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Changes in the indicators of connective tissue metabolism in the blood serum of experimental rats under the conditions of modeling the development of degenerative processes in paravertebral muscles

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Low back pain is a common health problem. To deepen the understanding of the pathogenesis of the disease, experimental studies on animals with modeling of the pathological process are necessary. Objective. Based on the analysis of biochemical markers of connective tissue metabolism in the blood serum of laboratory rats, the applicability of the studied models of degenerative muscle tissue damage to study the relationship between this condition and the development of disorders in spinal motor segments was assessed. Methods. Two models of reproduction of degenerative processes in the paravertebral muscles of white rats were tested: I (n = 5) — alimentary (diet-induced) obesity, by keeping it for 3 months on a high-calorie diet; II (n = 5) — ischemia, by tying the large rectus muscles of the back with suture material (45 days). Control group (n = 5) — intact animals of similar age and sex. The content of glycoproteins, total chondroitin sulfates (CS), hexosamines, protein-bound hexoses, seroglycoides, fractional distribution and total content of hydroxyproline and glycosaminoglycan sulfates (GAGs) were investigated in the blood serum of rats. The results. In the blood serum of rats of groups I and II, a significant increase compared to the control level of glycoproteins was determined, with a greater effect in the ischemia model, but no significant changes of protein-bound hexoses, hexosamines and CS were recorded. The level of inflammatory markers (sialic acids and seroglycoides) in the blood serum of animals of both groups did not differ significantly from the control, and changes in the parameters of hydroxyproline (except for the slightly changed protein-bound fraction) and GAGs were significant only for the ischemia model. Conclusions. Based on the analysis of biochemical markers of connective tissue metabolism in rats of groups I and II, changes characteristic of degenerative processes were determined, with a greater manifestation in the ischemia model. No significant increase in biochemical markers of inflammation was recorded. Both models can be used to reproduce dystrophic processes in osteochondrosis.

Біль у попереку — поширена проблема охорони здоров'я. Для поглиблення розуміння патогенезу хвороби необхідні експериментальні дослідження на тваринах із моделюванням патологічного процесу. Мета. На підставі аналізу біохімічних маркерів метаболізму сполучної тканини в сироватці крові лабораторних щурів оцінити придатність досліджуваних моделей дегенеративного ураження м'язової тканини для вивчення взаємозв'язку цього стану з розвитком порушень у хребтових рухових сегментах. Методи. Апробовано дві моделі відтворення дегенеративних процесів у паравертебральних м'язах білих щурів: I (n = 5) — аліментарного (дієт-індукованого) ожиріння, шляхом утримання упродовж 3 міс. на висококалорійному раціоні; II (n = 5) — ішемії, шляхом перев'язування великих прямих м'язів спини шовним матеріалом (45 діб). Контрольна група (n = 5) — інтактні тварини аналогічного віку та статі. У сироватці крові щурів досліджено вміст глікопротеїнів, загальних хондроїтинсульфатів (ХС), гексозамінів, гексоз, пов'язаних із білком, сероглікоїдів, фракційний розподіл і сумарний вміст гідроксипроліну та глікозаміноглікансульфатів (ГАГс). Результати. У сироватці крові щурів I і II груп визначено значуще підвищення порівняно з контролем рівня глікопротеїнів, з більшим ефектом у моделі ішемії, проте не зафіксовано значущих змін гексоз, пов'язаних із білком, гексозамінів і загальних ХС. Рівень маркерів запалення (сіалових кислот і сероглікоїдів) у сироватці крові тварин обох груп значуще не відрізнявся від контролю, а зміни показників гідроксипроліну (крім мало змінюваної білково-зв'язаної фракції) і ГАГс були значущими лише для моделі ішемії. Висновки. На підставі аналізу біохімічних маркерів метаболізму сполучної тканини щурів I та II груп визначено зміни, характерні для дегенеративних процесів, із більшим проявом у моделі ішемії. Не зафіксовано істотного підвищення біохімічних маркерів запалення. Обидві моделі можуть бути використані для відтворення дистрофічних процесів за остеохондрозу. Ключові слова. Сполучна тканина, дегенерація, біохімія, хребет, моделювання на тваринах, паравертебральні м'язи.

Key words. Connective tissue, degeneration, biochemistry, spine, modeling, muscle

Introduction

Lumbar pain is a common health problem, affecting more than 80 % of adults during their lifetime and is the leading cause of disability worldwide. This condition is often associated with lumbar disc degeneration, which should not be considered as an isolated event, but rather a continuum of events. Patients with significant intervertebral disc degeneration often have increased fatty infiltration in the multipartite and extensor muscles. In women, it is more severe at the level of L_{IV}–L_V and L_V–S_I. In men, fatty infiltration is greater in the lumbar muscle at the L_V–S_I level [1, 2].

The muscles that attach to the spine play a crucial role in the functioning of the spine and the entire body. A clear association between fatty infiltration and paravertebral muscle fibrosis and low back pain has been demonstrated in the scientific literature. Dysfunction/degeneration of muscles can be a factor in initiating the progression of spine abnormalities, in particular, disorders in the structure of the intervertebral disc [3]. Conversely, dysfunction of the lumbar paravertebral muscles due to pain, caused by structural and functional changes in the spinal motor segments, can trigger a violation of their structure. Macroscopically, it is manifested by a decrease in the cross-sectional area and an increase in fat infiltration in the paravertebral muscles at the lumbar level [1, 4]. In addition, there are microscopic changes, such as an impairment of the distribution of fibers [5].

Vertebral lesions are associated with fatty infiltration of the paravertebral muscles. It can be assessed qualitatively (e.g. Gutalier's classification) and quantitatively using software for calculating MRI scans or computed tomography [6, 7].

Assessing the state of the paravertebral muscles before surgery for degenerative spine diseases can be useful for the surgeon in terms of predicting the functional state and recovery of patients [5, 8].

The purpose of the study: based on the assessment of biochemical indicators of connective tissue metabolism in the blood serum of laboratory rats, to assess the suitability of the studied models of degenerative muscle tissue damage for further study of the relationship between this condition and the development of degenerative disorders in spinal motor segments.

Material and methods

Experimental studies were conducted in compliance with the requirements of humane treatment of experimental animals [9, 10] after the approval of the plan by the Bioethics Committee at the State Institution Professor M. I. Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine (Protocol No. 191 of 22.04.2019).

Within the framework of the study, two variants of reproduction of degenerative processes in paravertebral muscles were tested using female white laboratory rats (at the beginning of the experiment, aged 2 months, weight 130–210 g) of the population of the above experimental biological institution.

In the first group (*model of alimentary (diet-induced) obesity*) rats (n = 5) were kept for 3 months on a high-calorie diet adapted to the Teklad Custom Diet TD.10670 formulation (22.5 g or 40–45 % kcal fat), developed by the Envigo company for laboratory rats and mice [4, 11]. Food composition per 100 g: lard 18 g, pork liver 2 g, sunflower oil 3 g, wheat groats 45 g, cottage cheese 2 g, egg powder 10 g, milk powder 6 g, sugar 5 g, beetroot 3 g, carrot 2 g, meat and bone meal 4 g.

In the second group (*ischemia model*), rats (n = 5) had the large rectus muscles of the back tied with suture material through a skin incision (Fig. 1). The animals were removed from the experiment 45 days after manipulation.

The control group (n = 5) involved intact animals of similar age and sex, which were kept on a standard diet [12].

Euthanasia of all rats was performed by decapitation under open inhalation anesthesia with diethyl ether due to the need to obtain blood for biochemical studies, which, after natural coagulation, was freed from the formed elements by centrifugation for 15 min at 3000 rpm. The supernatant liquid was separated and the content of glycoproteins was determined according to the method of Steinberg and Dotsenko [13], total chondroitin sulfates according to the reaction with rivanol using the Nemeth–Csoka method as modified by L. I. Slutsky [13, 14].

Fractional analysis and research of the total content of hydroxyproline in the blood serum of experimental

Fig. 1. Appearance of the surgical wound after ligation of the rectus major muscles of the back in white rats

animals with the determination of fractions of free and protein-bound metabolites, which characterized anabolic and catabolic processes in the collagen-hydroxyproline system, was carried out with separation into free and protein-bound fractions [15].

The content of hexosamines in the cartilage tissue of experimental animals was determined by the method of N. F. Boas, the content of hexoses bound to protein by the orcinol method [16].

The fractional composition of glycosaminoglycan sulfates (GAGs) was determined by precipitation with resoquin and sequential separation from the sediment with sodium chloride solutions of increasing concentration. The composition of fraction I included easily soluble GAGs with a predominance of hyaluronates and chondroitin-6-sulfate, fraction II comprised moderately soluble GAGs with a predominance of chondroitin-4-sulfate, fraction III involved mainly highly sulfated glycosaminoglycan sulfates with a predominance of keratan sulfates, as well as dermatan sulfate, heparan sulfate, etc. [17]. The content of sialic acids [16], seroglycoides [18] in blood serum was also determined.

The results of biochemical studies were statistically processed according to the non-parametric Mann–Whitney test. After that, the level of error of the first kind was determined for comparing the indicators of the four groups among themselves (Kruskal–Wallis criterion), r_c — the level of the error of the first kind for comparing the indicators

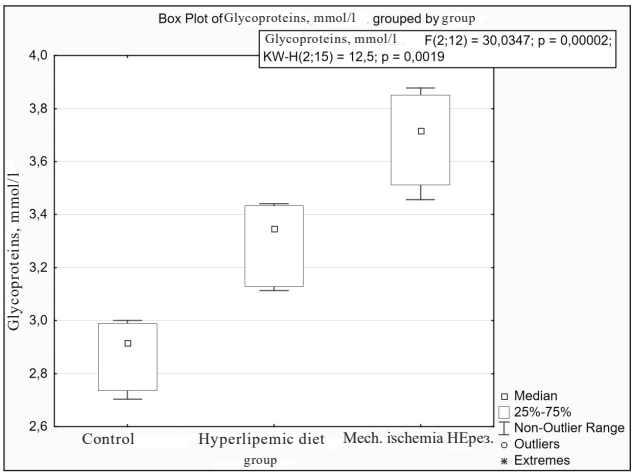
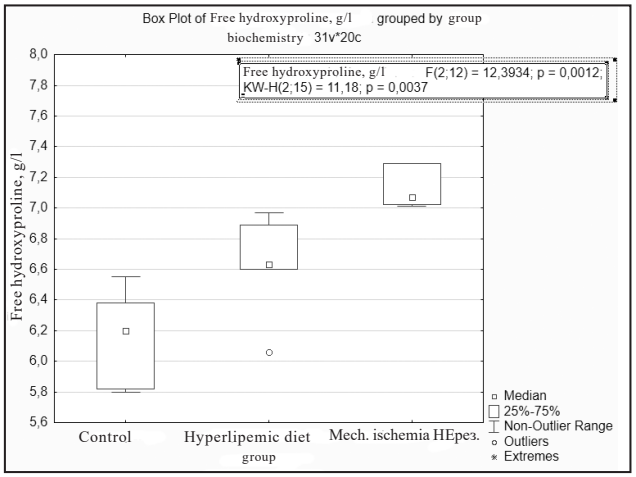
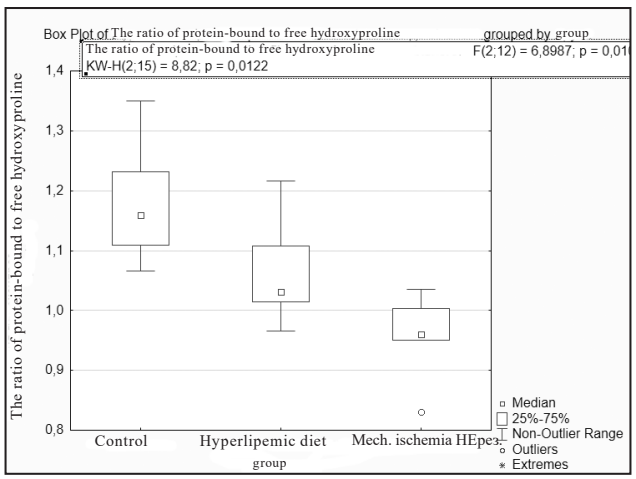


Fig. 2. The level of glycoproteins in blood serum of rats of the studied groups





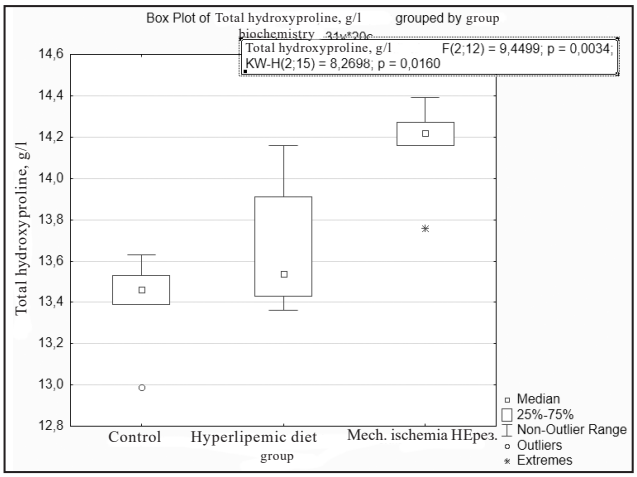


Fig. 3. Characteristics of the level of hydroxyproline in the blood serum of rats of the three studied groups (significant difference between the ischemia model and the control)

Table

Changes in the biochemical parameters of connective tissue metabolism in the blood serum of rats after simulation of the degenerative process in muscle tissue

Indicator		Group of animals		
		control (n = 5)	obesity model (n = 5)	ischemia model (n = 5)
Free hydroxyproline, g/l H(2.15) = 11.18; p = 0.0037		6.15 ± 0.15 6.20 [5.82; 6.38]	6.63 ± 0.16 6.63 [6.60; 6.89]	7.25 ± 0.16 7.07 [7.02; 7.29]
			$p_к$ = 0.060; p_i = 0.012	$p_к$ = 0.012
Hydroxyproline protein-bound, g/l H(2.15)=1.63; p = 0.443		7.25 ± 0.15 7.17 [7.08; 7.19]	7.05 ± 0.15 7.19 [6.73; 7.31]	6.91 ± 0.13 6.93 [6.74; 7.09]
			$p_к$ = 0.916; p_i = 0.531	$p_к$ = 0.210
			13.68 ± 0.15 13.54 [13.43; 13.91]	14.16 ± 0.11 14.22 [14.16; 14.27]
			$p_к$ = 0.403; p_i = 0.047	$p_к$ = 0.012
Ratio of hydroxyproline protein-bound to free H(2.15) = 8.820; p = 0.012		1.18 ± 0.05 1.16 [1.11; 1.23]	1.07 ± 0.04 1.03 [1.02; 1.11]	0.96 ± 0.04 0.96 [0.95; 1.00]
			$p_к$ = 0.095; p_i = 0.095	$p_к$ = 0.012
GAGs	Sum H(2.15) = 9.62; p = 0.008	0.29 ± 0.01 0.31 [0.28; 0.31]	0.34 ± 0.02 0.35 [0.30; 0.36]	0.41 ± 0.01 0.42 [0.39; 0.44]
			$p_к$ = 0.210; p_i = 0.022	$p_к$ = 0.012
	Chondroitin-6-sulfate, g/l H(2.15) = 9.95; p = 0.007	0.17 ± 0.01 0.18 [0.17; 0.18]	0.21 ± 0.01 0.21 [0.18; 0.22]	0.25 ± 0.01 0.26 [0.23; 0.26]
			$p_к$ = 0.070; p_i = 0.037	$p_к$ = 0.012
	Chondroitin-4-sulfate, g/l H(2.15)=8.06; p = 0.018	0.10 ± 0.003 0.10 [0.09; 0.10]	0.11 ± 0.006 0.11 [0.10; 0.12]	0.13 ± 0.007 0.14 [0.12; 0.15]
			$p_к$ = 0.210; p_i = 0.095	$p_к$ = 0.012
	Keratin sulfates, g/l H(2.15) = 9.14; p = 0.010	0.02 ± 0.001 0.02[0.017; 0.021]	0.02 ± 0.002 0.02 [0.020; 0.024]	0.028 ± 0.001 0.028 [0.028; 0.030]
			$p_к$ = 0.210; p_i = 0.034	$p_к$ = 0.012
Amount ratio of hydroxyproline to the amount of GAGs H(2.15) = 8.540; p = 0.0140 < 0.05		45.81 ± 1.60 43.93 [43.56; 46.90]	40.99 ± 2.02 39.33 [37.96; 44.77]	34.57 ± 1.28 34.02[32.65; 36.40]
			$p_к$ = 0.210; p_i = 0.060	$p_к$ = 0.012
Hexoses bound to protein, g/l H(2.15) = 5.46; p = 0.065		1.12 ± 0.03 1.14 [1.07; 1.19]	1.11 ± 0.04 1.14 [1.04; 1.19]	1.29 ± 0.06 1.32 [1.22; 1.37]
			$p_к$ = 0.835; p_i = 0.060	$p_к$ = 0.060
Hexosamines, g/l H(2.15) = 2,66; p = 0.265		0.95 ± 0.08 0.95 [0.77; 1.13]	1.05 ± 0.09 1.05 [0.87; 1.21]	1.18 ± 0.07 1.12 [1.06; 1.32]
			$p_к$ = 0.403; p_i = 0.296	$p_к$ = 0.210
Total chondroitin sulfates, g/l H(2.15) = 2.94; p = 0.229		0,46 ± 0,04 0.39 [0.39; 0.55]	0.56 ± 0.06 0.48 [0.47; 0.69]	0.58 ± 0.05 0.51 [0.49; 0.69]
			$p_к$ = 0.210; p_i = 0.403	$p_к$ = 0.210
Glycoproteins, mmol/l H(2.15) = 12.50; p = 0.002		2.87 ± 0.06 2.92 [2.74; 2.99]	3.29 ± 0.07 3.35 [3.13; 3.43]	3.68 ± 0.09 3.72 [3.51; 3.85]
			$p_к$ = 0.012; p_i = 0.012	$p_к$ = 0.012
Sialic acids, mmol/l H(2.15) = 2.00; p = 0.368		3.97 ± 0.14 3.99 [3.76; 4.12]	4.11 ± 0.13 4.18 [3.85; 4.25]	4.254 ± 0.155 4.309 [3.978; 4.533]
			$p_к$ = 0.403; p_i = 0.403	$p_к$ = 0.296
Seroglycoides, g/l H(2.15) = 3.27; p = 0.195		0.54 ± 0.03 0.50 [0.49; 0.61]	0.58 ± 0.04 0.53 [0.51; 0.68]	0.62 ± 0.04 0.57 [0.55; 0.71]
			$p_к$ = 0.246; p_i = 0.208691	$p_к$ = 0.209

Note. The level of error of the first kind: r — comparison of indicators of three groups among themselves (Kruskal-Wallis test), r_c — comparison of indicators of the control group (Mann-Whitney test), r_i — group with ischemia (Mann-Whitney test).

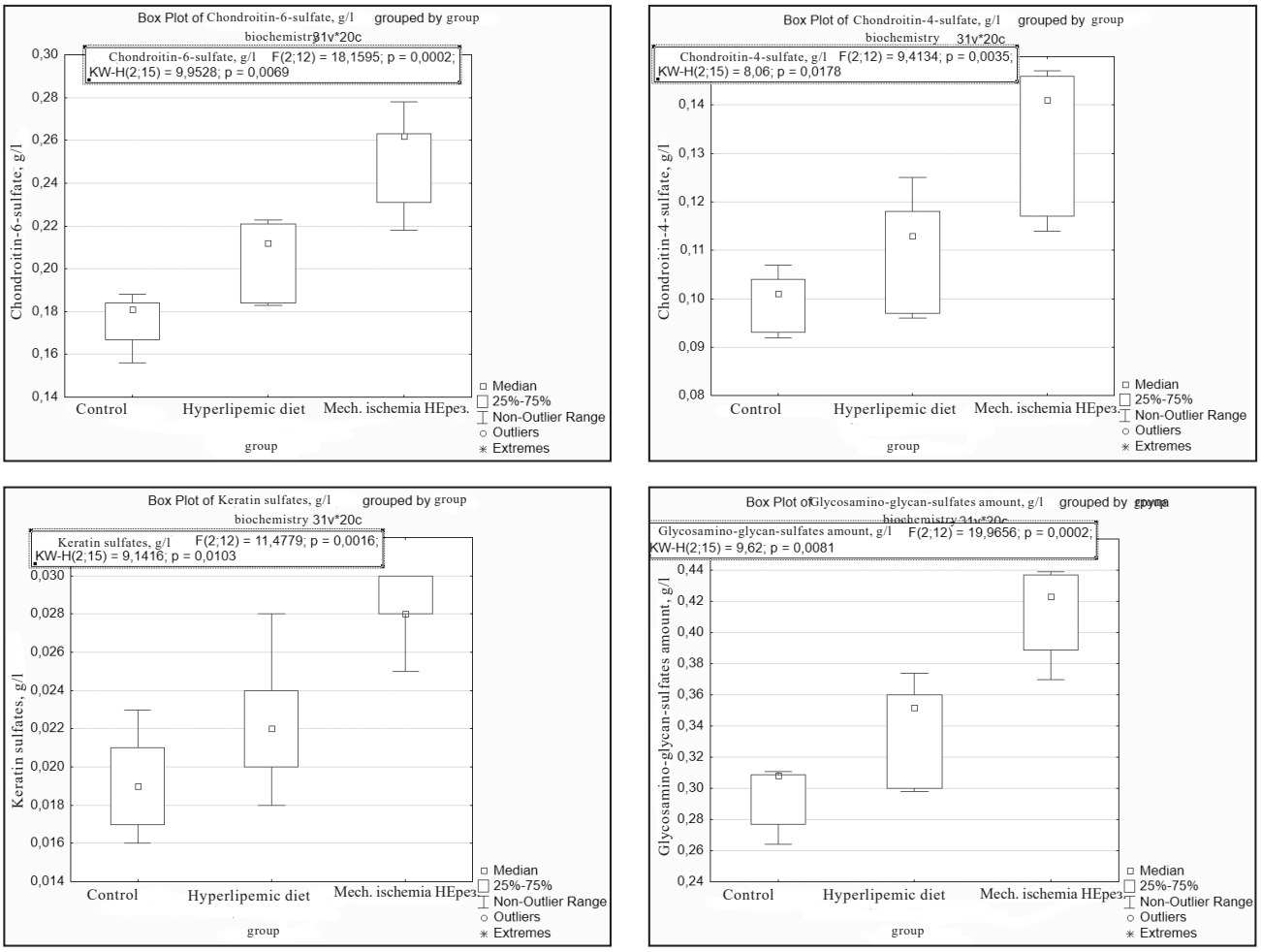


Fig. 4. Indicators of the GAGs system in rats of the three studied groups (significant difference between the ischemia model and the control)

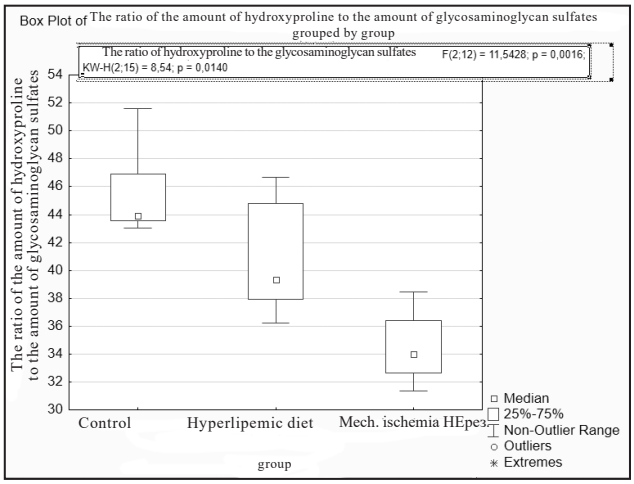


Fig. 5. The ratio of the amount of hydroxyproline in the blood serum to the amount of GAGs in experimental animals of three groups (significant difference between the ischemia model and the control)

of the group with the control, r_1 — for comparing the indicators of the group with mechanical ischemia of muscles with non-resident suture material, r_2 — indicators of the group with mechanical ischemia of muscles with resident suture material (Mann–Whitney criterion). The difference was considered significant at $p < 0.05$ [19].

Results and their discussion

In both studied models of paravertebral muscle degeneration, no significant changes were observed in such indicators of connective tissue metabolism as the level of protein-bound hydroxyproline, protein-bound hexoses, hexosamines, total chondroitin sulfates, sialic acids, and seroglycoids (see Table).

The only indicator that showed a statistically significant difference between the obesity model and the control group was the serum level of glycoproteins. It turned out to be higher in rats kept on a hyperlipidemic diet, which, in our opinion, indicated to a greater extent the elements of intoxication in the considered conditions. The level of glycoproteins was even higher in the blood serum of rats of the second group (ischemia model) (Table, Fig. 2). In conditions of unchanged other indicators of the inflammatory process, it should probably be considered as

manifestations of intoxication by products of dystrophy. The calculated Cohen's d standardized effect values allow us to characterize the effect of the obesity model on glycoprotein levels as very large (d = 1.982) and the ischemia model as great (d = 3.419).

In general, a much wider spectrum and a greater range of changes in connective tissue metabolism indicators were recorded in the blood serum of animals of the second group (ischemia model), suggesting a sharp disturbance of its metabolism. In particular, changes in the content of the fraction of free and total hydroxyproline in blood serum and the ratio of the concentration of the protein-bound fraction to free hydroxyproline were determined (Table, Fig. 3). This indicates the activation of catabolic processes in the collagen-hydroxyproline system and the development of connective tissue degeneration.

According to the ratio of the levels of protein-bound and free hydroxyproline, the effect of the ischemia model compared to the control can be characterized as very large (d = 1.669), according to the level of total and free hydroxyproline, it is great (d = 2.217 and d = 2.241, respectively).

A significant increase in the content of all studied fractions of GAGs in blood serum was also recorded (Table, Fig. 4). Regarding the sum of GAGs, fractions of chondroitin-6-sulfate and keratan sulfates, the largest value of the standardized effect was determined (d — 3.130; 2.662; 2.556, respectively).

The ischemia model had a rather large effect on the chondroitin-4-sulfate fraction (d = 1.921).

A corresponding decrease in the ratio of the amount of hydroxyproline to the amount of GAGs in the blood serum of experimental animals was determined (Table, Fig. 5). The value of the standardized effect (d = 2.455) suggests a great influence of the ischemia model on the ratio of the amount of hydroxyproline to the amount of GAGs.

Thus, in both models of paravertebral muscle degeneration, there was a significant increase in the level of glycoproteins in the blood serum of the animals, with the ischemia model having a greater effect than the obesity model.

Changes in the indicators of hydroxyproline (with the exception of protein-bound) and GAGs compared to the control group were significant only for the ischemia model, and the influence of the hyperlipidemic diet on these indicators could not be established.

Levels of inflammatory markers, sialic acids, and seroglycoides were not significantly different from those of the control group either in the obesity model or in the ischemia model, indicating minimal activation of the inflammatory component.

In both models, significant changes in the blood serum content of close metabolites of the glycosaminoglycan system — protein-bound hexoses and hexosamines — were also not recorded. An increase in the content of total chondroitin sulfates in the blood serum of experimental animals was also not detected.

Conclusions

Based on the assessment of biochemical indicators of the metabolism of connective tissue in rats with simulated degeneration of paravertebral muscles (obesity model and ischemia model), significant differences from animals of the control group were determined, namely: changes characteristic of the activation of degenerative processes. On the other hand, no significant increase in biochemical markers of inflammation was recorded in both experimental groups.

In the case of modeling degeneration of paravertebral muscles by ligation (ischemia model), more expressed changes in biochemical markers of dystrophy were revealed compared to a group of animals kept on a hyperlipidemic diet (obesity model).

Both models can be successfully used to reproduce dystrophic processes in osteochondrosis.

Conflict of interest. The authors declare no conflict of interest.

References

1. Age related content of different tissues in the lumbar spine paravertebral muscles with degenerative diseases / V. Radchenko, A. Skidanov, D. Morozenko [et al.] // Orthopaedics, Traumatology and Prosthetics. — 2017. — No. 1 (606). — P. 80–86. — DOI: 10.15674/0030-59872017180-86. (in Ukrainian)
2. Ozcan-Eksi E. E. Severe lumbar intervertebral disc degeneration is associated with modic changes and fatty infiltration in the paraspinal muscles at all lumbar levels, except for L_1-L_2: a cross-sectional analysis of 50 symptomatic women and 50 age-matched symptomatic men / E. E. Ozcan-Eksi, M. S. Eksi, M. A. Akcal // World Neurosurgery. — 2019. — Vol. 122. — P. e1069–e1077. — DOI: 10.1016/j.wneu.2018.10.229.
3. Noonan A. M. Paraspinal muscle pathophysiology associated with low back pain and spine degenerative disorders / A. M. Noonan, S. H. M. Brown // JOR Spine. — 2021. — Vol. 15, 4(3). — P. e1171–e1176. — DOI: 10.1002/jsp2.1171.
4. Alteration of lumbar muscle morphology and composition in relation to low back pain: a systematic review and meta-analysis / T. Seyedhoseinpoor, M. Taghipour, M. Dadgoo [et al.] // Spine Journal. — 2022. — Vol. 22 (4). — P. 660–676. — DOI: 10.1016/j.spinee.2021.10.018.
5. Structural changes of lumbar muscles in non-specific low back pain: a systematic review / D. Goubert, J. Van Oosterwijck, M. Meeus, L. Danneels // Pain Physician. — 2016. — Vol. 19 (7). — P. e985–e1000.
6. Assessment of paravertebral soft tissues using computed tomography / A. Skidanov, A. Avrunin, M. Tymkovych [et al.] // Orthopaedics, Traumatology and Prosthetics. — 2015. — No. 3 (600). — C. 61–65. — DOI: 10.15674/0030-59872015361-64. (in Ukrainian)
7. Assessing fatty infiltration of paraspinal muscles in patients with lumbar spinal stenosis: goutallier classification and quantitative MRI measurements / F. Mandelli, C. Nüesch, Y. Zhang [et al.] //

Frontiers in Neurology. — 2021. — Vol. 12. — Article ID: 656487. — DOI: 10.3389/fneur.2021.656487.
8. Skidanov A. Forecasting the results of surgical treatment of patients with degenerative diseases of the lumbar spine depending on the state of paravertebral muscles / A. Skidanov // Orthopaedics, Traumatology and Prosthetics. — 2018. — No. 4 (613). — P. 14–23. — DOI: 10.15674/0030-59872018414-23. (in Ukrainian)
9. European Convention for the protection of vertebrate animals used for research and other scientific purposes. Strasbourg, 18 March 1986: official translation. Verkhovna Rada of Ukraine. (In Ukrainian). URL: http://zakon.rada.gov.ua/cgi-bin/laws/main.cgi?nreg=994_137. 21.
10. On protection of animals from cruel treatment: Law of Ukraine №3447-IV of February 21, 2006. The Verkhovna Rada of Ukraine. (In Ukrainian). URL: http://zakon.rada.gov.ua/cgi-bin/laws/main.cgi?nreg=3447-15
11. Boas N. F. Metod for the determanation of hexosamines in tissues / N. F. Boas // The Journal of Biological Chemistry. — 1953. — Vol. 204 (2). — P. 553–562.
12. Scientific and practical recommendations for keeping laboratory animals and working with them [Naukovo-praktychni rekomendatsiyi z utrymannya laboratornykh tvaryn ta roboty z nymy] / Yu. M. Kozhemiakin, O. S. Khromov, M. A. Filonenko, G. A. Saifetdinova. — Kyiv : Derzhavnyy farmakolohichnyy tsentr MOZ Ukrayiny, 2002. — 155 p.
13. Morozenko D. V. Research methods markers of connective tissue metabolism in modern clinical and experimental medicine [Metody doslidzhennya markeriv metabolizmu spoluchnoyi tkanyny u klinichniy ta eksperymental′niy medytsyni] / D. V. Morozenko, F. S. Leontieva // Molodyy vchenyy. — 2016. — No. 2 (29). — P. 168–172. (in Ukrainian)
14. Clinical biochemistry : study guide [Klinichna biokhimiya : navchal′nyy posibnyk] / O. P. Tymoshenko, L. M. Voronina, V. M. Kravchenko [et al.]. — Kharkiv : Gold pages, 2003. — 239 p. (in Ukrainian)
15. Sharaev P. N. Method of determination of free and bound oxyproline in blood serum [Metod opredeleniya svobodnogo i svyazannogo oksiprolina v syvorotke krovi] / P. N. Sharaev // Laboratornoye delo. — 1981. — No. 5. — C. 283–285. (in Russian)
16. Medical laboratory technologies : A guide to clinical laboratory diagnostics, in 2 volumes [Meditsinskiye laboratornyye tekhnologii: Rukovodstvo po klinicheskoy laboratornoy diagnostike, v 2-kh t.] / [V. V. Alekseev and others]; ed. A. I. Karpishchenko. — 3rd ed. — Vol. 2. — Moscow : Geotar-Media, 2013. — 792 p. (in russian)
17. Pat. 29198 UA, MPK (2006) G01 N33/48. The method of determination of fractions of sulfated hexosaminoglycans [Sposib vyznachennya fraktsiy sul′fatovanykh heksozaminohlikaniv] / Leontyeva F. S., Filipenko V. A., Tymoshenko O. P. [et al.]; applicants and patent holders Sytenko Institute of Spine and Joint Pathology, Kharkiv State ZooVeterinary Academy. — No.u 2007 08505; appl. 24.07.2007; publ. 10.01.2008, Bul. No. 1. (in Ukrainian)
18. Kamyshnikov V. S. Clinical and biochemical laboratory diagnostics: reference book: in 2 volumes [Kliniko-biokhimicheskaya laboratornaya diagnostika: spravochnik: v 2 t.] / V. S. Kamyshnikov. — 2nd ed. — Minsk : Interpressservice, 2003. — Vol. 1. — 2003. — 495 p.; Vol. 2. — 2003. — 463 p. (in russian)
19. Lang T. A. How to describe statistics in medicine. A guide for authors, editors and reviewers [Kak opisyvat' statistiku v meditsine. Rukovodstvo dlya avtorov, redaktorov i retsenzentov] / T. A. Lang, M. M. Sesik. — Moscow : Practical Medicine, 2011. — 480 p. (in russian)

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CHANGES IN THE INDICATORS OF CONNECTIVE TISSUE METABOLISM IN THE BLOOD SERUM OF EXPERIMENTAL RATS UNDER THE CONDITIONS OF MODELING THE DEVELOPMENT OF DEGENERATIVE PROCESSES IN PARAVERTEBRAL MUSCLES

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