Original article:

Dynamics of biochemical markers of the connective tissue metabolism in the blood of rats after insertion of steel implants with diamond-like carbon coating into the femur

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Abstract:

Objective: It is known that the regeneration of bone tissue after implantation of any structures depends on the coating of their surface. Therefore, the research of the influence of steel implants with diamond-like carbon coating on regeneration of bone tissue in the dynamics after implantation is a topical issue. ***Material and Methods:*** The study was conducted on 61 male rats, 5 of them were intact animals. There were also 2 groups of rats with 28 animals in each group (the 1st group was the control one where implants were without diamond-like carbon coating, the 2nd group was experimental where the implants were without coating). We determined the content of glycoproteins, chondroitin sulfates, alkaline phosphatase, total calcium, oxyproline and osteocalcin in the blood of rats on the 7th, 14th, 30th and 90th days after implantation. ***Results and Discussion:*** Group 2 rats had a faster osteointegration compared with the group 1 rats, which was manifested by the normalization of biochemical markers of bone metabolism (glycoproteins, chondroitinsulfats, oxyproline and osteocalcin) on the 90th day of an experiment. ***Conclusion:*** Using steel implants with diamond-like carbon coatings showed that the content of glycoproteins and chondroitin sulfates increased only on the 7th day; oxyproline and osteocalcin increased on the 7th, 14th and 30th days after implantation; on the 90th day the rates of these biochemical markers reached the norm, indicating a high efficiency of osteointegration.

Keywords: biochemical markers, bone tissue, diamond-like carbon coating, implantation, osteointegration.

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Introduction

It is known that regeneration of bone tissue after implantation of any structures depends on the coating on its surface[1,2]. According to research results of F. E. Pinottiet al.[3], the hydrophilic surface implants improve osteointegration in the tibia of rats. To evaluate the effectiveness of implantation, modern laboratory markers of osteointegration (osteopontin, osteocalcin, osteoactivin) are used. They have positive effect on the course of bone tissue regeneration. Reducing the expression of osteopontin and osteocalcin over time after implantation of titanium implants, as well as their lowest level at the end of the postoperative period, allows us to assess the biocompatibility of the materials[4,5]. According toT. Haraet al. research results[6], rough surface implants under the periosteum contribute to the expression of alkaline phosphatase, bone sialoprotein and osteocalcin. The influence of chitosan-collagen composites that could induce invivo-formation of new bone tissue around the surface of the titanium implant was also studied. Theimmunohistochemical

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markers osteopontin and alkaline phosphatasewere determined for the evaluation of regeneration[7]. According to C. Hinüberet al.[8], unlike titanium diamond-like coatings show greater inertness, non-toxicity and biocompatibility, which is interesting to manufacturers of surgical implants. However, in modern medicine, scientific studies of using diamond-like carbon coating as orthopedic implants of medical steel are absent. There are mainly scientific publications on the application and biocompatibility of this coating of implants in dentistry[9-11]. Thus, the research of the influence of steel implants with diamond-like carbon coating on regeneration of bone tissue in dynamics after implantation is topical. Moreover, the evaluation of its course using biochemical connective tissue markers in the blood for further application in traumatology and orthopedics.

Study goal was to investigate the dynamics of biochemical markers of connective tissue metabolism to evaluate the osteointegration of steel implants with diamond-like carbon coating after their insertion into the femur.

Material and methods

The research was carried out on the basis of the departments of experimental modeling and transplantation in the experimental biological clinic and laboratory diagnostics and immunology of the state institution Sytenko Institute of Spine and Joint Pathology of the National Academy of Medical Sciences of Ukraine in 2018. In total, 61 male rats were used during the experiment, 5 of them were intact animals and 56 rats were divided into 2 groups with 28 animals in each group (the 1st group was the control one where implants were without diamond-like carbon coating, the 2nd group was experimental where the implants were without coating). The age of animals at the beginning of the experiment was 5-6 months; the body weight was 300-400 grams.In vivo testing of steel implants with diamond-like carbon coatings was carried out using an experimental model that created frontlateral access to the distal metaphysis of the left femur. With the help of dental bur, a standard holedefect with a diameter of 2 mm and a depth of 3 mm was created. It was followed by implantation of the test specimen (1-1.5 mm of the implanted specimen remained not immersed in the defect). Implants were made of medical stainless steel BÖHLERINTERNATIONAL, the standard EN 10204-2.2 / DIN 50049-2.2 (LLC LEP "LEOORTHOGROUP", Ukraine).On the surface of implant samples, a diamond-like carbon coating was applied (by the method of filtered vacuum-arc cathode plasma, the thickness of the layer was at least 1 μm).The manufacturer is the laboratory of super-hard amorphous diamond and polycrystalline diamond coatings of the National Scientific Center "Kharkiv Physical-Technical Institute", Ukraine. Test specimens of implants were without coating. The shape of the implants was cylindrical; the pins were 4 mm in length, 2 mm in diameter. Diamond-like carbon coating was applied on the surface ofone planeof the diameter and 2.5-3 mm of the pin length. The blood for research was taken from animals after decapitation on the 7th, 14th, 30th and 90th days after implantation.The serum was prepared from the blood by centrifugation. The content of glycoproteins, chondroitin sulfates, alkaline phosphatase, total calcium, oxyproline and osteocalcin was determined[12,13]in blood serum of rats. The statistical analysis of the data was carried out using the non-parametric Wilcoxon criterion with the median (Me) and percentile calculations (25% and 75%)[14].

Ethical approval: all studies were conducted in compliance with the relevant bioethical requirements in relation to experimental animals in accordance with the Law of Ukraine No. 3447-IV dated February 21, 2006 "On the Protection of Animals from Cruel Treatment" (Articles 26, 31), the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and the Order of the Ministry of Education and Science of Ukraine No. 249 dated March 1, 2012 "On Procedure of Carrying out Scientific Research and Experiments on Animals by Scientific Institutions". The work was reviewed and approved by the SytenkoInstituteofSpineandJointPathology Bioethics Committee, proceeding No. 174 dated January 29, 2019.

Results and discussion

Group 1 rats, which were inserted implants without diamond-carbon coating into the femoral bone, had the glycoproteins in the blood increased by 61.6% on the 7th dayafter implantation, by 51.2% on the 14th day after implantation compared with the same index in intact animals ($p < 0.05$). The content of chondroitin sulfates in the blood of ratswas 27.5% on the 7th day of observation, and 7.2% on the 14th day compared with the same biochemical markers in the intact animals ($p < 0.05$). On the 30th and 90th days after implantation, there were no further changes in the content of glycoprotein and chondroitin sulfate in the blood of group 1 rats. The alkaline phosphatase activity was elevated by 45.2% only on the 14th day of observation.The content of total calcium in the blood of rats in group 1 did not change during the experiment. The concentration of oxyproline in the blood of rats stayed increased throughout the observation period: on the 7th day it increased by 46.7%, on the 14th day – by 68.4%, on the 30th day – by 79.6%, on the 90th day – by 61.2% compared to the same indicator in intact animals (p <0.05). The content of osteocalcin in the blood of group 1 rats increased on the 7th dayby 27.0%, on the 14th day – by 26.0%, on the 30th day – by 30.0%, on the 90th day – by 31.3% compared to the same indicator in the intact animals (p <0.05), Table 1

Table 1. Dynamics of biochemical markers connective tissue metabolism in rats' blood after introduction of steel implants without coating – I group (Me, 25%–75%)

Markers	Intactrats, n=5	Dynamic of laboratory markers			
		7 days	14 days	30 days	90 days
Glycoproteins, g/l	1.25 1.07 – 1.30	2.02* 1.90 – 2.36	1.89* 1.80 – 1.96	1.22 1.15 – 1.30	1.26 1.25 – 1.30
Chondroitin-sulfates, g/l	0.320 0.287–0.320	0.408* 0.385 – 0.415	0.343* 0.328 – 0.359	0.267 0.248 – 0.281	0.190 0.179 – 0.194
Alkaline phosphatase, U/L	420.0 380.0–435.0	384.0 352.0 – 489.0	610.0* 564.0 – 673.0	496.0 404.5 – 522.0	317.0 312.0 – 348.0
Total calcium, mmol/l	2.40 2.38 – 2.40	2.38 2.37 – 2.39	2.38 2.37 – 2.39	2.38 2.38 – 2.38	2,38 2.38 – 2.39
Oxyproline, mg/l	15.20 14.50–15.50	22.30* 21.70 – 24.00	25.60* 24.50 – 28.50	27.30* 23.75 – 27.40	24.50* 22.85 – 25.70
Osteocalcin, ng/ml	48.00 47.00 – 53.00	61.00* 56.50 – 65.00	60.50* 59.35 – 63.00	62.40* 61.75 – 64.10	63.00* 62.00 – 64.55

*Note: * – authentically by Wilcoxon compared to the intact group, p<0.05*

Group 2 rats, which were inserted implants with diamond-carbon coating into the femoral bone,had the content of glycoproteins and chondroitin sulfates in the blood elevated only on the 7th day by 45.6% and 31.9%, respectively, compared to the same biochemical markers in the intact rats (p <0.05). The content of glycoproteins and chondroitin sulfates did not change on the 14th, 30th and 90th days. The alkaline phosphatase activity was increased by 11.7% on the 7th day, and by 62.6% on the 14th day compared to the intact animals (p <0.05). The total calcium content in the blood of group 2 rats did not change during the experiment. The concentration of oxyproline in the blood of rats was increased throughout the observation period: by 27.6% on the 7th day, by 71.1% on the 14th day, and by 26.3% on the 30th day compared with the same biochemical markers in the intact animals (p <0.05).The content of osteocalcin in the blood of rats increased by 15.4% on the 7th day, by 34.3% - on the 14th day, by 15.4% - on the 30th day compared with the same biochemical markers in the intact rats (p <0.05). On the 90th day of the experiment, the content of oxyproline and osteocalcin in the blood of rats did not differ from the content of these biochemical markers in the intact rats (Table 2).

Glycoproteins and chondroitin sulfate are biochemical markers of inflammatory-dystrophic and regenerative processes in bone tissue after implantation. Oxyproline is an amino acid that is a part of the bone marrow collagen structure. Osteocalcin is a non-collagen protein, which is mainly localized in the extracellular matrix and synthesized by mature osteoblasts and is a sensitive indicator of bone tissue metabolism.We observed an increase in the content of oxyproline and osteocalcin during the experiment in group 1 rats, indicating an increased duration of osteointegration of bone implants which delayed the formation ofdirect contact and functional connection between an implant and bone tissue.

On the contrary, group 2 animals had afaster osteointegration, which was manifested in the faster normalization of the biochemical markers of bone metabolism. Since the distribution of markers on bone formation and its resorptionrates are conditional, and the increased duration of its reorganization is characterized by oxyproline and osteocalcin increase in the blood of rats, it can be concluded that the presence of diamond-like carbon coating improves and accelerates the processes of osteointegration of the steel implants to the bone tissue after implantation.

Conclusion

After the insertion of steel implants without a diamond-like carbon coating into the femur of group 1 rats,the increase in the content of biochemical

Table 2. Dynamicsofbiochemicalmarkersconnectivetissuemetabolismin rats' blood after introduction of steel implants with coating – IIgroup (Me, 25%–75%)

Markers	Intactrats, n=5	Dynamic of laboratory markers			
		7 days	14 days	30 days	90 days
Glycoproteins, g/l	1.25 1.07 – 1.30	1.82* 1.74 – 1.91	1.36 1.27 – 1.45	1.22 1.19 – 1.44	1.33 1.24 – 1.37
Chondroitin-sulfates, g/l	0.320 0.287–0.320	0.422* 0.410–0.433	0,317 0.308 – 0.338	0.263 0.250 – 0.298	0.283 0.266 – 0.297
Alkaline phosphatase, U/L	420.0 380.0–435.0	469.0* 443.0 – 487.5	683.0* 582.0 – 753.0	307.0 258.5 – 343.0	396.0 373.5 – 504.0
Total calcium, mmol/l	2.40 2.38 – 2.40	2.38 2.37 – 2.41	2.40 2.38 – 2,46	2.38 2.30 – 2.38	2.38 2.38 – 2.39
Oxyproline, mg/l	15.20 14.50–15.50	19.40* 18,80 – 21,30	26.00* 24.85 – 27.80	19.20* 17.95 – 19.40	15.70 14.95 – 16.05
Osteocalcin, ng/ml	48.00 47.00 – 53.00	55.40* 54.75 – 57.85	64.45* 62.60 – 65.55	55.40* 53.77 – 59.30	48.10 46.85 – 49.40

*Note: * – authentically by Wilcoxon compared to the intact group, p<0.05*

markers of inflammatory-destructive and regenerative processes (glycoproteins and chondroitin sulfates) in the blood of group 1 rats on the 7th and 14th days, as well as the indices of osteointegration (oxyproline and osteocalcin) on the 7th, 14th, 30th and 90th days after implantation indicates a longer course of osteointegration of implants.Group 2 rats were inserted steel implants with diamond-like carbon coatings, and had the content of glycoproteins and chondroitin sulfates increased only on the 7th day, the content of oxyproline and osteocalcin increased on the 7th, 14th and 30th days after implantation. At the end of the experiment, on the 90th day of observation, all markers of bone metabolism in group 2 ratswere the same as those of the intact animals. This obviously proves high efficiency of osteointegration of steel implants with diamond-like carbon coatings.

Conflict of interest: None declared

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