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Features of Collagen Metabolism in Compact and Cancellous Bone Tissue in Rats with Alloxan Diabetes

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Abstract One of the chronic complications of diabetes mellitus is diabetic osteopathy, which manifests itself from moderate osteoporosis to spontaneous fractures of long bones [13]. The mechanisms of development of diabetic osteopathy in patients with diabetes have not been sufficiently studied [3]. At the same time, in the pathogenesis of the development of osteopathy, the following has been established: a) a decrease in the production of collagen and alkaline phosphatase by osteoblasts, caused by a deficiency of insulin, necessary for the formation of organic and inorganic bone matrix; b) indirectly, through insulin-like growth factors, a decrease in stimulation of osteoblasts; c) increased bone resorption by osteoclasts caused by the accumulation of glycation products [1].

Keywords Alloxan diabetes

1. Introduction

In patients with diabetes mellitus, the activity of biochemical markers of bone metabolism changes significantly. Thus, the synthesis of type 1 bone collagen is significantly reduced, derivatives of transverse collagen fibers specific to bones and cartilage tissue - pyridinoline and deoxypyridinoline, as well as hydroxyproline, an amino acid exclusively contained in collagen proteins, are intensively excreted in the urine [9]. Similar data were obtained in experimental diabetes [8]. However, one must take into account the fact that hydroxyproline is formed during the breakdown of not only bone collagen, but also collagen that is localized in other tissues. Thus, changes in the level of hydroxyproline should be considered as an indirect sign of the development of metabolic changes in the bones.

Bone is a constantly renewed tissue and there are two main metabolic processes continuously present in it - bone formation and bone resorption. Substances associated with these two processes are considered markers of bone collagen [2,4]. These markers, in addition to those listed above, include amino- and carboxyl-terminal properties of type 1 procollagen: PINP and PICP - markers of bone formation. Amino- and carboxy-terminal telopeptides of type 1 collagen associated with cross-links: β-CrossLaps are markers of bone resorption [11,14].

Diabetes can cause the development of irreversible complications in bone tissue; Further study of the pathogenetic mechanisms of osteopathy is necessary, including in experimental models of diabetes mellitus. Models of experimental diabetes in laboratory animals are varied, including the alloxan model, which is widely used. Numerous studies have established that the use of alloxan in rats causes a condition similar in manifestations to insulin-dependent diabetes mellitus: persistent hyperglycemia and hypoinsulinemia [1,15].

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2. Materials and Methods

Experiments were carried out on 48 white outbred male rats weighing 180-220 grams in compliance with the principles of humane treatment of animals set out in the Declaration of Helsinki (2000). The animals were kept on a standard vivarium diet with free access to water.

In rats of the experimental group (38 animals), diabetes mellitus was induced by a single subcutaneous injection of alloxan tetrahydrate (Sigma-Aldrich, USA) at a dose of 170 mg/kg body weight [5]. The reproduction of diabetes was monitored by the following indicators: the level of glycemia in blood plasma using the glucose oxidase method and the

level of glycated hemoglobin in whole blood (NycoCard-HbA1c on a NycoCard Reader 11 reflectometer, USA). The animals were taken out of the experiment on days 14 and 28 under short-term ether anesthesia. The mortality rate during the experiment was 36%.

In homogenates of compact bone tissue (femoral diaphysis) and cancellous bone tissue (body of the 2nd lumbar vertebra) the following was determined: the amount of total collagen [14], PINP (ELISA, ELISA; Cloud-Clone Corp., USA), beta-CrossLaps (ELISA, ELISA; IDS SERUM CrossLaps, UK), activity of collagenolytic enzymes (CA) according to E. Schalinatus modified (12). The amount of SA was expressed in millimoles of hydroxyproline per 1 kg of dry tissue mass (mmol/kg), PINP and b-CrossLaps - in picograms per 1 ml of homogenate supernatant (pg/ml), CA - in micromoles of hydroxyproline per 1 g of protein per hour (μmol /g*h).

Statistical processing of the obtained data was carried out using the Statistica software package from Stat Soft. In the sample groups, the median (Me) and interquartile range (25%; 75%) were determined. The statistical significance of differences between groups was assessed using the Wilcoxon -Mann-Whitney U test with a critical level of 0.05.

3. Results and Discussion

Administration of alloxan to animals caused the development of hyperglycemia and an increase in the level of glycated hemoglobin. Thus, by the 14th day of the experiment, the content of glucose and glycated hemoglobin increased by 89.7% (p = 0.0004) and 25.3% (p = 0.0006), respectively; by the 28th day of the study, the studied indicators were 152.1% (p = 0.0001) and 148.8% (p = 0.001), respectively, which suggests the development of diabetes in experimental animals.

The state of collagen metabolism is determined by the processes of accumulation and breakdown of this protein. The prevalence of synthetic processes of bone tissue collagen is indicated by an increase in the content of SA and the level of P1NP [6,11]. The intensity of the breakdown of the main protein of connective tissue is characterized by an increase in the concentration of b-CrossLaps [11,14], the level of CA and a decrease in the amount of SC [8].

By the 14th day of the study, in the diaphysis of the femur and the body of the 2nd lumbar vertebra, the level of the bone collagen resorption marker b-CrossLaps increased from 0 to 23.2 pg/ml (p = 0.0001) and from 0 to 62.25 pg/ml (p=0.008), respectively, compared to the control. On the 28th day of the experiment, the content of the studied markers decreased and amounted to 17.5 pg/ml (p = 0.00004) and 32.5 pg/ml (p = 0.00002), respectively. During the same period of alloxan diabetes, an increase in the level of KA was detected from 0 to 0.5508 μmol/g*h (p=0.0004) in compact bone and from 0 to 0.1695 μmol/g*h (p=0.00004) – in spongy bone tissue. There were no significant significant differences in the SC indicator compared to the control, either in the diaphysis of the femur or in the body of the 2nd lumbar vertebra (Table 1).

Changes in P1NP levels are controversial. On the 14th day of experimental diabetes, a significant increase in the studied indicator was noted from 0 to 405.2 pg/ml (p = 0.0003) in compact bone and from 30.5 to 265.6 pg/ml (p = 0.0004) in trabecular bone bones. The SC content increased according to the type of bone tissue by 9.6% (p = 0.04) and 16.8% (p = 0.03), followed by a return to the initial level on day 28. In the body of the 2nd lumbar vertebra on the 28th day of the experiment, there was a decrease in the level of bone collagen formation marker P1NP by 47.6% (p = 0.01) compared to the control. At the same time, no significant differences in the content of P1NP in compact bone in diabetes compared with controls were detected (Table 1), which is explained by more active processes of bone metabolism in trabecular bone [2].

Table 1. Indicators of collagen metabolism in rats with alloxan diabetes, Me [25%; 75%]

Index	Bone type	Control (n=10)	Alloxan diabetes, day 14 (n=8)	Alloxan diabetes, day 28 (n=11)
β-CrossLaps (pg/ml)	comp.	0 [0; 0]	23,2 [10,35; 29,6] p***=0,0001	17,5 [13,8; 20,5] p***=0,00004
	spongy	0 [0; 0]	62,25 [2,6; 134,3] p**=0,008	32,5 [26,4; 42] p***=0,00002
P1NP (pg/ml)	comp.	0 [0; 29,7]	405,2 [266,3; 531,7] p***=0,0003	23,5 [20,4; 50]
	spongy	30,5 [20,5; 41]	265,6 [151,6; 408,2] p***=0,0004	15,98 [3; 27,4] p*=0,01
SC (mmol/kg)	comp.	118,99 [102,54; 122,6]	130,43 [122,2; 163,86] p*=0,04	123,57 [105,26; 139,8]
	spongy	102,54 [91,53; 121,5]	119,78 [115,21; 137,3] p*=0,03	101,65 [91,53; 114,41]
KA (μmol/g*h)	comp.	0 [0; 0,15]	0 [0; 0,1216]	0,5508 [0,2358; 0,6356] p***=0,0004
	spongy	0 [0; 0,12]	0 [0; 0,1258]	0,1695 [0,1356; 0,4238] p**=0,005

Note: comp. – compact bone tissue; spongy – spongy bone tissue; p – statistical significance of differences with control.

The observed changes in bone collagen metabolism can be explained as follows. In the pathogenesis of bone tissue damage in diabetes, an excess of glucocorticoid hormones plays a significant role [10], which enhance bone resorption, which leads to a decrease in bone mass. This can explain the increase in b-CrossLaps content on days 14 and 28 of the experiment. On the other hand, according to the literature, glucocorticoids reduce calcium absorption in the intestine [7]. In this case, there is a decrease in the synthesis of calcium-binding protein, vitamin D deficiency and accelerated destruction of receptor zones for calcium triol in the mucosa [7]. Vitamin D deficiency causes an increase in the number of osteoblasts in rats and, consequently, an increase in the synthesis of bone collagen [4]. The latter is a probable confirmation of the increase in MC on the 14th day of diabetes.

The suppression of anabolic processes by the 28th day of the experiment, visible from the decrease in the level of bone formation marker P1NP, may be explained by the reduced formation of insulin-like growth factor 1 [13], which is probably mediated by hypoinsulinemia observed in alloxan-induced diabetes [15,16].

Thus, alloxan-induced diabetes causes an intensification of bone metabolism and leads to the following changes:

1) activation of the processes of breakdown of type 1 collagen in compact and spongy bone tissue on days 14 and 28 of observation;
2) acceleration of synthesis processes in trabecular and compact bone on the 14th day of experimental diabetes, followed by inhibition of anabolism in cancellous bone tissue on the 28th day of the experiment;
3) the predominance of anabolic processes over the processes of bone collagen breakdown on the 14th day of observation in both compact and trabecular bone.

REFERENCES

[1] Zholdoshbekov E.Zh., Zholdoshev B.N., Madumarov M.G. Bone and joint changes in diabetes mellitus // Bulletin of the Kyrgyz-Russian Slavic University. – 2010. – T. 10. No. 7. – P. 133-136.

[2] Kamilov F.Kh., Farshatova E.R., Menshikova I.A., et al. Osteoporosis: the influence of chemical factors of the industrial environment on bone tissue metabolism. – Ufa: Publishing House "World of Printing". – 2015. – 311 p.

[3] Manulenko V., Shishkin A.N., Mazurenko S.O. Clinical features of the development of osteopathy in patients with type 2 diabetes // Bulletin of St. Petersburg University. Episode 11. "Medicine." – 2009. - No. 2. – P. 7-13.

[4] L.M. Obukhova, E.I. Erlykina. Biochemistry. Metabolic aspects of childhood biochemistry: textbook. – St. Petersburg: SpetsLit, 2023 – P. 353-373.

[5] Palchikova N.A., Selyatitskaya V.G., Shorin Yu.P. Quantitative assessment of the sensitivity of experimental animals to the diabetogenic effect of alloxan // Problems of endocrinology. - 1987. - No. 4. – P. 65-68.

[6] Pobel E.A., Bengus L.M., Dedukh N.V. Markers of bone metabolism during the healing of long bone fractures // Osteoporosis and Osteopathy. – 2012. - No. 2. – P. 25-32.

[7] Porovoznyuk V.V., Mazur I.P. Skeletal system and periodontal diseases. – Kyiv, 2004. – 446 p.

[8] Savinova N.V., Danilova O.V., Butolin E.G., Vyatkin V.A. Collagen metabolism and mineral content in the bone tissue of animals with experimental diabetes // Molecular Medicine. – 2020. – T. 18. No. 2. – P. 27-32.

[9] Slutsky L.I. Biochemistry of normal and pathologically altered connective tissue. – Leningrad: Medicine, 1969. – 376 p.

[10] Tkach S.N., Shcherbak A.V. Damage to the osteoarticular system in diabetes mellitus // Clinical medicine. – 1986. - No. 5. – P. 21-26.

[11] Toroptsova N.V., Nikitinskaya O.A. Forecasting the effectiveness of therapy using biochemical markers of bone metabolism // Ukrainian Rheumatological Journal. – 2011. – T. 3. No. 45. – P. 35-38.

[12] Sharaev P.N., Ivanov V.G., Tolstolutskaya T.O., et al. Methods of laboratory research of connective tissue biopolymers: a textbook. – Izhevsk, 2009. – 44 p.

[13] Shishkin A.N., Manulenko V.N. Diabetic osteopathy // Bulletin of St. Petersburg University. Episode 11. "Medicine." – 2008. - No. 3. – P. 70-79.

[14] Gerdhem P., Ivaska K.K., Alatalo S.L., et al. Biochemical markers of bone metabolism and prediction of fracture in elderly women // J. Bone Miner Res. – 2004. – Vol. 19. No. 3. – P.386-393. doi: 10.1359/JBMR.0301244.

[15] Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes // Diabetologia. – 2008. – Vol. 51. N2. – P. 216-226.

[16] Szkudelski T. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas // Physiol. Res. – 2001. – Vol. 50. – R. 536-546.