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Changes in morphological and biochemical blood parameters in the postoperative period in experimental orthopedic surgeries in rabbits, treated with bone marrow aspirate, platelet-rich plasma and hydroxyapatite

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ABSTRACT

The evaluation of the morphological and biochemical blood parameters is extremely helpful after orthopedic procedures as it could be used as an indicator for the quality of the regeneration process. There were significant changes in the RBC count, amount of Hb and Hct in the postoperative period. We observed an increase in the total number of platelets as a result of the soft tissue and bone trauma, destruction of blood vessels, etc. All the changes in the post-surgical period regarding the biochemical parameters reflect the changes that occur in the macro-organism after anesthesia and surgery. Serum alkaline phosphatase differences in all groups is because of different activation of osteoblasts, producing specific bone alkaline phosphatase.

KEYWORDS: anesthesia, BMA, fracture, rabbit, hematology, PRP

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I. INTRODUCTION

Fracture healing is a continuous physiological process for achieving connection between the two bone ends. It is characterized by the production of osteoid tissue, which subsequently mineralizes. Critical-length fractures are known to be challenging and difficult to heal, and sometimes impossible, using traditional methods of repair [1].

Research in the field of bone tissue engineering is aimed at studying various methods and means to fill the defect, to stimulate osteogenesis, the rate of bone healing and its adequate remodeling [2]. It is known that in slow-healing fractures (non-union) the use of auto- and allografts or biomaterials are one of the best methods that lead to good results. Due to some limitations in the use of bone grafts (graft rejection, incompatibility, donor limit), research is aimed at finding other substances (auto- and / or allogeneic) to support the healing process. The use of autogenous platelet-rich plasma (PRP), bone marrow aspirate (BMA) or autologous stem cells is very promising and it is a reason for further research [3]. Rabbits, as an experimental model in orthopedics, are widely used in both human and veterinary medicine due to their poor aggression, relatively easily handled anesthesia and cost-effectiveness compared with larger animal species.

On the other hand, each operation is accompanied by anesthesia and a correspondingly different level of stress in the experimental animals. Changes in the main indicators of the blood, as well as changes in some stress hormones can adversely affect the recovery process in the postoperative period and the healing of bone fragments [4].

The aim of the present study was to follow the changes in the main morphological and biochemical parameters of the blood in the postoperative period after obtaining platelet-rich plasma and bone marrow aspirate in the experimental treatment of radial defects with a critical length in rabbits.

II. MATERIALS AND METHODS

All experimental procedures and management of animals were conducted in accordance with European Community guidelines m. 86/609/EEC regarding the protection of animals for experimental purpose.

Hematologic parameters were measured using a fully automatic hematological analyzer BC - 2800 Vet, MINDRAY, China. Biochemical parameters were measured using a fully automatic biochemical analyzer BS - 200, MINDRAY, China.

Animals

Twenty-one rabbits at the age between 12 and 18 months, weighing 3,2 ± 0.6 kg, New Zeeland breed, were included in the study. The surveys were performed in the period from 2018 to 2021, in the University Clinic for Small Animals at the Faculty of Veterinary Medicine, University of Forestry, Sofia. The animals were housed in cages and pre-adapted to the environment for a period of one month before the start of the experiment. The diet included concentrated feed for rabbits and hay.

All animals participating in the experiment after preliminary studies were defined as clinically healthy and were divided into one control and two experimental groups (n=7). After anesthesia with *xylazine hydrochloride* (Xylazine 2%, Bioveta®) (5 mg/kg) and *ketamine hydrochloride* (Anaket 10%, Richterpharma®) (50 mg/kg), a critical length defect was created in the middle third of the radius by ostectomy. In the control group hydroxyapatite (**HA**) and 0,2 ml vit. AD_3E were placed in the defect area; in the platelet rich plasma (**PRP**) group the defect was filled with HA, 0,2 ml vit. AD_3E and PRP, obtained from 10 ml of whole blood, 24 hours in advance from the same animal. In the **BMA** group, HA, 0,2 ml vit. AD_3E and autologous concentrated bone marrow aspirate obtained immediately before ostectomy was put in the defect.

Bone healing process was monitored for a period of 90 days, during which blood samples were obtained for hematological and biochemical examination. Blood counts were monitored in dynamics - immediately before anesthesia and surgery, on the 24^{-th} hour after surgery, 15^{-th}, 30^{-th}, 60^{-th} and 90^{-th} day post surgically. *Whole blood count (WBC $x10^9/l$), red blood count (RBC $x10^{12}/l$), hemoglobin (HB, g/l), hematocrite (Hc, %), total platelet count (PLT $x10^9/l$);* biochemical indicators - *alanine aminotransferase (ALAT, U/l), aspartate aminotransferase (ASAT U/l), alkaline phosphatase (ALP U/l), glucose (Glu nmol/l), total protein (TP g/l), urea (Ur nmol/l), and creatinine (CREAT µmol/l)* were examined. Blood was obtained in sterile vacutainers from v.cephalica antebrachii.

III. STATISTICAL ANALYSIS

All the data was expressed as mean and standard deviation. Differences between the three groups were analyzed using analysis of variance (ANOVA). The data was analyzed using SPSS software (Version 19). Data were represented as mean±SD. All data were assessed for normality and all were normally distributed. Friedman Test were used and a P value of less than 0.05 was considered statistically significant.

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IV. RESULTS

When comparing the obtained values of the examined analyses, no significant differences in the initial values between the three groups were determined.

During the studied periods, changes in the morphological indicators of the blood were found in all three groups, reflected in Table 1.

Table. 1. Morphological blood examinations (mean±SD)

Indicator	group	Before surgery	24h after surgery	15d after surgery	30d after surgery	60d after surgery	90d after surgery
WBC ($x10^9/l$)	**HA**	10,3±2.2	**9,9** ±2,8	10,4± 2.7	10,4± 2,9	11,5±2,6	11,6± 1,9
	PRP	8,1± 1,6	**7,7**± 1,2	11,5 ±0,8	**15,2**±0,7	9,3± 1,6	9,9±0,6
	BMA	9,8± 1,9	**9,7**± 3,1	9,2± 1,2	8,5± 1,4	**10,0** ±1,8	9,04 ±0,7
RBC ($x10^{12}/l$)	**HA**	7,2 ±0,4	6,9± 0,5	7,0 ±0,8	6,6 ±0,5	6,7± 0,9	6,8± 0,2
	PRP	6,4± 0,5	6,0± 0,4	6,3± 0,1	6,7 ±0,2	6,6± 0,6	6,9± 0,6
	BMA	6,3± 0,4	6,09± 0,3	6,2± 0,5	6,6± 0,4	6,7± 0,4	6,8± 0,2
HGB (g/l)	**HA**	161,8 ±11,7	142,4 ±5,3	139,2± 7,3	143,4 ±5,5	144,2±8,3	141,0± 3,9
	PRP	143,8±7,0	147,2±2,3	142,0± 6,2	139,4± 6,8	142,0±8,6	143,6 ±6,5
	BMA	144,4±7,0	130,4±56,1	134,0±10,3	139,8±5,1	140,0±5,0	143,0± 2,8
HCT (%)	**HA**	47,6±4,5	47,8±5,1	49,3±5,2	49,6±4,8	46,7 ±4,1	44,2± 1,1
	PRP	43,6±5,0	**42,6**±4,4	44,2±3,3	43,5±2,	43,4±2,0	**42,3**± 2,1
	BMA	43,1±5,4	41,9±5,0	**40,8**±3,8	42,7±1,7	44,8± 2,7	44,6± 2,5
PLT ($x10^9/l$)	**HA**	280,2± 21,6	**364,4**±48,9	**264,0**±26,9	277,2±40,4	287,6±37,1	**331,6**±58,8
	PRP	294,0± 65,6	377,8±42,6	**400,4**±39,9	392,2±14,92	366,6±59,3	**354,6**±64,1
	BMA	310,0± 27,5	**654,2**±129,2	577,8±105,4	306,0±35,6	395,0±84,4	**398,6**±49,0

The data obtained for the changes in the total number of leukocytes show significant changes during the study period in all three groups. After a transient decrease in the total leukocyte count in 24h in all three groups, a significant increase in their number was observed on the 30th day in the **PRP** group - 15.22± 0.75 $x10^9$, ($p<0,001$) and 60th day in the **BMA** group - 10.04±1.77 $x10^9$, ($p<0,05$). At the end of the study period (90th day), the leukocyte count in all three groups was similar to baseline.

The total number of erythrocytes and the hemoglobin amount was decreased in 24h in all three groups, with the lowest values observed in the **BMA** group (RBC 6.09±0.31 $x10^{12}$, $p<0.01$, HB 130.4±6.1g/l, $p<0,05$). In contrast, hematocrit changes showed a different trend in different groups - the lowest hematocrit levels in the **PRP** group were found at 24h (42.60±4.40 l/l) and 90th day (42.30±2.14 l/l); in **BMA** group - on the 15th day (40.82± 3.81, $p<0,05$). In group **HA** there were no pronounced changes in hematocrit during the studied period.

The total platelet count in the **HA** group increased significantly at 24h, when the highest levels were found for the whole study period – 364± 49 $x10^9$, ($p<0,05$). On the 15th day, they are significantly lower (264± 27 $x10^9$, $p<0,05$), compared with the initial values (280± 21 $x10^9$). In subsequent study periods, the total platelet count gradually increased, and on 90th day they were 332 ± 59$x10^9$.

In the **PRP** group, the total platelet count increased insignificantly until the 15th day, when the highest values were found - 400±40$x10^9$, then it slowly decreases and on the 90th day are 354± 64 $x10^9$.

A statistically significant increase in total platelet count was observed in **BMA** group at 24h, 654± 129,2 $x10^9$, ($p<0,001$), compared with the initial values (310± 27,5 $x10^9$), then gradually decreases until the 90th day (398± 49 $x10^9$).

Changes in the levels of biochemical parameters are reflected in Table 2.

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Table 2. Biochemical analysis of blood (mean±SD)

Indicator group		Before surgery	24h after surgery	15d after surgery	30d after surgery	60d after surgery	90d after surgery
ALAT (U/l)	HA	40,6 ±7,00	**70,5** ±7,8	41,1 ±7,59	43,3± 7,8	55,5± 5,7	43,4 ±8,6
	PRP	56,7± 10,6	**108,4** ±19,3	34,9±4,41	33,25±4,96	26,6±4,38	29,2±1,65
	BMA	42,0± 7,35	**150,8**±7,89	67,8±5,12	29,4 ±4,8	72,5 ±4,6	56,6± 4,7
ASAT U/l	HA	27,6± 5,7	**71,28**±4,4 *	53,0 ±2,6	55,7± 4,6	72,5± 4,67	56,6± 4,7
	PRP	55,3± 5,3	**99,3**± 7,2	64,1± 2,8	45,0± 4,1	14,6± 2,2	19,4± 1,6
	BMA	15,9± 4,4	**87,8** ±8,6	13,8± 3,2	17,0± 3,2	18,5± 3,1	19,2± 2,3
ALP (U/l)	HA	19,4 ±2,5	176,8± 20,6	**267,2**±14,01	**283**± 43,2	**265,6**±43,6	**197,4**±20,7
	PRP	35,4± 7,4	198,8± 10,6	352,5±3 5,2	417,7±32,1	317,3±21,9	218,2±21,2
	BMA	20,9±4,2	170,9± 10,9	525,8±35,0	**628,0**±44,0	328,7±26,7	88,8±12,9
Glu (nmol/L)	HA	5,6± 0,4	**17,9**± 0,5	6,3± 0,58	6,1 ±0,5	5,1± 0,2	5,3± 0,3
	PRP	5,4± 1,1	**15,8** ±2,7	6,1± 0,2	5,7± 0,2	5,4± 0,2	5,3± 0,8
	BMA	5,8± 0,7	**16,8** ±1,3	5,4 ±0,36	6,4± 0,7	5,4± 0,4	5,6 ±0,5
TP (g/l)	HA	68,8± 5,9	55,6± 4,6	59,3± 3,5	66,2± 3,9	69,6± 3,8	67± 4,5
	PRP	62,4± 5,5	54,1± 7,0	59,4± 4,7	62,8± 4,9	55,8± 4,2	67,9± 2,2
	BMA	61,4± 3,0	45,4± 3,8	27,8± 5,2	54,5± 1,2	54,9± 3,1	54,6± 1,8
Ur (mmol/l)	HA	7,3± 1,9	**21,8**± 2,2	10,4± 2,5	8,0± 1,6	8,2± 1,7	9,4± 0,6
	PRP	7,5± 1,1	**18,9**± 1,5	8,9± 1,5	8,5± 1,5	8,2± 1,2	8,1± 1,3
	BMA	6,8± 0,6	**19,2**± 1,9	7,1± 0,3	6,9± 0,2	7,0± 0,3	7,5± 0,3
CREAT (µmol/l)	HA	90,0± 5,4	**160,7**± 8,2	93,6± 5,4	87,2± 4,9	89,3± 6,4	83,9± 6,0
	PRP	85,7± 3,3	**165,3**± 4,6	80,7± 4,9	87,2± 4,8	89,3± 6,4	83,9± 6,0
	BMA	86,7± 5,3	**148,9**± 5,1	69,6± 2,6	69,5± 6,5	70,3± 6,3	76,3± 1,7

The activity of liver enzymes changes in all three groups, with a significant increase in 24th hour (**HA** - ALAT 70,5 ±7,8; ASAT 71,28±4,4; **PRP -** ALAT 108,4 ±19,3, ASAT 99,3± 7,2; **BMA –** ALAT 150,8±7,89; ASAT 87,8 ±8,6).

ALP in group HA, after an initial increase, remains elevated but lower than in the other groups - (15th day - 267,2± 14,01 U/l; 30th day - 283± 43,2U/l; 60th day - 265,6± 43,6; 90th day- 197,4± 20,7U/l). In PRP and BMA groups, serum alkaline phosphatase is elevated until 30th day, when the highest levels are observed in the BMA group - 628,0±44,0. After that it gradually decreases, but at the end of the study period in all three groups remains higher than the initially established activity levels.

Elevations in glucose levels were found only 24th hour after surgery, with changes similar in all three groups - **HA** (17,9± 0,5), **PRP** (15,8 ±2,7), **BMA** (16,8 ±1,3).

No significant changes in total protein levels were observed during the study periods.

Urea increased significantly at 24th hour in all three groups in a similar manner (**HA** - 21,8±2,2**; PRP** - 18,9±1,5; **BMA** - 19,2±1,9), as in the following periods its levels return within the initially established ones. Creatinine levels change in a similar way. The highest levels are established 24 hours post-surgically, after which in the following measurement periods they are similar to the initially established ones.

V. DISCUSSION

The monitoring of the changes in the morphological and biochemical composition was performed with one type of surgical intervention and the same anesthesia, which minimizes the influence of anesthesia and surgery on the blood changes.

Changes in the erythrocyte count, amount of hemoglobin and hematocrit in the postoperative period can be a result of many factors. It has been found that in the early postoperative period, in addition to blood loss, the decrease in erythrocyte count may be because of their aggregation due to cellular changes (morphological changes, changes in membrane potential and surface glycocalyx composition) and changes in plasma factors (eg fibrinogen concentration). Free radicals formed as a result of surgical and anesthetic stress, the development of the inflammatory process, ischemia, mechanical damage to tissues and cells, as well as the change in the micro-environment (pH, osmorality) also create conditions for erythrocyte aggregation [5].

Our results show that the most pronounced decrease in RBC and HCT is observed in the PRP group, which is probably due to the receipt of a larger volume of blood before surgery in order to obtain the plasma. According to Meurrens et al. (2016) [6] obtaining a larger volume of blood for donation or for the purpose of obtaining blood components, despite being less than 1% of the total blood volume, leads to an immediate decrease in the basic hematological parameters. Recovery to baseline levels is observed within 4 weeks, which is confirmed by our studies. At the same time, it should be noted that receiving blood or low-grade blood loss does not lead to conditions similar to anemia, because the indicators remain within normal ranges. Meurrens et al., (2016) [6] reported that even with repeated blood donation, ferritin and hematopoietin levels remain within normal ranges without risk of anemia. This indicates that blood loss in receiving blood to separate PRP, together with blood loss during surgery do not pose a risk of anemia in the early postoperative period.

It is essential for the quantitative ratio between the concentration of hemoglobin and the total number of erythrocytes is the fact that these parameters can be affected by the general condition of the body - fluid balance, physical activities, oxygen deficiency, etc.

The observed increase in hemoglobin levels on the one hand may be due to an increase in its concentration in erythrocytes (due to hypoxia, dehydration, erythropoietin stimulation, etc.), but on the other hand this condition can be interpreted as false due to decreased levels of hematocrit, which corresponds with similar studies performed by Otto et al. (2017) [7] in humans. For this reason, they recommend measuring total hemoglobin mass and plasma volume as a more reliable study compared to the hemoglobin concentration.

The change in the total number of platelets in the early postoperative period, in the absence of severe blood loss, is usually expressed in an increase in their total number, which was also confirmed by our studies. The reasons for this are many - soft tissues and bone trauma and destruction of blood vessels during surgery lead to the release of large amounts of tissue and plasma mediators of inflammation and activation of hemostasis and thrombosis. This is the so-called reactive or secondary thrombocytosis. In these cases, the levels of thrombopoietin, IL-6 and catecholamines are usually very high and are responsible for the increased platelet count. It is believed that the pathophysiology behind secondary thrombocytosis (reactive thrombocytosis) is because of elevated inflammatory cytokines levels, such as interleukins (IL-1, IL-6, and IL-11) [8]. Secondary thrombocytosis is self-limiting and the total platelet count returns to normal within a relatively short period of time, as found in our study.

Changes in biochemical parameters in the early postoperative period reflect the changes that occurred in the body after anesthesia and surgery. The increase in plasma ALT and AST concentrations observed after ketamine-xylazine administration is due to the hypotonic effect of xylazine. Ketamine hydrochloride does not significantly alter hepatic blood flow in rabbits and is unable to compensate for hypotension, especially in hepatic circulation, which results in increased activity of both liver enzymes [9]. Hypotension, as a result of anesthesia, also leads to a decrease in blood flow to the kidneys, thus reducing the clearance of nitrogen compounds. According to González et al. (2002) [10], these anesthetics reduce the glomerular filtration rate and consequently lead to an increase in creatinine and urea levels in the first 24 hours after anesthesia, which is confirmed by our results.

Serum alkaline phosphatase is a collection of isoenzymes originating from various tissues - liver, bones, intestines, kidneys, etc. The changes in alkaline phosphatase levels in the three groups were probably due to the varying degrees of activation of osteoblasts, which produce specific bone alkaline phosphatase, especially at the end of the first month. It is estimated that 50% of total serum alkaline phosphatase in patients with normal liver function is due to osteoblasts and is bone alkaline phosphatase, and the remaining 50% is due to liver activity [11].

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Singh et al. (2013) [12] concluded after extensive research that changes in serum alkaline phosphatase reflect bone metabolism and its serial examination during bone healing can be used as a prognostic sign for the rate and tendency of healing. Similar are the results obtained by Human Nazht et al. (2019) [13], which compare the results of the changes in serum ALP with radiological data on bone healing of the femur in rabbits. Our results for the dynamics of serum alkaline phosphatase are in agreement with these data. The fact that with increased osteogenesis the activity of serum alkaline phosphatase increases is confirmed. The highest levels of sAF, found by us, correspond to the radiological changes in our patients, accompanied by the formation of bone bridges between the two bone fragments.

VI. CONCLUSION

Changes in morphological and biochemical parameters mainly affect the early postoperative period with the exception of alkaline phosphatase. The pre-obtaining of blood for PRP separation does not pose a risk of developing anemia in the early postoperative period. Serum alkaline phosphatase is an enzyme whose bioactivity can be used as a marker for the stages of bone healing. The study of this indicator is of great prognostic importance regarding the healing processes, it is also easily accessible and can be used in clinical practice.

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