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Original Article

Effect of hafnium and titanium coated implants on several blood biochemical markers after osteosynthesis in rabbits

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Abstract: Purpose: An experimental study comparing the dynamics of several biochemical markers before and after osteosynthesis, utilizing implants coated with titanium and hafnium nitrides and non-coated implants on rabbits' bones. Materials and methods: The Study has been conducted on 30 rabbits of both sexes, at the age of 6-7 months, weighing 2526.5±74.4 gm. Animals underwent open osteotomy of the tibia in the middle third of the diaphysis followed by the intramedullary nailing. The level of alkaline phosphatase, calcium, phosphorus, total protein, glucose, ALT and AST were monitored for 60 days. Results: the use of implants coated with titanium and hafnium nitrides, which have high strength, thermal and chemical stability, was not accompanied by the development of additional negative reactive changes compared to non-coated implants. Conclusion: Nanotechnology used in manufacturing bioinert coatings for implants for osteosynthesis, has made the post-operative period less complicated as reflected by less expressed changing in the markers of bone metabolism and hepatotoxicity.

Keywords: Implants, bioinert coating, titanium and hafnium nitrides, osteosynthesis

Introduction

One of the fundamental problems of traumatology and orthopedics is the persisting frequency and severity of reactive inflammation and infection. Much of it is linked to the quality of implants and the material from which they are made. The term "metallosis" has consistently pursued each of orthopedic surgeons. Reactions to the materials used in modern designs often depends on the patient's tolerance, but, isolating them from each other gives a chance to avoid the development of severe complications.

One of the solutions to this problem of the new generation of implants is coating their surfaces with titanium. The coated implants become biocompatible, have low coefficient of friction and adequate osteointegration [1].

Potentially promising are the implants coated with hafnium nitride, which is characterized by chemical inertness, excellent resistance to oxidation in extreme conditions [2], as well as biological compatibility (bioinert) with the tissues of the body [3, 4], but this type of coating is rare. The combination of titanium and hafnium nitride coating which is manufactured by using nanotechnological methods presents a particular interest, as this combination allows preventing possible intolerance of the organism to the metal or any post-operative complications.

The aim of the study consisted in the comparative evaluation of dynamics of reactive changes in the blood of experimental rabbits after using implants made of medical steel (comparison group) and implants coated with a mixture of hafnium and titanium Nitrides (experimental group).

Material and methods

The experiment was conducted in accordance with the requirements of the "European Con-

Table 1. Dynamics of changes of alkaline phosphatase, calcium and phosphorus level in the blood of experimental animals

	Alkaline phosphatase		Calcium		Phosphorus	
Normal values for rabbits	100-700 U/l		1.4-3.1 mM/l		0.81-1.13 mM/l	
Group	Experimental	Control	Experimental	Control	Experimental	Control
Pre operative	270.95±27.81	249.96±40.80	2.90±0.15	3.10±0.08	1.63±0.08	1.67±0.10
1st day post operative	235.35±27.79	217.79±29.71	3.10±0.04	3.16±0.05	1.48±0.06	1.42±0.05
5th day post operative	166.16±18.56*	146.36±18.63	3.22±0.04*	3.29±0.05	1.53±0.05	1.51±0.06
10th day post operative	175.94±19.23	138.44±22.02	3.22±0.04*	3.29±0.05**	1.58±0.07	1.54±0.07
20th day post operative	179.88±24.90	122.77±18.27	3.14±0.03	3.18±0.04	1.54±0.07	1.57±0.08
30th day post operative	180.56±24.10	125.52±23.38	3.22±0.06	3.17±0.06	1.54±0.05	1.52±0.06
60th day post operative	136.27±15.87	142.41±21.62	3.41±0.08*	3.25±0.10	1.54±0.10	1.40±0.14

*Significant differences with the pre-operative values of $p < 0.05$; **$p < 0.01$.

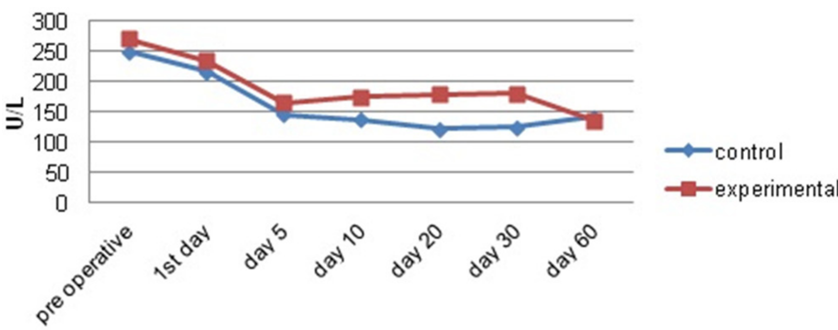

Figure 1. Dynamics of ALP during the experiment.

vention for the protection of vertebrate animals used for experimental and other scientific purposes". Studies have been conducted on 30 rabbits of both sexes, at the age of 6-7 months, weighing 2526.5±74.4. An open femoral osteotomy of the tibia in the middle third of the diaphysis has been done followed by intramedullary osteosynthesis.

The surgery was carried out under general anaesthesia (Rometar 2%, 0.15-0.2 ml/kg, Zoletil 100, 10-15 mg/kg). In the experimental group intramedullary osteosynthesis was performed with medical steel 12X18H9T coated with titanium and hafnium nitrides, with a diameter of 2 mm. In control group non-coated medical steel 12X18H9T (alloy composition: C-0.2%; Si-0.8%; Mn-2%; Ni-[8-9.5]%; S-0.02%; P-0.035%; Cr-[17-19]%; Cu-0.3%; Fe-67%), with the same diameter was used. Postoperative immobilization of the operated limb in a cast for up to 10 days followed.

Blood collection was performed by venipuncture of v. Saphena lateralis before surgery, and on the 1st, 5th, 10th, 20th, 30th and 60th days after the intervention. We evaluated the markers of bone metabolism: the overall activity of alkaline phosphatase (ALP) (kinetic colorimetric method using ALP DGKC system test (Audit Difgnostics, Ireland)), level of calcium (Ca) (photometric method (CCF, HUMAN)) and phosphorus (P) (spectrometric method (HUMAN)), as well as markers of potential hepatotoxicity: total protein (Randox, UK), aspartate aminotransferase and alanine aminotransferase (AST, ALT), the level of glucose (test system GLUC-PAP) (Randox, UK).

Statistical processing was carried out using batch SPSS v. 13.0. Student's t-test with a Bonferroni correction was used for all analysis. Differences were considered significant at a significance level of $p < 0.05$.

Results

The level of serum alkaline phosphatase decreased in both comparison and experimental group. However, on the 5th day after surgery, a significant difference was revealed only in experimental group (**Table 1**, **Figure 1**). In the control group ALP had tendency to decrease at all stages of observation with a minimum value on the 20th day. At the same time, the reduction of ALP activity in experimental group was less pronounced and reached the level of the con-

Table 2. The changes of glucose and total protein level in the blood of experimental animals

	Glucose		Protein	
Normal values for rabbits	4.1-8.5 mM/l		54-83 g/l	
Group	experimental	control	experimental	control
Pre operation	6.86±0.28	7.56±0.46	56.42±1.48	61.10±1.82
Day 1	7.85±0.19	7.67±0.37	59.17±1.14	60.77±1.32
Day 5	7.09±0.24*	6.17±0.63	59.30±0.90	59.33±2.32
Day 10	7.39±0.28$^{\sim\sim}$	6.95±0.25	62.13±1.37	62.47±1.15
Day 20	6.85±0.17	6.45±0.21	64.49±3.56*	64.79±1.37
Day 30	7.01±0.42	7.01±0.42	64.96±1.58**	64.72±1.23
Day 60	5.75±0.32$^{\sim\wedge}$	5.66±0.47*	58.84±0.68	66.23±1.88^{++}

*Significant differences with the pre-operative values of $p < 0.05$; **$p < 0.01$. $^{\sim}$Significant differences with the values of the 1st day $p < 0.05$; $^{\sim\sim}p < 0.01$. $^{\wedge}$Significant differences with the values of the 10th day $p < 0.05$. $^{++}$Significant differences of the experimental group, $p < 0.05$.

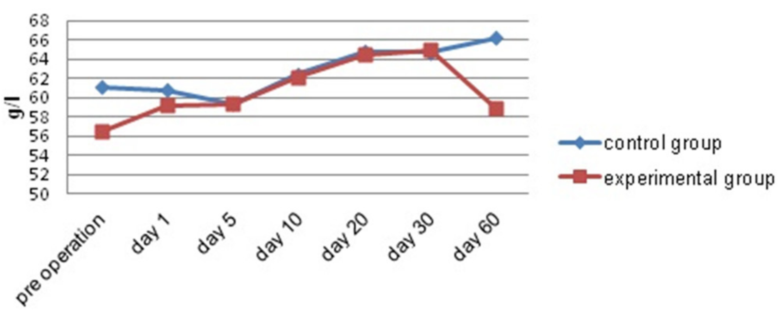

Figure 2. Dynamics of total protein for both groups.

trol group on the 60th day. At this period (60th days), ALP level was severely different from the pre-operative level, but the statistical significance was not established. The reason was the difference in the number of the animals, as it reduced with time: 5 rabbits were discarded in each period of the experiment.

The results are consistent with those of other authors who have registered decrease in the activity of ALK, from the first to the fourth week after the osteotomy and osteogenesis in dogs [5]. Moreover, according to Akesson K., the dynamics of bone metabolism and regeneration of the bone tissue after osteotomy involves the natural processes of resorption that predominated in the early months, while the markers of bone formation, including osteocalcin and alkaline phosphatase rose only 4-7 months after the start of the experiment. In general, the process of bone tissue formation is returned to baseline values during the year [6].

The level of serum calcium increased in both groups of animals with significant differences in dynamics. In the control group the calcium concentration in the serum had a tendency to decrease at all stages of the experiment while in the experimental group the level of calcium on day 10, significantly exceeded the pre operative value by 12% ($p = 0.004$) and on the 60th day-by 15% ($p = 0.015$). These results are consistent with the bone regeneration concept after surgery. Regeneration, is a dynamic process, starting with local resorption and formation of bone by assembly of discrete temporary anatomic structures - multicellular units (Basic Multi-cellular Unit (BMU)) including osteoclasts, osteoblasts, active mesenchymal cells and capillary loops, functioning to maintain skeletal balance. The bone regeneration is influenced and regulated by cells of BMU through local signals of growth factors and other cytokines. Activation of osteoclasts in bone resorption process is followed by the release of calcium into the extracellular space.

There is no unified consensus on the duration of the period of enhanced bone resorption in the process of bone tissue regeneration. Increased bone resorption and hypercalcemia after femoral fracture can be observed in the first week and last up to 3 months [7]. In experiments on rabbits, it has been proven, that bone formation process after osteotomy takes at least 12 weeks [8].

Our study found no significant difference in concentrations of phosphorus in the blood serum of both groups (**Table 1**). The values did not differ from the preoperative level and ranged within normal limits throughout the experiment.

Table 3. The changes of AST and ALT level in the blood of experimental animals

	AST		ALT	
Normal values for rabbits	48-80 U/L		14-113 U/L	
Group	Experimental	Control	Experimental	Control
Pre operation	45.67±11.96	49.16±8.74	70.74±8.31	82.61±8.17
Day 1	28.09±5.60	38.70±3.42	78.65±10.50	78.59±9.45
Day 5	15.44±1.89*	30.96±8.43	63.01±6.53	59.18±8.79
Day 10	27.87±3.49	32.85±4.22	66.96±5.75	73.57±7.40
Day 20	23.10±3.21	18.88±1.39	57.87±6.80	56.16±5.81
Day 30	39.03±6.28	36.20±7.65	66.08±3.94	69.51±11.31
Day 60	19.45±2.24	26.97±7.74	75.86±8.02	69.70±11.38

*Significant differences with the pre-operative values of $p < 0.05$.

The study showed no sign of hepatotoxic effect or liver cells cytolysis. The comparative analysis showed that the next day after the intervention the glucose concentration in the blood serum in both groups tended to increase: in the control group by 2%, and by 13% in the experimental group (**Table 2**). Reliable changes of glucose level were noticed in the experimental group with a decrease on day 10 by 36% ($p = 0.001$). On day 60 compared to the first day, an increase in glucose level by 28% was noticed ($p = 0.030$). In the control group glucose level significantly decreased ($p = 0.020$) on day 60 compared to pre-operative levels.

Insignificant changes in glucose level but within physiological range for this type of animals (rabbits) are, apparently, the consequence of postoperative stress. As result of an injury, body tissues (skeletal muscles cells, hepatocytes, fat tissue) produce resistance to the insulin which is in part due to the release of anti insulin hormones and anti-inflammatory cytokines (reaction to stress) [9]. Anti-inflammatory cytokines are of significant importance in the stress induced hyperglycemia after operative intervention [10]. With the mechanical injury the reason explaining the hyperglycemia can also be an increase in the production of glucose in the liver, but not the disturbance of its utilization by tissues.

The absolute values of the total protein during the experiment in both groups of animals did not exceed the physiological limits (**Table 2**, **Figure 2**). Some differences were observed only in the dynamics of the parameter being investigated: in the experimental group, the level of total protein on day 20 ($p = 0.024$) and day 30 ($p = 0.013$) reliably exceeded initial values by 14% and 15%, whereas in control group, the concentration of total protein on day 60 reliably exceeded this index of experimental group by 12% ($p = 0.006$).

Osteotomy and osteosynthesis are accompanied by the development of natural defending inflammatory reaction with the rise of the level of acute phase proteins, in particular, the C-reactive protein (results are not included in this publication), which can explains the observed raising level of total protein and this, however, was within the limits of normal values.

The levels of ALT and AST (**Table 3**) were evaluated as indicators of possible hepatotoxicity by different types of implants. In both groups, the result of ALT remained within the limits of the average values and did not differ from preoperative values. AST was at the lower end of normal values even at the pre-operative blood analysis. The quantitative dynamics of this evaluation criterion (AST) in both groups during the whole experiment remained poorly understood. In this case, the statistical reliability of blood changes was established only in the experimental group on the 5th day of experiment. Thus, the quantitative and qualitative analysis of the biochemical indices of the blood after use of implants coated with the mentioned above compounds requires additional studies.

Discussion

Osteotomy and osteosynthesis by coated implants with titanium and hafnium and non coated implants is accompanied by local reconstruction of the bone tissue in experimental rabbits. During the first phase of bone regeneration, the activity of the osteoclasts which demineralize the bone matrix and prepare a surface for osteoblasts for adhesion predominates. The markers at this phase in our experiment were characterized by a reduction of the ALP level and a rising of serum calcium level.

The direction of the shifts of these parameters in both experimental groups was the same. Only the rise of the calcium level was reliably expressed in the experimental group. Concerning the question about the duration of the restoration period of the bone tissue after implantation, it is necessary to continue the observation.

During the whole experiment we did not observe any essential systemic damage, neither because of operative intervention, nor used implantation materials. There was no disruption of glucostatic, protein synthetic function of the liver. The stable level of the routine enzymatic markers (ALT, ACT) excluded any sign of cytolysis.

Conclusions

The use of coated with titanium and hafnium nitrides implants, that possess high strength, thermal and chemical stability, is not accompanied by the development of additional reactive changes in the organism of experimental rabbits in comparison with the implants made of non coated medical steel.

Analyzing the systemic reaction of the body to the intramedullary osteosynthesis, the present experiment confirms the prospect of the use of nano-technologically coated implants with a bioinert combination of titanium and hafnium nitrides for the purpose of prevention of the possible complication, such as individual intolerance of patient to the implants.

Disclosure of conflict of interest

None.

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