PECULIARITIES OF OSTEOARTHRITIS IN RATS

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Was received model of osteoarthritis in the rat line Wistar, by a one-time injection of CH3COOI acid into the knee joint. There changes in joint studied histological and clinical methods. On the 7-th day of the experiment was installed resemblance to similar changes in the joints of humans with acquired osteoarthritis

Keywords: osteoarthritis, modeling, rats, CH3COOI acid, knee, cartilage

1. Introduction

Among the diseases of bones that attract particular attention should be called osteoarthritis, which is quite common among the population of Ukraine. Treatment of the disease and its related complications ineffective because the use model of osteoarthritis (OA) in animals enables new approaches to treat disease. Osteoarthritis on rats is modeled in different ways: assurgically as hormono- and antibiotic injection and various acids that can cause a variety of an anatomical and biochemical changes in the joints of animals [1–3].

Quick and inexpensive method of obtaining the model of osteoarthritis in rats by injection of iodinesodiumacetate in to the joint [4]. But the issue of getting osteoarthritis model rats in this manner remains open. Little known pathophysiological theory of osteoarthritis development of rats. Unknown whet her changes of the joint in development of osteoarthritis in animals and in humans aren't different. In this regard, it is important t o carry out morphological and functional studies to obtain reliable data on comparative pathomorphosis induced osteoarthritis of rats and acquired osteoarthritis of humans.

2. Literature review

Osteoarthritis is an acquired musculoskeletal disorder, as is commonly believed, noninflammatory origin, which develops when cartilage degradation velocity exceeds the speed of his recovery, leading to erosion of cartilage, subchondral bone thickening and joint damage. With the thinning of cartilage integrity of the surface can be lost, can form cracks when moving joints cartilage tends to be facilitated erosion. In the formation of new cartilage it tends to more fibers and reduced ability to withstand mechanical stress. With the passage of time can bare the surrounding bone, which is less suited to withstand mechanical stress, which leads to the formation of microcracks. Localized bone necrosis can oc cur under the surface of the bone, leading to cysts, which further weaken the bone supporting the cartilage [5].

Subchondral bone, periosteum, synovial membrane, ligaments and articular bag many innervated and contain nerve endings that can be a source of nociceptive stimulus [3, 5].

With the development of osteoarthritis it can ultimately affect the structures surrounding the joint. There may be local inflammation such as synovitis, for exam-
ple, in response to inflammatory mediators that are released in the process of cartilage degradation. Articular bag susceptible to thickening and movement of nutrients into and metabolic products of the joint may be limited. Ultimately, it may be a noticeable increase in periacicular muscle tissue in the development of osteoarthritis, and joint or abnormally used less frequently.

It is believed that the pain caused by osteoarthritis cartilage degradation is not as such, but to changes in the surrounding structures, including bone, cartilage as not innervated [6].

A change in the joint development of osteoarthritis in animals? Osteoarthritis is also more likely to develop in older animals - cartilage is destroyed and dries it becomes like an old ragged piece of foam, which is no longer a spring. Under the influence of enzymes it is covered with cracks, and in severe cases disappears completely, eliminating any bone protection [7]. When the cartilage disappears, there is pain, joints "ossify" deteriorating mobility. This frustrating condition progresses, affecting one joint after another.

Pathophysiological theories of osteoarthritis describe the disruption of normal joint structure changes capsule and cartilage damage as a result of violations of normal tissue remodeling joint. Osteoarthritis results from interaction of mechanical and biological factors. This process starts as changes in cartilage or subchondral bone, or as a result of disease within most of these tissues (eg gene defects of 11th type of collagen – chondrogenesis) or from external abnormal mechanical stress (eg, unstable joints, increased load, injuries). As the progression of osteoarthritis these changes become more pronounced [1].

Recently, attention has been paid biochemical changes that contribute to osteoarthritis. Obviously, the disease develops when the enzymes that cause degradation of cartilage (such as proteases, cytokines, ahrekanazy, substance P, nitric oxide), its effects outweigh the function of proteins responsible for maintaining the integrity of the cartilage (such as tissue inhibitor of metalloproteinases, kininogena, –1 inhibitor plasminogen activator, transforming growth factor, insulin-like growth factor-1, gamma interferon). Matrix metalloproteinases, including collagenase, stromelizina, zhelatynazy, membrane protease and metalloelastaza - found in cartilage in osteoarthritis, and their concentrations are usually correlated with the degree of histological damage. Various cytokines, including interleukin-1 and tumor necrosis factor, may also cause damage to joints and cartilage loss through activation of enzymes metalloproteinasyn degeneration and through other mechanisms [1, 2].

We know that under CH3COOI acid activated hlyseraldihid-3-phosphate dehydrogenase chondrocytes, and consequently a violation of glycolysis and hybel cells. The death of chondrocytes results in histological and morphological changes [2]. Liu X. X. with collaborators at the induction osteoarthritis the model line Wistar rats confirmed atrophy of chondrocytes piknotichnym cell nucleus and cell membrane destroyed [8]. Cells exposed to action osteoarthritis produce collagen type I. Fickert S indicates genes COL1 (collagen type I), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), OCN (osteocalcin gene) as markers of chondrogenesis abuse and degeneration of cells [9].

Regarding the models of osteoarthritis, caused by iodine sodium acetate in animals of different species and age Bendele A.M. compares guinea pigs and rats .. Regarding guinea pigs, he said that the best model for males is 6 months 900 gr., For Age rats person for simulation models must be at least 6 months but not more than 15 months. Counting cartilage damage in rats and hamsters can be difficult because the cartilage has relatively few layers of cells compared to larger species [1]. But rat – is a very common subject of laboratory research, as explored histological changes that arising under CH3COOI acid and compared with changes that occur in humans naturally is the task of our study.

3. Aimof research

Get the model of osteoarthritis in rats and spend histological and clinical studies to produce reliable data that osteoarthritis model rats and the natural human osteoarthritis alike.

4. Materials and methods

Research is performed at the Department of "Regulation of Cellmechanisms Institute of Molecular Biology and Genetics, NAS of Ukraine. Modeling of osteoarthritis received on male Wistar rats aged 6 months and weighing 300–400 g by a single injection into the middle joint of the right hind limb with ciprofloxacin (at a dose of 0.05 cm3 of drug) and iodine acetic acid (6 mg iodine acetic acid in 100 mkl of 0.9 % sodium chloride) under the control of video roentgenograph [4, 6]. At the beginning of the research all rats were measured weight, hindlimb joints were shaved with razor, their sizes were measured; smear of blood were made for counting leukocyte formula and X-rays, ultrasound. All manipulations with animals were carried out under anesthesia and in sterile conditions (Fig. 1).

The animals were conducted a daily clinical observation. With the development of OA observed in the dynamics of 7, 14, 21 and 28 day by methods: cytological, anatomical, histological, clinical (X-ray, ultrasound). Results of experiments were processed by methods of mathematical statistics. The calculation of results performed using Microsoft Excel 2003.
Fig. 1. Modeling of osteoarthritis on rats of line Wistar: a – intramuscularly injected anesthesia; b – clearing the joint of hair; c – measuring the size of the joint with beam compass; d – making an injection into the middle of the joint

5. Results

On the third day after injection of iodine acetic acid in the animals right joint with modeling OA knee joint size increases by 5.8 mm (p<0.01) (Table 1). On the background of a sharp increase in size of the right joint significantly increases the number of microxyphil neutrophils (by 40.06 % (p<0.01) compared to intact animals before the start of research) (Table 2) and the weight of animals is reducing (in 33.72 grams compared to intact animals before the start of research). This pattern of physiological changes in weight, size of knee and leukocytal blood form of animals with modeling OA is typical for a sharp inflammatory process resulting mass apoptosis of chondrocytes [1]. In the of animals with modeling OA on the 7th day is observed a decrease in size of the right joint – 13.5 mm., which is lower by 4.75 mm. than on day 3 (p<0.1), but higher by 2, 05 mm. than in control animals (p<0.1) (Table 1); number of lymphocytes increases and becomes in the normal range – 70.33 %. On the background of reduction of microxyphil cells there is also a reduction of monocyte percentage by 1.16 %, which is 1.18 % lower than in control rats (p<0.001) (Table 2). Reducing the percentage of monocytes in humans demonstrates the suppression of the immune system [10]. Weight of animals with model OA is significantly reducing compared with control rats on day 7 by 35.22 grams (p<0.001).

Researching the development of OA after iodine acetic acid by ultrasound was found that already on day 7, there is distinct changes typical for the disease: narrowing of joint space and evident degeneration and thinning of the articular cartilage (Fig. 2).

Roentgenologic research of the development of model OA in dynamics have shown that changes in the joint, found on day 7 resemble changes typical for OA in humans (Fig. 3) On 7 day of research in rats in the control group of animals on histological preparations at low magnification indicated a typical structure of the joint (Fig. 4).
**Dynamics of changes of physiological parameters of Wistar rats line under the influence of iodine acetic acid**

| Time of experiment | number of animals (n) | Weight (g) | Left joint (mm) | Right joint (mm) |
|--------------------|-----------------------|------------|-----------------|------------------|
| 0                  | 13                    | 423,22±12,97*** | 11,4±0,19**    | 11,45±0,19*,**   |
| 0 C                | 2                     | 468,2±0,19   | 11,2±0,01       | 11,6±0,06       |
| 3                  | 6                     | 389,5±30,5   | 11,5±0,8        | 17,25±1,75**,**  |
| 7                  | 10                    | 388±14,66*** | 11,75±0,25*     | 12,5±0,2*       |
| 7 C                | 2                     | 444,0±0,02   | 11,4±0,03       | 11,6±0,03       |
| 9                  | 6                     | 417,0±12,0   | 11,8±0,2*       | 13,8±0,8        |
| 14                 | 9                     | 408,8±8,35   | 12,1±0,28*      | 12,9±0,06*      |
| 14 C               | 2                     | 469,2±0,01   | 11,8±0,10       | 12,2±0,02       |
| 16                 | 5                     | 398,0±0,03   | 11,5±0,02       | 11,6±0,04       |
| 21                 | 8                     | 421,75±13,21 | 11,8±0,22       | 12,5±0,12*      |
| 21 C               | 2                     | 467,5±0,06   | 11,8±0,02       | 11,8±0,01       |

Note: C – control animals; *** – P<0,001 (between indicators for change in weight of the animals on day 0 and 7); ** – P<0,01 (between indicators for change in size of the right joint in animals on 0 and 3 days); * – P<0,05 (between indicators for change in size of the left joint in animals on day 0 and 7, between indicators for change in size of the left joint in animals on 7 and 9 days, between indicators for change in size of the right joint in animals on 0 and 3 days, the left joint size in animals on 0 and 14 days; between indicators of size of the left joint of animals on 7 and 14 days; between indicators of size of the left joint of animals on 9 and 14 days; between indicators of size of the left joint of animals on 9 and 14 days; between indicators of size of the right joint of animals on 0 and 14 days; between indicators of size of the right joint of animals on 9 and 14 days; between indicators of size of the right joint of animals on 3 and 21 days).

**Dynamics changes in indicators of leukocytic blood forms of Wistar rats under the influence of iodine acetic acid**

| Time of experiment | number of animals(n) | Lymphocytes (%) | MP (%) | SN (%) | Mont (%) |
|--------------------|-----------------------|-----------------|--------|--------|---------|
| 0                  | 13                    | 61,55±11,86*    | 9,44±2,57**,*** | 3,33±1,04* | 2,44±0,55*** |
| 0 C                | 2                     | 78,0±0,0        | 17,0±0,0 | 3,0±0,5 | 2,0±0,0  |
| 3                  | 6                     | 47,5±10,5*      | 49,5±10,5*** | 1,5±1,5  | 1,5±1,5  |
| 7                  | 10                    | 70,33±2,74*     | 19,33±4,8***,*** | 9,0±2,92* | 1,16±0,60*** |
| 7 C                | 2                     | 76,0±1,0        | 14,5±1,5 | 6,0±1,0 | 2,0±0,5  |
| 9                  | 6                     | 65,5±3,5*       | 27,5±0,5*** | 6,5±2,5  | 0,5±0,5*** |
| 14                 | 9                     | 71,5±4,05*      | 21,0±3,34*** | 4,0±1,22 | 3,25±0,85 |
| 14 C               | 2                     | 82,5±0,5        | 14,0±0,5 | 1,5±0,5 | 2,0±1,0  |
| 16                 | 5                     | 81,0±0,0        | 19,5±0,5 | 0,0  | 0,0  |
| 21                 | 8                     | 73,3±3,84*      | 19,0±3,46 | 5,66±2,66 | 2,0±1,0  |
| 21 C               | 2                     | 76,0±0,5        | 19,0±1,0 | 30±0,0 | 2,5±0,5  |

Note: C – control animals; MP – microxyphil neutrophils, SN – stab nuclear neutrophils; Mon – monocytes; *** – P<0,001 (between the percentage of MP cells in leucocytal form in animals on day 0 and 7, between the percentage of MP in leucocytal shape in animals on day 0 and 9, between Mon percentage of cells in leucocytal shape in animals on day 0, 7, between Mont percentage of cells in leucocytal shape in animals on day 0 and 7, between Mont percentage of cells in leucocytal shape in animals on day 0 and 9, between indicators for change in percentage of MP cells in leucocytal shape in animals on day 0 and 7, between indicators for change in percentage of MP cells in leucocytal blood form in animals on 3 and 14 days); ** – P<0,01 (between indicators for change in percentage of MP cells in leucocytal blood form in animals on day 0 and 9, between the percentage of MP cells in leucocytal shape of animals on 3 and 7 days); * – P<0,05 (between indicators for change in percentage of lymphocytes in the total leucocytic form in animals on day 0 and 3, between indicators for change in percentage of lymphocytes in the total leucocytic form in animals on 7 and 9 days, between the percentage of SN cells in the general leucocytal shape in animals on day 0 and 7, between indicators for change in percentage of lymphocytes in the leucocytic form in animals on 3 and 14 days, between indicators for change in percentage of lymphocytes in the leucocytic form in animals on 3 and 21 days).
And in rats with induced osteoarthritis cartilage of joint surface is damaged by chemical destruction. Histologically, there is a distinct dystrophic process of cartilage – it has a rough surface with deep erosion, cracks and uneven thickness. Meniscal cartilage thickens and may grow on the surface (Fig. 5).

![Fig. 2. Ultrasound of experimental rats with OA on day 7 after the injected iodine acetic acid:](image)

*a* – the right joint of an intact rat – normal joint space (2.0 mm), the condition of the cartilage unchanged;  
*b* – the right joint of rat with OA on day 7 after injection of iodine acetic acid: clearly evident narrowing of joint space (1.7 mm) and evident thinning and degeneration of joint cartilage;

![Fig. 3. Radiographs of the knee joints of experimental rats of line Wistar](image)

At greater increase of the knee joints of control animals are clearly distinguished superficial and intermediate zones of hyaline cartilage, which testifies to its zoning. Obvious damage is not noticed, revealed only rare minor defects of cartilage surface area (Fig. 6).

In experimental animals, in the large increase of the knee joints cartilage zoning varies. In the surface zone – erosion, cracks, reaching areas endochondrial bone. Over a short distance focially cell free plate detected with the presence of fibrillar sites on the surface on the background of erosion. There are empty lacunae, foci of dissociation matrix (Fig. 7).

At the cellular level, an intermediate zone of cartilages in the control animals consisted of the clearly formed mononuclear chondrocytes and intercellular substance, permeated by elastic fibers. Chondrocytes in the formulation are placed singly or gathered in isogenic groups (Fig. 8).

In animals with induced osteoarthritis on day 7 of the experiment revealed the presence of large amounts of chondrocytes in lacunae being in a state of apoptosis as evidenced by condensation of chromatin and cytoplasm compaction, fragmentation of the nucleus and cytoplasm with formation of apoptic cells with intact cell membrane (Fig. 9).

Dynamics of changes in animals with model OA indicates that on day 7 evident changes in the structure of the right knee joint were noticed that anatomically, physiologically, clinically, histologically, radiographically are very similar to human acquired osteoarthritis [11], that in perspective gives ability to use this model in the development of more effective methods of treatment of this disease.
Fig. 4. Morphological and anatomical structure of the knee joint of control animal (hematoxylin-eosin stain)

Fig. 5. Morphological and anatomical structure of the knee joint of animal with induced osteoarthritis (hematoxylin-eosin stain)
Fig. 6. Histological research of the knee joints cartilage zoning of control animals (hematoxylin-eosin stain)

Fig. 7. Histological research of cartilage zoning of the knee joints of animals with induced osteoarthritis (hematoxylin-eosin stain)

Fig. 8. Cellular structure of the intermediate zone of cartilages in the control group of animals (hematoxylin and eosin stain)
To study the therapeutic effect it should be chosen the 7 day of experiment because on the third day of the experiment inflammatory process is observed that disappears on the 7th day of the experiment. Osteoarthritis is non-inflammatory disease, so it is necessary first to decrease inflammation. After 7 days we have observed for two more weeks animals with selected biometric markers. As well as on the 7th day changes in the knee joints of experimental animals were similar to human acquired induced osteoarthritis.

6. Conclusions

1. With the injection of iodine acetic acid in right joint of animals already on the 7 day of experiment are observed evident changes in the structure of the right and left joints that for physiological, histological and radiological indicators partly reproduce changes in bone and cartilage tissue during the development of osteoarthritis in humans.

2. Zones in the right joints of experimental animals are similar to the acquired human osteoarthritis, which is confirmed by X-ray examination of the extremities, by histological study of the structure of joints, joints size and structure of osteoarthritis.

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