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HYALUAL ARTRO

«The outcomes of the experimental study of efficacy of hyaluronic acid, combined with sodium succinate in the «Hyalual-ARTRO» formulation as compared to the monopreparation of hyaluronic acid».

> Based on the Scientific Research Project Report UDC 616. 728.48-001-06-084-08-089/2011 The Ministry of Healthcare of Ukraine The O.O. Bogomolets National Medical University The Department of Trauma and Orthopedics



The methods of study – morphologic and histological examination; biochemical assays; studies of the antioxidant protection system (spontaneous and induced chemiluminescence as an index of free-radical peroxidation intensity), the methods of statistical analysis.

The experiments involved 32 adult rabbits, weighting 2.9 ± 0.15 kg, employing the model of the transcartilage lesion of the articular cartilage in the knee joint. Hyaluronic acid/sodium succinate combination («Hyalual®-ARTRO» preparation) and the monopreparation of hyaluronic acid, both agents administered intraarticularly, were compared concerning their influence upon development (preventive action) and progression (therapeutic action) of osteoarthrosis of the knee joint and the reparative chondrogenesis in the articular cartilage. Also, the activities of the free-radical lipid peroxidation system (FRLP) in plasma and homogenates of hepatic and myocardial tissues were assessed, as well as alterations of the biochemical markers of cartilaginous and osseous metabolism, as detected in blood serum in articular injuries.

Six groups of experiments were performed.

The animals of the **1st Group** were six intact rabbits, which were later sacrificed in groups of three animals each, in 45 and, respectively, 90 days. n the animals of the 2nd (control) group (six rabbits) after obtaining the trans-cartilage lesion in the joint cartilage the operated knee joints were injected with 0.5 ml of normal saline (placebo) on a weekly basis, starting from the 2nd (three rabbits) and the 46th day (other three rabbits) postoperatively; these were sacrificed in 45 and 90 days postoperatively, respectively. **The 3d** and **the 4th groups** included 5 rabbits each, which, effective the next day after the artificial trans-cartilage defect were injected intra-articularly 0.5 ml of «Hyalual®-ARTRO» (1% hyaluronic acid solution with sodium succinate) – 3d group and 1% hyaluronic acid solution – 4th group, respectively, on a weekly basis to prevent osteoarthrosis. In order to evaluate therapeutic influence of «Hyalual®-ARTRO» and 1% hyaluronic acid on the clinical course of osteoarthrosis in the animals of **the 5th** and **6th group** (5 rabbits in each) the preparations were injected into the operated joints 46 days after creation of the trans-cartilage defect in the same way as in the animals of the 3d and the 4th group.

The experiments were carried on in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, CETS No. 123 (Strasbourg, March 18. 1986).

The extent, to which the destructive and dystrophic alterations in the knee joint have developed, was evaluated by the pattern and the expression of the pathological changes, found in the structure of the articular cartilage. 'The guidelines on experimental studies and clinical trials of anti-arthritis medications', approved by the Pharmacological Committee of the Ministry of Healthcare of Ukraine and the evaluation scale proposed by O'Driscoll S. W. et al./ Frenkel S.R. et al. were employed as standard evaluation tools.

The comparative morphological and histological analysis of the prophylactic influence on the development of post-injury osteoarthrosis exerted by the combined preparation «Hyalual-ARTRO» and the monopreparation of hyaluronic acid.

According to the macroscopic inspection of preparations, in application of «Hyalual-ARTRO» (hyaluronic acid with sodium succinate) in 80% of observations the bony-cartilaginous defect was completely filled by the regenerate in 45 days post-injury; partial filling was found in 20% of cases. However, in application of the monopreparation of hyaluronic acid complete filling of the cartilaginous defect occurred only in 40% of observations.

Fig.1,2

When «Hyalual-ARTRO» preparation (hyaluronic acid with sodium succinate) was applied, the microscopic examination revealed predominance of chondroid cellular elements of uneven density in the regenerate. Focal proliferation of chondrocytes and spacious cell-free areas were noted within the regenerate.

Fig.3

Homotypic morphological changes were observed in administration of the monopreparation of hyaluronic acid. However, in these cases the regenerate was dominated by the cells of the fibroblastic pool; areas of disorganization were noted as well as formation of layers of fibrous connective tissue.

Fig.4



The anterior surface of the native macroscopic preparations of the thigh-bone (distal extremity) in 45 days post-injury



Fig. 1 The complete filling of the bonycartilaginous defect by the regenerate in intra-articular administration of hvaluronic acid with sodium succinate («Hyalual-ARTRO» preparation).



Fig. 2 The partial filling of the bony-cartilaginous defect by the regenerate in intra-articular administration of the monopreparation of hyaluronic acid.

Microscopic examination of the regenerates. 45 days post-injury. Haematoxylin-eosin stain. x10





Fig. 3 The chondroid fibrous regenerate in administration of hyaluronic acid with sodium succinate («Hyalual-ARTRO» preparation).



Fig. 4 Fibrous and cartilaginous regenerate in administration of the monopreparation of hyaluronic acid.



The comparative morphological and histological analysis of the therapeutic influence on the progression of post-injury osteoarthrosis, exerted by the combined preparation «Hyalual-ARTRO» and the monopreparation of hyaluronic acid.

In macroscopic examination of the native preparations in 90 days after the trans-cartilage injury the supra-patellar surface of the thigh-bone (distal extremity) was observed to have signs of progression of pathological changes in the articular cartilage, which were more pronounced in the animals administered the monopreparation of hyaluronic acid. These changes were characterized by the loss of lustre and appearance of the localized roughness of the articular cartilage.

Fig. 5,6

The microscopic examination in animals, which were administered «Hyalual-ARTRO» preparation (hyaluronic acid with sodium succinate) the bony-cartilaginous defect was completely filled with the regenerate; the latter being well-integrated and formed by densely packed chondroid cells. The shape and the surface of the regenerate acquired the shape, typical for the joint; the regenerate provided for a tight contact, being continuous with the edge of the articular cartilage defect.

Fig. 7,8

In intra-articular administration of the monopreparation of hyaluronic acid (without sodium succinate) the formation of the regenerate within the defect occurred in a more slow fashion; the degenerative and dystrophic changes in the articular cartilage being more prominent.

The anterior surface of the native macroscopic preparations of the thigh-bone (distal extremity) in 90 days post-injury



Fig. 5 Complete filling of the bony-cartilaginous defect by the regenerate in intraarticular administration of hyaluronic acid with sodium succinate («Hyalual-ARTRO» preparation).



Fig. 6 Partial filling of the bony-cartilaginous defect by the regenerate in administration of monopreparation of hyaluronic acid. Loss of lustre and appearance of roughness of the articular cartilage.

The microscopic examination of the regenerates. 90 days post-injury. Haematoxylin-eosin stain. x10





Fig. 7 The chondroid regenerate in administration of hyaluronic acid with sodium succinate («Hyalual-ARTRO» preparation).



Fig. 8 The chondroid cells of the regenerate. The regenerate of the previous figure is partially shown.



The combined formulation «Hyalual-ARTRO» and the monopreparation of hyaluronic as prophylaxis against the development of experimental osteoarthrosis: the results of the comparative study.

Thus, in application of hyaluronic acid preparations the morphological data of the reparative restoration of the articular cartilage in post-injury trans-cartilage defects indicate that the application of «Hyalual-ARTRO» preparation (hyaluronic acid in combination with sodium succinate) causes a more active formation of the cartilaginous regenerate inside the defect compared to the monopreparation of hyaluronic acid. This allows speculating that combination of the solution of hyaluronic acid with sodium succinate optimizes differentiation of skeletogenous cells into chondrocytes and promotes integration of the cartilaginous regenerate.

The results of the histological study have shown that in animals of both groups the process of reparative restoration and filling of the articular cartilage defect for 45 days after sustaining a trans-cartilage injury is accompanied by the development of degenerative and dystrophic changes, which are not limited to the forming regenerate alone, but spread to the articular cartilage beyond the defect. These pathological changes in the articular cartilage, which are typical for the initial stage of the post-injury osteoarthrosis deformans, were less expressed in the animals which were injected «Hyalual-ARTRO» into the kneejoint (combined preparation of hyaluronic acid with sodium succinate).



The results of the quantitative morphological evaluation of the regenerate, filling the trans-cartilage defect and the articular cartilage at the edges of the defect in application of hyaluronic acid preparations.





The preparation of hyaluronic acid + sodium succinate (Hyalual-ARTRO)

* - prophylactic influence upon the development of the osteoarthrosis of the knee-joint.

** - prevention of progression of the already present osteoarthrosis of the knee-joint and the influence upon the reparative chondrogenesis of the articular cartilage.



THE RESULTS OF THE EXPERIMENTAL STUDY OF THE ACTIVITY OF FREE-RADICAL LIPID PEROXIDATION (FRLP) AND THE SYSTEM OF THE ANTIOXIDANT PROTECTION OF THE ORGANISM

The previously reported disturbances of the FRLP system activity in rabbit plasma, the latter being an integrative medium of the animal organism, were confirmed by the biochemical investigations of the antioxidant protection system in test animals. The outcomes of the research work demonstrate alterations of plasma lipid composition due to the prolonged previous activation of the FRLP system and lack of readily oxidized lipid fractions. Besides, impairments of the FRLP system activity were detected in hepatic and myocardial homogenates of rabbits with the experimental injury of the knee-joint. As a whole, the above data have evidenced a substantial structural damage to the liver tissue and an insignificant activation of FRLP system in the myocardium.

In intra-articular administration of «Hyalual-ARTRO» and the monopreparation of 1% hyaluronic acid in rabbits, both aimed at prevention and treatment of osteoarthrosis, the indices of FRLP system activity have indicated differing efficacy of these two medications both in the area of direct intended action and in selected biological substrates under study (plasma, hepatic tissue and myocardium).



THE RESULTS OF THE EXPERIMENTAL STUDY OF THE ACTIVITY OF FREE-RADICAL LIPID PEROXIDATION (FRLP) AND THE SYSTEM OF THE ANTIOXIDANT PROTECTION OF THE ORGANISM

In particular, when the above preparations were applied with a prophylactic intent, the earliest stages of experimental osteoarthrosis were characterized by the following findings:

- Partial normalization of FRLP system activity in plasma with a distinctly higher efficacy
 of hyaluronate/succinate combination;
- The inhibited condition of the hepatic FRLP system was notably improved under the influence of «Hyalual-ARTRO» formulation, whereas hyaluronic acid as a monopreparation has significantly deepened the detected derangements, probably due to the additional metabolic burden to the liver.

When the aforementioned preparations were applied as therapies for the already present experimental osteoarthrosis, the following effects were noted:

- a substantial excessive antioxidant action of the «Hyalual-ARTRO» preparation and lack of the same effect in plasma when hyaluronic acid was administered as monopreparation;
- the absence of any substantial influence of the hyaluronic acid monopreparation on the expression of structural and functional manifestations of hepatic damage, and, on the contrary, prevention of such derangements by the «Hyalual-ARTRO» formulation.

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THE RESULTS OF BIOCHEMICAL STUDIES OF THE MAIN MARKERS OF OSSEOUS AND CARTILAGINOUS METABOLISM IN THE STUDY GROUPS

The serum concentrations of glycoproteins, chondroitine sulphate and changes in the fractional composition of GAG have indicated a higher efficacy of «Hyalual-ARTRO» preparation when compared with the conventional monopreparation of hyaluronic acid. This phenomenon is attributed to the fact that apart from the solution of hyaluronic acid «Hyalual-ARTRO» includes sodium succinate – an inhibitor of free-radical oxidation and a membrane protector with an antihypoxic action, capable of inhibiting lipid peroxidation and increasing the activity of superoxide dismutase. Since the «Hyalual-ARTRO» preparation contains hyaluronic acid, which prevents and treats arthrosis, it accelerates reparative processes, reducing inflammatory and destructive changes in the cartilaginous and bony tissue of joints in test animals. «Hyalual-ARTRO» is an effective treatment modality for the degenerative lesions of the cartilages. The unique combination of hyaluronic acid with so-dium succinate allows for a simultaneous action upon several pathogenetic mechanisms, causing degeneration of cartilaginous tissue.



THE RESULTS OF BIOCHEMICAL STUDIES OF THE MAIN MARKERS OF OSSEOUS AND CARTILAGINOUS METABOLISM IN THE STUDY GROUPS

The results of biochemical blood tests in experimental rabbits

Indices	Groups of test animals					
	Group I, n=6 Intact, control	Group II, n=6 Injury to the joint	Group III, n=5 Prophylaxis, «Hyalual®-ARTRO»	Group IV, n=5 Prophylaxis, Mono- preparation	Group V, n=5 Treatment, «Hyalual®-ARTRO»	Group VI, n=5 Treatment, Mono- preparation
Glycoproteins, g/l	0,60±0,04	1,00±0,12 *	0,68±0,02 ◊	0,82±0,02	0,78±0,02 ∩	0,88±0,01 ∆
Crude protein, g/l	69,5±1,81	66,3±1,32	67,7±1,68	67,7±1,19	68,1±2,58	68,5±2,42
Chondroitine sulphate, g/l	0,124±0,003	0,505±0,041*	0,129±0,007	0,123±0,012	0,112±0,002	0,166±0,013∆
Total GAG, IU	10,8±0,43	27,9±3,51 *	12,7±0,86	16,4±1,52	10,0±0,71	11,1±0,40
GAG, Fraction I	6,7±0,33	16,0±2,14 *	7,2±0,51	9,6±0,74	6,2±0,41	6,9±0,20
GAG, Fraction I I	3,3±0,20	9,0±1,01 *	4,6±0,43	4,2±0,87	2,0±0,25	1,9±0,28
GAG, Fraction III	0,8±0,05	2,9±0,54 *	0,9±0,15 ◊	2,6±0,37	1,7±0,11 △	2,4±0,13 ∆
Alkaline phosphatase, U/I	383,0±21,6	113,0±11,5 *	271,0±53,3	315,0±18,7	285,0±56,2	279,0±50,6
Total calcium, mmol/l	3,18±0,03	2,51±0,10	2,95±0,05	2,87±0,04	2,90±0,06	2,72±0,06
lonized calcium, mmol/l	1,45±0,03	1,19±0,11	1,50±0,06	1,56±0,09	1,40±0,05	1,20±0,03
Potassium, mmol/l	9,0±0,19	8,95±1,07	8,26±0,16	8,93±0,18	7,16±0,06	6,57±0,13
Sodium, mmol/l	154,6±2,20	135,9±1,37	132,5±1,73	126,7±1,32	134,1±0,67	131,6±1,21

Notes: * - p < 0.05 in comparison with the 1st and the 2nd groups of animals; $\delta - p < 0.05$ 05 in comparison with the 3^d and the 4th groups of animals; $\Delta - p < 0.05$ 05 in comparison with the 3^d and the 5th groups of animals; $\Delta - p < 0.05$ in comparison with the 3^d and the 5th groups of animals.

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THE INFLUENCE OF «HYALUAL-ARTRO» PREPARATION UPON THE MAIN PATHOGENETIC LINKS OF OSTEOARTHROSIS

The main pathogenetic links of osteoarthrosis	THE INFLUENCE OF SODIUM SUCCINATE IN THE «Hyalual-ARTRO» PREPARATION	
ΗΥΡΟΧΙΑ	Possesses a pronounced anti-hypoxia activity, resulting from the influence on the transport of mediator aminoacids ^{1,2}	
DEFICIENCY OF ENERGY	Acts as a stimulator of synthesis of reparative elements within the cell. The system of succinic acid oxidation bypasses the slow stages of Krebs cycle, which allows for a significant acceleration of energy production process ^{3,4}	
ACIDOSIS	Decreases the concentrations of lactate, pyruvate and citrate, which accumulate in cells in early stages of hypoxia ^{5,6}	
FREE-RADICAL LIPID PEROXIDATION	Enhances the compensation activation of aerobic glycolysis and decreases the intensity of inhibition of oxidative process in the mitochondria ^{7,8}	

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THE FIRST **CARTILAGE-MODIFYING**



Hyaluronic acid	Sodium Succinate		
 Symptomatic action Possesses an analgesic action ^{1, 2} Provides an anti-inflammatory effect ^{3, 4} 	 Pathogenetic action Normalizes intra-cellular metabolism in chondrocytes ⁵ Stimulates the synthesis of components of the extra-cellular cartilage matrix ^{6,7} 		
 Decreased patient requirements in NSAID and intra-articular steroids² Improvement of the functional condition of the joint Improved quality of life for the patient² 	 Prolonged life of the cartilage ⁸ The option to delay endoprosthetic surgery ⁸ 		
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