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Laboratory Monitoring of Bone Tissue Remodeling after Augmentation of Impression Intraarticular Fracture with Different Types of Bone Graft

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The effects of bone graft materials on the inflammatory response and biochemical markers of bone remodeling were studied on a rabbit model of fracture augmentation with the following grafts: β-tricalcium phosphate, demineralized bone matrix, nanostructured carbon implant, and porous titanium implant made by additive 3D printing. The markers of bone remodeling and the blood system response in the postoperative period were studied. It was found that porous titanium implant and β-tricalcium phosphate induced osteogenesis and minimized osteoclastic resorption. Augmentation with nanostructured carbon implant and demineralized bone matrix stimulated the processes of osteoclastic resorption.

Key Words: *fracture; osseointegration; augmentation; bone remodeling*

Replacement of bone tissue defects occurring in surgical treatment of intra- and periarticular fractures, partial osteochondral defects in degenerative joint diseases, nontraumatic orthopedic pathology, and operative oncology of the musculoskeletal system is a priority field of operative orthopedics [3-5,7,8].

Here we compared the effect of different osteoplastic materials on the inflammatory reaction of the blood and markers of bone tissue remodeling during augmentation of impression intraarticular fracture in the animal experiment.

MATERIALS AND METHODS

The study was performed on 30 female Chinchilla rabbits weighing 3-3.5 kg. All manipulations were carried out in accordance with the principles of humane methodology of biomedical experiments. The animals were divided into the main (n=24) and control groups (n=6). The control group included animals with modeled fracture without augmentation with osteoplastic materials.

Intraarticular impression fracture of the tibia was modeled as follows [6]: a rectangular fragment (7×8 mm) was cut from the tibia with a diamond metal cutting disc and then, dynamic force displacement of the proximal fragment to the distal direction was performed to ensure the incongruency of the joint surface. After that, augmentation of the impression fracture was performed. The animals were anesthetized with Rometar (2%, 8 mg/kg, SPOFA) and Zoletil (6 mg/kg, Virbac Sante Animale).

Rabbits of the main group were divided into 4 subgroups (6 animals each) according to the type of the bone graft material: β-tricalcium phosphate (βTCP), xenoplastic material (demineralized bone

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matrix, DBM), nanostructured carbon implant (NCI), and porous titanium augment (PTI) (Ti4Al6V SLM) obtained by additive manufacturing using selective laser melting technology.

For evaluation of nonspecific systemic reactions of the body, clinical blood tests were performed on a MEK-6400 analyzer (Nihon Kohden). Smears were stained after Romanowsky and differential leukocyte count with nuclear neutrophil index was assessed. For evaluation of the bone metabolism, serum concentration of osteocalcin (OC), activity of the bone isoenzyme of alkaline phosphatase, and type I collagen C-telopeptide (CTX, cross-laps) were determined by sandwich ELISA. The concentration of rabbit C-reactive protein was measured similarly. Commercial kits from Cloud-Clone Corp. and Multiskan GO microplate reader (Thermo Fishes Scientific) were used. The blood was analyzed before and on days 1, 3, 7, 14, 45, 90, and 180 after surgery.

Statistical analysis of the results included descriptive statistics and Student's *t* test to assess the reliability of the differences. The data were expressed as $M \pm SEM$, the differences were significant at $p<0.05$.

RESULTS

Remodeling of the bone tissue is closely associated with the reactions of hemopoiesis and immunopoiesis

TABLE 1. Hematologic Parameters in the Postoperative Period

Parameter	Day after surgery	Control	βTCP	NCI	PTI	DBM
Erythrocytes, $\times10^{12}$/liter	0	5.67±0.18	6.08±0.20	6.02±0.23	5.19±0.12	5.24±0.14
	1	5.10±0.02	3.90±0.63*	5.24±0.23	4.19±0.19*	4.41±0.15*
	3	4.71±0.18	3.32±0.39*	5.54±0.19*	4.67±0.25	4.94±0.06
	7	5.11±0.36	4.91±0.12*	5.08±015*	4.94±0.21	5.38±0.12
	14	5.08±0.12	5.42±0.13	5.76±0.60	5.00±0.12	5.44±0.21
	45	4.76±0.12	4.69±1.26	5.52±0.84	5.44±0.44	5.48±0.11
	90	4.87±0.14	4.79±0.88	6.29±.97	6.20±0.43	5.86±0.54
Hemoglobin, g/liter	0	115.0±5.0	124.0±3.0	129.0±7.0	129.0±3.0	124.0±4.0
	1	103.0±3.0	88.0±13.0*	107.0±5.0	89.0±5.0*	106.0±5.0
	3	109.0±2.0	101.0±5.0*	103.0±2.0	103.0±4.0	108.0±1.0
	7	107.0±2.0	107.0±4.0	121.0±4.0	105.0±3.0	108.0±3.0
	14	108.0±2.0	116.0±10.0	120.0±13.0	116.0±9.0	117.0±4.0
	45	101.0±17.0	126.0±13.0	116.0±17.0	117.0±10.0	119.0±12.0
	90	121.0±12.0	124.0±4.0	132.0±19.0	133.0±12.0	121.0±16.0
Leukocytes, $\times10^{9}$/liter	0	8.9±0.9	5.6±0.6	6.6±0.6	5.9±0.4	6.8±0.8
	1	12.3±0.4*	11.6±1.4	13.8±1.0	12.9±0.7	11.9±0.4
	3	9.0±0.10	12.9±0.3*	12.9±0.5*	10.8±0.8*	11.2±0.3*
	7	10.6±0.10	12.2±0.8*	12.2±1.2*	9.7±0.7	10.5±0.3
	14	8.3±0.10	7.8.0±1.9	13.8±1.6*	8.3±0.5	6.9±0.7
	45	10.4±0.1	6.9±1.1	11.6±0.59*	6.6±0.9	7.8±0.9
	90	10.0±0.0	6.4±0.4	10.9±0.6	6.8±1.1	6.4±0.2
Platelets, $\times10^{9}$/liter	0	359±14	262±12	265±21	327±12	316±14
	1	347±27	204±26	321±17*	390±10	348±19
	3	396±5	359±23	423±33*	323±18	399±25*
	7	398±16	422±15	419±24*	343±25	394±27
	14	314±10	336±5	594±34*	318±21	394±13*
	45	298±14	238±22	575±23*	300±35	241±31
	90	325±21	283±12	487±23*	325±17	221±19

Note. Here and in Tables 2 and 3: $p<0.05$ in comparison with the control.

[1,2], which provided the basis for evaluation of blood reactions after grafting of various osteoplastic materials as augments in intraarticular fracture management. In animals of all groups, stereotyped reaction of the blood system was observed in the postoperative period: moderate anemia and neutrophilic leukocytosis with maximum changes on days 3-7 (Table 1). In two weeks after surgery, the hematological parameters generally returned to normal and remained at this level until the end of the experiment. The only exclusion was the NCI group: leukocytosis in these animals persisted longer and thrombocytosis was noted. Presumably, this material is not biologically inert and induces reactive changes in the blood. However, no significant

TABLE 2. Content of C-Reactive Protein in the Postoperative Period (ng/ml)

Day after surgery	Control	βTCP	NCI	PTI	DBM
0	0.15±0.15	0.15±0.15	0.11±0.05	0.15±0.15	0.15±0.15
1	0.10±0.10	1.35±0.13*	1.50±0.36*	1.09±0.08*	0.70±0.12
3	0.15±0.15	1.38±0.13*	1.87±0.51*	1.29±0.11*	1.14±0.15*
7	0.08±0.04	0.88±0.22*	2.63±1.08*	0.63±0.14*	0.66±0.09*
14	0.05±0.03	0.41±0.07	1.44±0.28*	0.33±0.10	0.35±0.03
45	0.01±0.01	0.22±0.05	1.79±0.52*	0.17±0.06	0.26±0.13
90	0.08±0.04	0.04±0.02	0.95±024*	0.01±0.01	0.06±0.04

TABLE 3. Biochemical Markers of Bone Metabolism

Parameter	Day after surgery	Control	βTCP	NCI	PTI	DBM
Osteocalcin, ng/ml	0	6.33±0.88	5.33±0.88	2.33±0.81	6.43±0.78	2.33±0.88
	1	5.33±1.85	12.00±1.73	11.83±3.68	10.00±2.25	23.25±2.79
	3	6.33±0.88	12.57±2.40*	9.83±0.72	11.33±2.35	9.62±1.17
	7	6.67±3.67	18.42±1.82*	15.16±2.68*	10.83±1.27	13.00±1.75
	14	2.66±0.66	35.14±6.98*	12.50±4.11	19.16±4.36*	14.12±1.21*
	45	2.66±0.66	30.28±5.34*	13.00±1.41	25.66±3.10*	14.12±1.21*
	90	7.66±2.66	15.00±1.96	10.83±1.70	7.50±1.05	11.75±1.01
	180	9.00±1.00	10.71±0.96	10.63±2.49	7.33±1.30	11.00±1.60
CTX, ng/ml	0	0	0	0	0	0
	1	0	0.025±0.018	0.038±0.023	0.048±0.012*	0.033±0.016
	3	0.004±0.002	0.154±0.017*	0.016±0.010	0.143±0.012*	0.160±0.047*
	7	0.003±0.003	0.092±0.015*	0.008±0.002	0.078±0.013*	0.130±0.026*
	14	0.036±0.020	0.064±0.06*	0.160±0.067*	0.080±0.012*	0.168±0.039*
	45	0.040±0.020	0.004±0.002	0.208±0.082*	0.025±0.019	0.183±0.080*
	90	0.006±0.006	0.000±0.000	0.155±0.060*	0.011±0.011	0.015±0.015
	180	0.003±0.001	0	0	0	0
Alkaline phosphatase, U/liter	0	9.16±3.54	4.66±3.54	9.66±3.41	5.66±1.52	9.66±3.52
	1	9.66±3.17	26.68±4.27	24.33±3.30	12.13±6.41	12.07±1.55
	3	10.33±1.66	21.57±7.17	20.10±3.85	13.96±9.40	11.78±0.20
	7	6.33±1.76	42.35±4.35*	10.31±3.42	34.76±5.24	13.75±3.20
	14	9.66±3.52	41.24±8.39*	21.93±7.69	61.00±13.20*	14.21±0.81
	45	8.00±0.00	22.58±3.03	7.78±1.42	84.50±10.77*	41.93±5.48*
	90	9.00±3.51	13.74±1.72	9.10±0.57	19.43±1.05	20.96±2.74
	180	9.00±2.64	10.62±0.96	9.78±0.61	10.47±0.48	14.30±2.23

eosinophilic reaction indicating the development of an allergic reaction were noted.

The inflammatory response of the blood corresponded to shifts in the serum concentration of C-reactive protein (Table 2). Its concentration slightly increased on days 1-7 in comparison with the control and then remained elevated in animals with NCI. These data also correspond to the nuclear index of neutrophils: on day 7 after surgery, this parameter was 0.07 in the NCI group, while in other groups, it decreased to 0.02 ($p<0.05$).

Osteocalcin and bone fraction of alkaline phosphatase are common markers of bone formation, and CTX is a marker of osteoclastic resorption [11,12].

In the control group, the marker of bone remodeling did not change over 6-month follow-up (Table 3).

In experimental groups, the content of osteocalcin increased on days 3-45 after augmentation, reaching the maximum value in the βTCP group. In the NCI group, the values of this biomarker were lower. Alkaline phosphatase activity increased on days 7-45 with maximum on day 7 after implantation of βTCP. In animals of PTI or DBM groups, alkaline phosphatase activity was maximum on day 45 and surpassed the control level by 10.5 times ($p=0.04$). These findings suggest that βTCP and PTI most markedly stimulated osteoblastic processes, while NCI was less effective. The CTX content reached the maximum on days 3-45 after augmentation. An exception was NCI subgroup in which the level of CTX was the highest and remained elevated up to day 90, but in 6 months, it returned to the preoperative level. Thus, NCI and to a lesser extent DBM induced pronounced osteoclastic resorption.

Judging from the values of bone metabolism markers, βTCP and PTI were optimal materials capable for inducing osteogenesis and minimizing the phenomena of osteoclastic resorption. In view of ambiguous reports on the osteoinductive properties for these materials [9,10], our findings can shed light on this issue.

NCI did not exhibit osteogenesis-inducing properties, but augmentation with material, similar to DBM, stimulated the processes of osteoclastic resorption. The nature of xenoplastic material, native bone, presumably induced the resorption processes. It can be assumed that the use of NCI and DBM in the surgical management of intraarticular fractures can lead to improper integration of the augment and impair treatment outcome.

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