee joint of the rat. Articular surfaces. Single cells in the superficial zone. Injection of HMCD into the joint. Hematoxylin and eosin staining. x400

No distinguishing features in the organization of the meniscus under the conditions of drug and physiological solution administration were revealed. The lateral meniscus is made of fibrocartilaginous tissue and consists of dense bundles of collagen fibers with sparsely arranged fibrochondrocytes; the peculiarity of the medial meniscus organization is the presence of a section of bone tissue in the central part surrounded by fibrous cartilage. The presence of bone tissue is a characteristic feature of meniscus structure in rats.

The periarticular muscles were without abnormalities. Longitudinal sections showed bundles of muscle fibers with interlayers of loose connective tissue in the endomysium. Numerous elongated nuclei are located in sarcoplasm on the periphery of the fibers.

5 days after the end of the drug administration. No destructive or inflammatory changes in the joint were detected. Articular surfaces had a normal structure, no delamination or cracks were detected (Fig. 4).

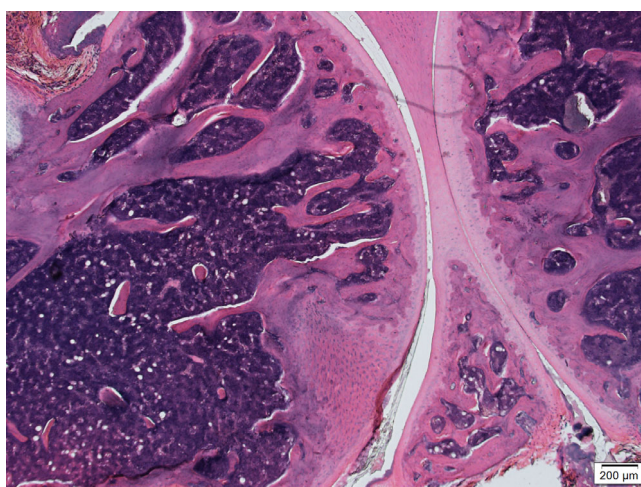


Fig. 4. Knee joint of the rat. Articular surfaces. Meniscus. Injection of HMCD into the joint. Hematoxylin and eosin staining. x40

The menisci in all rats had typical organization; no degenerative changes were detected. The number of cell layers (up to 3) in the synovial sheath was increased in two series of experiments and a slight overgrowth of subsynovial tissue was recorded. The periarticular muscles had a characteristic organization.

Morphometric study. Using the OARSI guidelines regarding the evaluation of articular cartilage and synovial membrane in rats, a morphometric study was performed to objectify the morphological findings (Table). No statistically significant differences were found between the two study groups.

Assessment of articular cartilage (points) and synovial membrane (points) according to OARSI recommendations [13]

Drug	Day 1 after injection (points) M±m	Day 5 after injection (points)
Articular cartilage		
Physiological solution (control, 15 samples)	0.867±0.192	0.733±0.206
HMCD (15 samples)	1.0±0.218* Ustat 102.5>Ucr 64 P=0.678302	0.8±0.2** U stat 107.5>Ucr 64 P=0.835705
Synovial membrane		
Physiological solution (control, 15 samples)	0.6±0.19	0.733±0.206
HMCD (experimental group, 15 samples)	0.533±0.165* Ustat 109>Ucr 64 P=0.884574.	0.8±0.28** Ustat 91.5>Ucr 64 P=0.383733.

Notes: HMCD – Hydroxymethyl-quinoxaline dioxide; * – Comparison was made with control and HMCD groups 1 day after injection; ** – Comparison was made with control and Hydroxymethyl-quinoxaline dioxide groups 5 days after injection; As calculated U stat >= U cr, we accept the Null Hypothesis.

Thus, using the OARSI guidelines, the minimum changes in the articular cartilage and the synovial membrane, which in average fall under one point, have been established.

Topical antiseptics are widely used in orthopedics and traumatology, including in the presence of periprosthetic infection or arthroscopic intervention [15]. The requirements for antiseptic preparations are primarily their bactericidal and bacteriostatic action. In addition, these drugs should not cause tissue damage and irritation, have neither resorptive action, nor allergic one, must not disrupt tissue regeneration processes in the areas of application [3]. According to ten studies, one of which was a randomized clinical trial, eight – retrospective cohort studies, and one – case series, it was confirmed the effectiveness of povidone-iodine (betadine®) and chlorhexidine compared with saline [16]. Diluted betadine prior to surgical wound closure in revision total knee and hip arthroplasty is a simple procedure, but is used in irrigation for no more than 3 minutes followed by saline washing [4]. No more than one minute is recommended to rinse the wound with chlorhexidine in total joint arthroplasty [17]. This is due to the fact that in the culture of fibroblast cells and mesenchymal stromal cells it has been proven that chlorhexidine has cytotoxic qualities in the range from undiluted (200 g/l) and diluted to a concentration of 0.4 g/l [6].

Under the action of octenidine dihydrochloride, polyhexanide and hydrogen peroxide their cytotoxicity depending on the concentration was also noted. However, studies highlighting the effects of modern antiseptics during long-term use on articular tissues under *in vivo* conditions are limited. While the bactericidal and bacteriostatic effects of some drugs have been studied in detail [2], their effects on tissues when used require further research. In our earlier study, it was noted that decamethoxin has no adverse effect on articular tissues when used for a long time [18]. To expand the range of antiseptic drugs, in this study we investigated the effect of hydroxymethyl-quinoxaline dioxide that is widely used. So far, the effect of the drug on articular tissues under conditions of its long-term use has not been determined. Histological studies with semi-quantitative assessment of the articular cartilage and synovial membrane according to the OARSI recommendations developed for rats, proved weakly pronounced changes in these structures, which did not differ significantly compared with the introduction of physiological solution into the knee joint of rats.

CONCLUSIONS

Introduction of hydroxymethyl-quinoxaline dioxide (dioxydine) into the rat knee joint showed weakly pronounced reactive changes in the articular cartilage and synovial membrane. The data of morphometric

study using Osteoarthritis Research Society International recommendations confirmed the safety of long-term use of this drug for articular tissues as an antiseptic, which allows us to recommend it for further studies in clinical conditions in total joint arthroplasty.

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