Analysis of the dynamics of the structural changes development in the humerus of guinea pigs under modeling biomechanical disturbances

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Today, the role of the traumatic factor and inflammation in the development and progression of osteoarthrosis is generally recognized, but the available research results do not allow to establish the role of impaired biomechanics as a monofactor in the development of deforming osteoarthrosis of the shoulder joint. Violation of the function of the bone and bone-cartilage elements of the joint, which is compensated by soft tissue formations, leads to overloads of the joints, upsets the normal balance of the load forces in the joint, creates abnormal biomechanics and the resulting pathological manifestations of deforming osteoarthrosis. The aim of the study is research of the dynamics of the disturbed biomechanics influence of the shoulder joint on the development of deformation osteoarthrosis and the features of the development of its structural changes. The experiments were conducted on guinea pigs weighing 380-420 grams at the age of 5 months. A model of surgical restriction of joint mobility was reproduced, which caused the formation of contracture. Using the methods of histology and scanning electron microscopy, we studied the relief of the articular surface, the topography of degenerative changes, and structural changes in the articular cartilage and subchondral bone. A statistical evaluation of the obtained data samples was carried out using Student t-test. The results were considered reliable at \( p < 0.05 \). The results of an experimental study demonstrated a decrease in the thickness and structure of articular cartilage when modeling deforming osteoarthrosis and confirmed the hypothesis that pathological limitation of the mobility of the shoulder joint and violation of biomechanics is an independent factor in the formation of osteoarthrosis. After surgery on day 30, degenerative changes and their progression with the formation of contracture on day 90 of observation were found in the articular cartilage. The features of the development of articular surface degeneration, the dynamics of the pathological changes and topography, which can expand the understanding of the pathogenesis of the disease, were established. The loss of the superficial zone caused the progression of dystrophic changes in the articular cartilage and sclerosis of the subchondral bone at 60 and 90 days.

Keywords: osteoarthrosis, humerus, topography, dynamics, lesion area.

Introduction
Deforming osteoarthrosis is a group of diseases of joints of different etiology with degeneration of hyaline cartilage and subchondral bone layer, obvious or hidden synovitis and subsequent loss of joint functionality [2, 16]. Numerous modern methods of treatment of the pathology of the shoulder joints are often not effective enough, which may be due to untimely treatment and insufficient assessment of pathogenetic components in the appearance and progression of pathological changes [17, 18]. However, the place of biomechanical disorders in the development of osteoarthrits of the shoulder joint as a monofactor of the pathological condition remains unknown. Experimental studies on animals allow us to determine the pathogenetic links of this complex process on the basis of reproduction of different models of osteoarthrosis [17, 27].
In clinical practice, there is often a correlation between impaired biomechanics of the shoulder joint and the development of shoulder-scapula arthrosis [8, 21, 22]. This may be due to a violation of the balance of forces on the shoulder, resulting in a change in rotation of the humerus head and subsequent formation of an abnormal load on the joint surfaces [13, 17].

Today, the role of traumatic factor and inflammation in the development and progression of osteoarthritis is generally recognized [14, 17]. The results of experimental studies have shown the similarity of the main pathogenetic units of these processes in animal models with those that develop in the most common forms of slowly progressing human arthrosis [4, 18, 24]. However, the available research results do not allow us to establish the role of impaired biomechanics as a single factor in the development of deforming osteoarthritis of the shoulder joint [6]. In view of this, it became necessary to develop another methodological approach in the study of the site of abnormal loading in the appearance and progression of osteoarthritis. The shoulder joint is known to be the most mobile in the human body due to its superiority in the biomechanics of the soft tissue elements over the bone [10].

The peculiarities of the morphological structure cause the possibility of powerful compensation in the case of pathological changes of one of the joints by other biomechanical units [7, 19, 28]. However, the violation of the function of the bone and cartilage elements of the joint, which is compensated by soft tissue formation, leads to overload of the latter, disrupts the normal balance of forces of the joints, creates abnormal biomechanics and caused by pathological manifestations of deforming osteoarthritis [3]. Reducing the thickness of the cartilage, its elasticity in the future becomes a cause of instability of the joint and the progression of osteoarthritis [18, 24, 25]. The metabolic flexibility of the joint chondrocytes normally allows to generate energy and maintain cell viability under the initial stages of pathology by increasing the regulation of mitochondrial respiration and reducing the rate of formation of reactive nitrogen and oxygen [11], however, at later stages of the disease, chondrocytes lose this metabolic flexibility [15].

The purpose of the study was to investigate the dynamics of the impact of disturbed biomechanics of the shoulder joint on the development of deformity osteoarthrosis and the features of its structural changes.

Materials and methods

The studies were performed on Guinea pigs (Cavia porcellus) weighing 380-420 grams at the age of 5 months. Animals were divided into groups according to the objectives of the experiment. The control group (n=5) and the main experimental group (n=19) were formed. The experimental group included operated animals, which were withdrawn from the experiment at 30 day (n=5), 60 day (n=5) and 90 day (n=9). Before operative reproduction of the model of osteoarthritis of the shoulder joint of the animals was anesthetized with sodium thiopental (50-60 mg/kg, intraperitoneally). The sequence of surgical procedures was performed according to the technique described in 2013 by E.J. Kramer et al. [9]. First, the skin and subcutaneous tissue were cut, and then the interval of the capsule was separated between the anterior margin of the supraspinatus muscle and the upper edge of the subscapular muscle. On the edge of the foregoing muscles, the sutures were sutured with vicryl № 2.0 and stitched together to form a capsular contracture. The cavity of the joint was not opened. The wound was sutured in layers.

Animals were removed from the experiment at 30, 60 and 90 days after surgery by administering a lethal dose of sodium thiopental.

Histological examination. The histological material (humerus) was fixed in 10% formalin solution on 0.1M phosphate buffer (pH=7.4) for 24 hours at 4°C, then washed with running water. In order to investigate the structural changes of the shoulder joint, the test specimens were decalcified according to the Freiman method in a 5% aqueous solution of EDTA calibrated with sodium hydroxide to pH 6.0-6.5. Decalcification in the first 24 hours was performed at 4°C, then at room temperature for 20-30 days [20]. The solution was changed every 5 days. From decalcified specimens of the humerus were made cryosections 12-15 μm thick (cryostat-microtome MK-25, USSR). Cryosections were stained with picrofuxin, toluidine blue, hematoxylin and eosin [26].

Morphometric study. Morphometric analysis consisted of quantitative assessment of the thickness of the articular surface of the humerus. To do this, the histological sections of all experimental animals from the surface to the subchondral measured its thickness at 20 points. Microphotographs were obtained on an Olympus BX 51 microscope. Morphometric analysis was performed using CarlZeiss software (AxioVision SE64 Rel.4.9.1) at magnification x200 and x400.

Scanning electron microscopy. For the methods of scanning electron microscopy SEM, the samples underwent a number of methodological procedures. The samples were dehydrated with ethanol solution in increasing concentrations (25%, 50%, 75% and 100%). The material was then dried in a Samdri-780A installation to pH 6.0-6.5. Decalcification in the first 24 hours was performed at 4°C, then at room temperature for 20-30 days [20]. The solution was changed every 5 days. From dehydrated specimens of the humerus were made cryosections 12-15 μm thick (cryostat-microtome MK-25, USSR). Cryosections were stained with picrofuxin, toluidine blue, hematoxylin and eosin [26].
- articular cartilage changes: change in cartilage thickness (loss of superficial and/or deep cartilage layer), reduction of cartilage cellular composition;
- subchondral bone changes: the appearance of foci of bone reorganization or reduction.

**Statistical processing.** Statistical evaluation of the obtained data samples was performed using Student's t-test. Results p<0.05 were considered valid. Data are presented as mean ± standard deviation.

**Bioethics.** All experimental manipulations were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council of Europe Directive 86/609/EEC (1986), Law of Ukraine No. 3447-IV "On protection of animals from ill-treatment", General Ethical Principles of Animal Experiments, approved by the First National Congress of Ukraine on Bioethics (2001).

**Results**

By surgical limitation of the biomechanics of the shoulder joint in the experimental animals, an abnormally altered (disturbed) projection of rotation of the humerus head in the hollow of the scapula was achieved.

Proliferation changes of the articular capsule and tendons of the skeletal muscles, as well as the increase of the microvascular density, were found in the study of the structural changes of the shoulder joint. These changes are the result of traumatic damage and reorganization of the connective tissue of the capsule of the joint (Fig. 1).

The results of histological examination confirmed our working hypothesis that surgical limitation of the biomechanics of the shoulder joint is an isolated factor in the appearance and progression of deforming osteoarthritis.

Morphometric analysis allowed us to quantify the structural changes of the shoulder joint. The value of the thickness of the cartilage surface of the humerus in the group of operated animals was statistically significantly smaller compared to the control group by an average of 48% (127.9±38.3 μm² versus 255.9±26.7 μm² in control, p<0.01). In general, the quantitative indicators were lower in the lower area of the joint surface of the humerus, which is associated with the formation of non-physiological (abnormal) load on this area of the joint.

Histogram 1 shows the number of structural change criteria identified in each comparison group. On the 60th day most of the samples confirmed the presence of structural changes in the joint, and on the 90th day progression or no difference on the 60 day of the experiment.

However, the histological method made it possible to establish the appearance and frequent localization of degenerative changes of hyaline cartilage, but for a more complete assessment of the topography of the established changes, a scan of the surface of the humerus was performed using the SEM method. The application of the method yielded the following results.

On the 30th day after surgery, focal destructive changes of the cartilage surface area at the lower or anterior pole were observed on the articular surface of the humerus (mean lesion area 8.3% of the articular surface area). The thickness of the cartilage surface at 60 day decreased significantly at the anterior lower pole and to a lesser extent at the posterior lower (lesion area averaged 25.9%, p<0.05). At 90 day, the deformation zone increased in area (an average of 54.2%, p<0.05), and deformation changes of the articular surface progressed from the central zone of

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**Fig. 1.** Native preparation of Guinea pig humerus head on 90 day after modeling of deforming osteoarthritis of the shoulder joint. (→) - the area of deforming osteoarthritis.
the articular surface to the outer contour, which was previously described at 60 day. The scan results of the humerus head are clearly shown in Figure 2.

Figure 2 shows histological sections of the head of the humerus, reflecting the change in thickness of hyaline cartilage on different surfaces of the head. Thus, at 60 day marked dystrophic changes more developed on the anterior and/or posterior surface of the head. These data should be confirmed by scanning the articular depression of the scapula.

In the study of the topography and the degree of degenerative changes, considerable attention was paid to the study of the surface area of the articular surface, because of its localization, the latter experiences the bulk of the load in anomalous biomechanics and therefore rapidly loses elasticity, density and degenerates.

Histological picture of the anterior and posterior articular lower surfaces was characterized by signs of focal or diffuse destruction of cartilage. A decrease in the thickness of the articular cartilage over a considerable area of the surface of the humeral head was noted. The deep area of the cartilage surface contained newly formed foci of paravascular ossification. In the resorptive cavities of the articular cartilage, foci of loose connective tissue were located next to the vessels, the density of such areas was increased in calcified cartilage and deep sections of non-calcified cartilage. The density of chondrocytes was low, they were unevenly located within the matrix. The expressed changes were observed in the cellular composition of the articular cartilage. Most isogenic chondrocyte groups were in a state of edema and necrosis. The ossification zones extended from the epiphyseal foci of the subchondral bone to its surface; in the formed gaps blood vessels with osteogenic cells grew; surrounding matrix with signs of compaction and ossification; the contour of the border of cartilage and bone tissue towards the epiphyseal cartilage has changed. On the opposite side, that is, the surface of the epiphysis, the structural changes of the cartilage were characterized by a sharp change in the relief of the articular surfaces, as confirmed by SEM studies.

At the ultrastructural level, the loss of the superficial zone of cartilage was established against the background of its deformation, matrix and cell structure. Since the 60th day of observation, there has been a sharp increase in the degree of degenerative changes of the shoulder joint, which progressed from the central zone of the articular surface to the outer contour. On average, reduction of the epiphyseal...
cartilage was 15.9%, 25.3%, and 49.3% (p<0.05) according to the observation period.

**Discussion**

The experimental study evaluated the role of immobilization of the shoulder joint as a monopathogenetic factor in the development of osteoarthritis. Today there is a considerable amount of research on the analysis of the nature and features of the development of dystrophic changes in the joint. Researchers focus on the mechanical and histochemical factors of these disorders [2, 16]. Mechanical factors include traumatic compression, mechanical overload, etc. [17, 18], and enzymatic dysfunction, microcirculation, denervation, and metabolic disorders, including genetically predetermined ones, are quite conditional on histochemical [3, 6].

The analysis of literary sources revealed quite similar features of the development of histostructural changes in the shoulder joint in different models of osteoarthritis reproduction in experimental animals [4, 14]. In particular, a decrease in the content of collagen, proteoglycans and other polysaccharides and proteins has been observed, providing strength and resistance to compression and loading [19, 25, 28]. The authors explain this by increasing the activity of local collagenases and metalloproteinases, i.e. enzymes of the catabolic (proteolytic) plan [7]. Together with abnormal loading and non-physiological biomechanics of the joint, all these factors cause changes in the cartilage surfaces of the humerus head and are considered as a prerequisite for joint instability [21, 22].

The development of osteoarthritis is accompanied by increased expression of vascular endothelial growth factor (VEGF) in articular cartilage [29]. VEGF is an angiogenic factor directly or indirectly involved in all links of angiogenesis. Under normal physiological conditions hypertrophied chondrocyte can secrete VEGF, which promotes angiogenesis in the area of calcified articular cartilage and bone formation.

The vascular growth, ossification foci formation, resorption cavities revealed in our study are signs of osteoarthritis, which is also accompanied by increased production of inflammatory cytokines, change in macromolecular structure of the articular cartilage matrix coincides with other researchers [12].

**Conclusions**

1. The results of the experimental study showed a decrease in the thickness and structure of articular cartilage in the modeling of deforming osteoarthritis. The features of the development of joint surface degeneration, the dynamics of the pathological changes and the topography established and which can expand the understanding of the pathogenesis of the disease.

2. The results of the experimental study confirmed the hypothesis that pathological limitation of shoulder joint mobility and disturbance of biomechanics is an independent factor in the formation of osteoarthritis. At the same time, according to the results of dynamic observation, the most critical is the period from 30 to 60 days, because it is at this interval of time that degenerative phenomena reached the most vulnerable to ossification of articular cartilage. The exposure of the latter in the projection to the area of loading of the articular surface and concomitant sclerosis caused a rapid progression of degenerative changes in the shoulder joint. Given these data, the first 30 days can be considered as a therapeutic window to correct the pathological process.

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Сегодня роль травматического фактора и воспаления в развитии и прогрессировании остеоартроза является общепризнанной, однако имеющиеся результаты исследований не позволяют установить роль нарушенной биомеханики как монофактора в развитии деформирующего остеоартроза плечевого сустава. Нарушение функции костных и костно-хрящевых элементов приводит к их перегрузкам, нарушая равновесие сил нагрузок в суставе, и создает аномальную биомеханику. Мета работы: дослідити динаміку впливу порушеної біомеханіки плечового сустава на розвиток деформуючого остеоартрозу та порушення його структурних змін.

АНАЛІЗ ДИНАМІКИ РОЗВИТКУ СТРУКТУРНИХ ЗМІН ПЛЕЧЕВОЇ КІСТИ МУРЧАКІВ ПРИ МОДЕЛЮВАННІ ПОРУШЕННЯ БІОМЕХАНІКИ

Сергеєнко Р.А., Страфун С.С., Савосько С.І., Макаренко О.М.

Сьогодні роль травматичного чинника і запалення у розвитку і прогресуванні остеоартрозу є загальновизнаною, проте існуючі результати досліджень не дозволяють встановити роль порушеної біомеханіки як монофактора у розвитку деформуючого остеоартрозу плечевого сустава. Порушення функції костних та костно-хрящових елементів суглоба призводить до їх перевантаження, порушує цілісність структур суглоба, і створює аномальну біомеханіку. Мета роботи: дослідити динаміку впливу порушеної біомеханіки плечевого сустава на розвиток деформуючого остеоартрозу та особливості розвитку його структурних змін.

АНАЛИЗ ДИНАМИКИ РАЗВИТИЯ СТРУКТУРНЫХ ЗМЕНИ ПЛЕЧЕВОЙ КОСТИ МОРСКИХ СВИНОК ПРИ МОДЕЛИРОВАНИИ НАРУШЕНИЙ БИОМЕХАНИКИ

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Сегодня роль травматического фактора и воспаления в развитии и прогрессировании остеоартроза является общепризнанной, однако имеющиеся результаты исследований не позволяют установить роль нарушенной биомеханики как монофактора в развитии деформирующего остеоартроза плечевого сустава. Нарушение функции костных и костно-хрящевых элементов сустава приводит к их перегрузкам, нарушая равновесие сил нагрузок в суставе, и создает аномальную биомеханику. Цель работы: исследовать динамику влияния нарушенной биомеханики плечевого сустава на развитие...
деформирующего остеоартроза и особенности развития его структурных изменений. Эксперименты проведены на морских свинках весом 380-420 грамм в возрасте 5 месяцев. Воспроизвели модель хирургического ограничения подвижности сустава, что вызвало формирование контрактуры. Методами гистологии и сканирующей (растровой) электронной микроскопии исследовали рельеф суставной поверхности, топографию дегенеративных изменений, структурные изменения суставного хряща и субхондральной кости. Статистическую оценку полученных выборок данных проводили с использованием t-критерия Стьюдента. Достоверными считались результаты при условии p<0,05. При моделировании деформирующего остеоартроза установили уменьшение толщины и изменение структуры суставного хряща. Получено подтверждение гипотезы: самостоятельным фактором в формировании остеоартроза является патологическое ограничение подвижности плечевого сустава и нарушение его биомеханики. После оперативного вмешательства на 30 сутки в суставном хряще обнаружены дегенеративные изменения и их прогрессирование с формированием контрактуры на 90 сутки наблюдения. Таким образом, установлены особенности развития дегенерации суставных поверхностей и динамика развития патологических изменений. Потеря поверхностной зоны способствовала прогрессированию дистрофических изменений в суставном хряще и склерозу субхондральной кости в сроки 60 и 90 суток.

Ключевые слова: остеоартроз, плечевая кость, топография, динамика, площадь поражения.