MEDICINE

INFLUENCE THE OCTENIDINE DIHYDROCHLORIDE ON THE TISSUES OF KNEE JOINTS OF IMMATURE LABORATORY RATS

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ABSTRACT
An effect of antiseptics on the joints tissue growing organism requires further study, which is primarily due to the need of foresight and reducing the consequences of carried diseases. The aim. of the reserch is to determine the concentration of the octenidine dihydrochloride ("Octenisept"), antiseptics, whose effectiveness is proved concerning to a wide range of microorganisms, in which it can be used in septic diseases of the musculoskeletal system of the growing organism.
The research of influence on the joints tissue the octenidine dihydrochloride in different partings studied in experiments on 40 immature 2-month-old white laboratory rats. Reactive and inflammatory changes, which expressed in different degrees, noted in the synovial layer of the animals' joint capsule, virtually in all animals in an experimental and control group. Significant differences observed in animals of the first control group (6 hours after washing the joint) where the thickness index of synovial layer, concerning the intact animals were by 16, 8 % higher. The thickness of the capsule was significantly higher than in the capsule of the intact animals and animals in the knee joint study group. Thus, in the group of animals where the joint was washed on octenidine dihydrochloride at 1:4 dilution, the thickness of the capsule was by 33.6 % more, in the group of animals with joint lavage octenidine dihydrochloride at a dilution of 1:3 through 6:00 capsule thickness values were (about intact animals) by 38.8 % higher, and after 12 hours - by 22.5 %. Based on the results obtained on the effect of octenidine dihydrochloride (taken at different dilutions) in the tissue of the knee joint, revealed that it has no toxic effect on articular cartilage and synovial membrane and other components of the joint. Using of the preparation at the 1:4 dilution does not cause inflammatory changes in the tissues of the joints, laboratory animals, allowing its using in this concentration in the treatment of inflammatory diseases of the musculoskeletal system in the growing organism.

Introduction. An effect of antiseptics on the tissue joints growing organism requires further study, which is primarily due to the need of foresight and reducing the consequences of carried diseases. Actuality of the problem is determined by the fact that despite improving of methods of diagnosis and treatment [1,3], the problem of mortality of children metaepiphyseal osteomyelitis (MEO) with is still very high. The disease is a medical and social problem because of severe clinical course and many adverse effects that may occur during the period of growth of the child and lead to disability [2].

The aim of the research is to determine the concentration of the octenidine dihydrochloride ("Octenisept"), antiseptics, whose effectiveness is proved concerning to a wide range of microorganisms, in which it can be used in septic

diseases of the musculoskeletal system of the growing organism.

Materials and methods. The research of the influence on the tissues of the knee joint the octenidine dihydrochloride ("Octenisept") in various dilutions studied in experiments on 40 immature 2-month-old white laboratory rats (live weight 150 ± 20 g).

Three parts of experiments (5 animals at time of observation) were held: 1. part is control, knee lump flushing with water for injection, the 2. part is research, single flushing knee with the octenidine dihydrochloride solution at a dilution of 1:4 (1 part drug, 4 parts water for injection), the 3. part is experiment, single flushing knee with the octenidine dihydrochloride solution at a dilution of 1:3 (1 part drug, 3 parts water for injection).

The solution was injected into the joint (under the kneecap) of making a shot from the exteriority of the joint, and the inside was introduced into the joint tube to remove solution from the joint. Thus, the joint lavage was modeled with punctures of two points. As an additional control used the five joints of intact rats of similar age and weight. Euthanasia of animals was performed by an overdose (inhalation) of diethyl ether at 6 and 12 hours after lavage joint by the octenidine dihydrochloride. In the 3rd part of experiments, the knee joint of rats was further investigated in 24 hours.

Results and discussion. *1) Intact rats.* Knee joint is represented by two bones in histological sections - the femur and tibial condyles of which are covered with articular cartilage. In the joint menisci are - medial (narrow) and lateral (wide), which are cut into a triangular shape. Menisci are fibrous tissue with high density of fibrohondrocyte. The peculiarity of the lateral meniscus rat is the presence in the central cancellous bone. Articular cartilageof bone ‘s condyle of the knee joint of rats of this age (immature animals) have no apparent characteristic of the articular cartilage of adult animals, zonality in the arrangement of chondrocytes. During the research of knee drugs in polarized light after toluidine blue staining revealed a bright metachromasia in inter-territorial matrix of cartilage non calcified and refraction, which is - 22,3 ± 1,4 nm. Metachromasia were low in surface area and brightness errors. Joint’s capsule represented with synovial and fibrous layers. Synovial layer is narrow, consisting of 1-2 layers synoviocells - large cells, round shape. Subsynovial located fibrous layer penetrated by vessels of capillary type. Fibrous layer consists of fibrous and fatty tissue.

2) The control of the animals’ series. At macroscopic examination at 6 and 12 hours after flushing joint control animals, water for injection, used as part of drug of the octenidine dihydrochloride in various dilutions, pathological changes in the joints were not found. Joint had no signs of swelling. With the opening of the capsule, articular cartilage has a whitish appearance and retains luster. Synovial joint was without destructive disorders in inversion with small hemorrhages point to another area of its surface. Microscopic examination in the light microscope after 6 hours of joint lavage revealed that the articular cartilage covering the femoral condyles and tibial bones, keeps the characteristic structural organization. The density of cells across the articular surfaces of the femoral and tibial bones is 135,9 ± 21,6 nm, which did not differ (p> 0,05) from that of intact animals. After 12 hours of joint lavage in the control group animals articular cartilage of the femoral condyles and tibial bone and kneecap and had meniscal lesions. In the study of GAG articular cartilage of femoral condyles and tibial bone disorders in the matrix organization is not defined. GAG were relatively evenly distributed in the matrix of articular cartilage. Maximum brightness metachromasia was in capsule cells - in matrix. Size refraction was - 20,5 ± 1,5 nm, which did not differ from the previous figures intact, control and experimental animals first.

3) Experimental animal series. Dilution of octenidine dihydrochloride 1:4. Six hours after washing the joint. During the macroscopic examination of the articular cartilage of animals' joints after flushing them with octenidine dihydrochloride (dilution of 1:4) from those of the control series, who were injected with water for injection used for octenidine dihydrochloride, distinctive features were not found. Articular cartilage was lustrous. The synovial membrane is thin, with mild symptoms of edema in the joint inversion. There are single hemorrhages in this area. Microscopic examination of the articular cartilage of femoral condyles and tibial bones found neither degenerative nor destructive disorders in cells and matrix. During the checking GAG reaction there was detected bright metachromasia in capsule cells and inter-territorial matrix of the intermediate zone. In the surface zone metachromasia was slightly lower. However, in general, the allocation of metachromasia showed that the matrix of articular cartilage retains the characteristics typical of the control group animals. Indices of GAG refraction differed neither from the control nor from intact animals (p>0,05) and compounded 22,4 ± 1,7 nm. Allocation of collagen was relatively uniform. In 12 hours after washing the joint with octenidine dihydrochloride. Macroscopic examination didn’t find any pathological disorders. Swelling in the joints and

periarticular tissues is absent. Articular cartilage is lustrous, with no signs of degeneration. A bright metachromasia was detected in capsules chondrocytes, mainly in the central parts of non-calcificated articular cartilage. Topography of GAG metachromasia and collagen errors did not differ from intact and control animals. Refraction GAG did not differ from those in intact animals and animals prior control and the first experimental series (20,1 ± 1,5 NM). Joint capsule was almost pathological changes. Thus, flushing the knee joint of rats with octenidine dihydrochloride solution at a dilution of 1:4 leads to reactive changes in the synovial layer of the capsule (in 6 hours after manipulation). In 12 hours there were neither reactive nor destructive disorders in the joint capsule observed. In other parts of the joint (meniscus, patella, articular surface of the candle) there are no changes in the structural organization.

4)Experimental animal series. Dilution of octenidine dihydrochloride 1:3. In six hours after washing the joint macroscopic study showed that the joint does not increase in volume, what means the absence of edema. Articular cartilage is lustrous and without evidence of pathological disorders. There are hemorrhages in the synovial membrane in the joint inversion. The density on the mainland non - callificied cartilage chondrocytes is high. The cells of this zone had representative structural organization. Only in the marginal parts of the joint (input area and outflow of the drug) articular cartilage was reactive with mild disabilities. In surface area were detected "shadow cells" and smaller cell-free area. Octenidine dihydrochloride probably destroy tinctured properties of the cells and they lose the ability to accept dye. The animals in this series of experiments observed a significant reduction in metachromasia and refraction of GAG in the surface area. However, topography and brightness of metachromasia on the rest part of articular cartilage does not differ from pre-series animals and intact animals (19,3 ± 1,4 NM). Damage of collagen refraction across the articular cartilage has not been found. "Shadow cells" were found in the marginal parts of the meniscus. In the rest of meniscus cells retained the characteristic structure. In the synovial membrane of the joint were recorded inflammatory manifestations. In marginal parts it was thickened by edema; synoviocytes are disconnected. Among them are macrophage cells with dense cytoplasm, indicating about the activation of phagocyte activity. In 12 hours after washing the joint microscopic examination of articular cartilage showed single cells in the superficial zone of articular cartilage.

In the joint capsule preserved signs of inflammation, but its expression was lower than in the previous survey period. In connection with the identified reactive changes in the synovial membrane of the knee joints of animals in this series of experiments, as well as a sharp decrease in metachromasia GAG in the surface zone of articular cartilage of femoral condyles and tibial bone in 6 hours after joint lavage there was set additional series in which the observation period was increased to 24 hours. Analysis of the structural organization of articular cartilage candles of both bones of the knee joint, patella and meniscus did not find any destructive and degenerative disorders. GAG metachromasia in the surface area and across non- cartilage was bright and refraction values did not differ from either intact animals or from controls (21,7 ± 1,8 nm). Destructive and reactive changes in the joint capsule at that time were not found. So flushing joint with octenidine dihydrochloride in dilution 1:3 caused reactive changes in the synovial membrane of the knee joint, which were at 6 and 12 hours after flushing joint. Within 24 hours after manipulation articular membrane corresponds to this in control and intact animals. So, for the objectification of conducted descriptive morphological examination of the knee joints of rats in the flushing them with octenidine dihydrochloride in various dilutions, and to quantify the relative changes in the tissues of the joints there was performed morphometric analysis of indicators, whose variability was noted in the qualitative morphological eximination.

Counting the number of chondrocytes of femoral condyles and tibial bones in the non - callificied cartilage's area showed that significant differences between the number of chondrocytes in animals of different series (one control and two experimental) as well as on the performance of intact animals were not find.

Number of chondrocytes in articular cartilage varied a bit, but significant changes in the application of flushing joint, both with water and octenidine dihydrochloride in various dilutions were not found. However, it should be noticed that using octenidine dihydrochloride at a dilution 1:3 in 6 hours causes single "shadow cells" in the superficial zone of articular cartilage and meniscus in the marginal parts. There is also a small area in which no chondrocytes were found.

The analysis of GAG refraction, measured across non - callificied articular cartilage, it was found that their indices in a series of experiments (control and two experimental groups) also were not significantly different .No differences were found in comparison with intact animals too.

However, it should be noted that in the group of animals in which the joints of animals were washed with octenidine dihydrochloride at a dilution of 1:3 in 6 hours after manipulation metachromasia and refraction GAG in the surface zone of articular cartilage were almost absent.

Reactive and inflammatory changes, expressed in varying degrees, have been noted in the synovial layer of the joint capsule animals, almost in all animals in experimental and control groups.Significant differences were observed in animals of first control group (6 hours after washing the joint) where rates of synovial layer thickness, relative to intact animals, were higher by 16.8 %.The thickness of the capsule was significantly higher than in the capsule intact animals and animals in the knee joint study groups.Thus, in the group of animals where the joint washed with octenidine dihydrochloride at 1:4 dilution, the thickness of the capsule was more by 33.6 %. In the group of animals washed with joint lavage octenidine dihydrochloride at a dilution of 1:3 a capsule thickness values were higher in 6 hours (about intact animals) by 38.8 %, and after 12 hours - by 22.5 %.The thickness of the capsule in animals of the experimental group in 6 hours after joint lavage was significantly higher (26.4 %) compared with control animals of the same time. After 24 hours, the thickness of the capsule in animals of the experimental group did not differ from intact animals.

To analyze the structural organization of the synovial capsule in which changes were recorded in 12 hours after joint lavage with octenidine dihydrochloride in a dilution of 1:3 there was given an additional series of experiments in which the tissues of the joint analysis performed in 24 hours after washing. It was found that in this term in the synovial layer of the joint capsule and articular cartilage of femoral condyles and tibial bone structural no changes were observed.

Conclusion. Based on the results obtained on the effect of octenidine dihydrochloride (taken at different dilutions) in the tissue of the knee joint, revealed that it has no toxic effect on articular cartilage and synovial membrane and other components of the joint. Using of the preparation at the 1:4 dilution does not cause inflammatory changes in the tissues of the joints, laboratory animals, allowing its using in this concentration in the treatment of inflammatory diseases of the musculoskeletal system in the growing organism.

Prospects for further research. This study demonstrates the need for further search of antiseptics, using which in a growing body will cause a minimum of side effects from the body at their maximum antimicrobial effectiveness

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