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Research Article

Biochemical Parameters of Rabbit Blood Serum in the Treatment of Limb Bone Fracture with Autologous Platelet-rich Plasma

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Abstract

Introduction into the body of autologous platelet-rich plasma (APRP) is one of the most promising procedures in tissue restoration. After platelet destruction, APRP contains α-granules, from which, after activation, transforming growth factor-beta, vascular endothelial growth factor and epidermal growth factor are released.

This study investigated the effect of autologous platelet-rich plasma on biochemical markers of rabbit blood during bone fracture regeneration. Changes in the concentrations of aspartate aminotransferase, alanine aminotransferase, total bilirubin, alkaline phosphatase, total protein and urea were found. Disorders of enzyme activity and protein metabolism allow indirectly assessing the therapeutic effect of autologous platelet-rich plasma.

Keywords: Autologous Platelet-rich Plasma; Blood Serum; Biochemical Parameters; Activity of Enzymes; Protein Metabolism

Relevance

Currently, the technology of treatment with autologous platelet-rich plasma (APRP) is widely used in medicine. Due to the natural properties of platelet-rich plasma, its introduction into the body is one of the most promising procedures in tissue restoration. Platelet destruction activates α-granules contained in APRP, from which many factors such as transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) are released [1].

The effectiveness of APRP has been proven in wound healing and tissue restoration in maxillofacial surgery, cardiac surgery, ophthalmology, orthopedics, plastic surgery, sports medicine and cosmetology [2,3]. APRP-therapy is also widely used in orthopedics, for example, in the treatment of degenerative-dystrophic diseases of large joints, and as one of the components in a synthetic or mineral graft usage to replace bone tissue defects [4]. However, the possibility of using platelet-rich plasma in fresh limb fractures remains poorly understood which determined the relevance of this study. The therapeutic effects of APRP are still controversial due to the lack of optimized and standardized protocols for optimal whole blood centrifugation conditions.

As tests for bone fractures, the researchers determined the activity of lactate dehydrogenase (LDH), the enzyme of carbohydrate catabolism, the ratio of LDH isoenzymes, the concentrations

of the end products of glycolysis, lactate (L) and pyruvate (PVC). Determined the activity of creatine kinase (CK) and the content of adenosine triphosphate (ATP) to assess the level of accumulation and release of energy [5-7]. Shevtsov., *et al*. studied the dynamics of the activity of alkaline and acid phosphatases, lactate dehydrogenase, and the content of lactate, pyruvate, total calcium, magnesium, inorganic phosphates, and serum chlorides to stimulate bone regeneration in the experiment [8]. Nakoskin A.N. and Nakoskina N.V. investigated the effect of local administration of an extract of low molecular weight proteins of bone tissue on the biochemical parameters of bone metabolism in rats. Osteoreparative processes during the experiment were assessed by the level of activity of serum enzymes: total alkaline phosphatase (TAP) and tartrate-resistant isoenzyme of acid phosphatase. The state of energy metabolism was assessed by the content of total protein (TP) in blood serum [9]. Melnikov S.A., *et al*. studied the content of inorganic phosphate, total calcium, total protein, the activity of total alkaline phosphatase, tartrate-resistant isoenzyme of acid phosphatase with intraperitoneal administration of drugs of low molecular weight non-collagen proteins of bovine bone tissue [10]. Blazhenko A.N., *et al*. (2019) investigated the effect of APRP therapy on reparative bone tissue regeneration in fresh limb fractures. The authors believe that platelet-rich plasma (PRP) for stimulating reparative osteogenesis is an inexpensive, simple to perform and effective procedure [3]. The use of autologous platelet-rich plasma is a promising direction in the management of regenerative processes in fractures of long bones.

Aim of the Study

To study the biochemical parameters of rabbit blood in the dynamics of therapy of experimental bone fractures using autologous platelet-rich plasma.

Materials and Methods

In studies, therapy with autologous platelet-rich plasma was tested on an experimental model of rabbit limb bone fractures in order to stimulate bone regeneration and healing of postoperative wounds. We have adapted a technique for obtaining platelet-rich plasma by two-stage centrifugation of autologous blood from the rabbit ear vein. An injectable form of platelet-rich plasma (PRP) was obtained by double centrifugation of autologous blood for 10 minutes on a tabletop centrifuge TDZ4-4Ws (China) according to the protocol proposed by J. Araki., *et al*. [11]. At a temperature of 22°C, the acceleration of the first centrifugation of peripheral blood was carried out at 250xg, the second at 2300xg for 10 minutes, the initial platelet concentration was 233.00 ± 6.55×10^9/L, in PRP it averaged 590.58 ± 25.69. The effectiveness of APRP therapy was preliminarily assessed based on the results of studies of biochemical parameters of blood serum. On a BA-88 A biochemical analyzer (Mindray, PRChina, 2014), blood serum parameters were determined by standardized methods: total protein - biuretic using a GOT (AST) kit (CYPRESS Diagnostics, Belgium), asparagine aminotransferase (AsaT) and alanine aminotransferase (AlaT) -using standardized Reitman-Frankel methods, creatinine - by the Jaffe method, alkaline phosphatase - by a unified method with nitrophenyl phosphate using a commercial ALP Kit (Cypress Diagnostics, Belgium), total bilirubin - by the Endrassic-Grof method using reagent kits from CYPRESS Diagnostics (Belgium) and Human (Germany)) and urea - by the urease method. Biochemical markers were examined twice in order to obtain standard indicators in control and experimental animals 6 days before the start of the experiments and before removing rabbits from the experiment on days 7, 14, 30, 60 and 90 of the experiments. All experimental studies were carried out in accordance with the requirements set forth in the "European Convention for the Protection of Vertebrates used for Experiments or for Other Scientific Purposes" (1986), in compliance with ethical standards and humane attitude towards the objects of study [12-15]. These studies were approved by the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan (Protocol No. 7 dated February 6, 2020).

The experiments were carried out on 66 outbred male chinchilla rabbits weighing 2.5 - 3.5 kg at the age of 11 - 12 months. All experimental animals were divided into 3 groups. The 1st control group consisted of 6 healthy individuals, the 2nd and 3rd experimental groups were divided into 30 individuals with diaphyseal fractures of the femur. In group 2, APRP was not used, in group 3 therapy included intramuscular administration of APRP.

Statistical studies were performed based on standard clinical guidelines. Quantitative data are presented as the arithmetic mean (M) ± standard deviation (SD) in the case of a normal

distribution and as the median (Md) and quartiles (Q) or (SD) for other distributions. The level of reliability P <0.05 was taken as statistically significant changes.

The results of the clinical examination were processed on a Pentium-IV personal computer using Microsoft Excel office software applications. Microsoft Access and the biostatistics program STATPLUS (2009), with the calculation of the arithmetic mean of the studied indicator (M), its standard error (m), reliability indicators (P) and the Student's criterion. At the same time, the methods and existing guidelines for statistical data processing in clinical and laboratory studies were taken into account [16].

Research Results and Discussion

Clinical and laboratory studies of the activity of the enzymes AlaT, AsaT, alkaline phosphatase, the contents of total protein, total bilirubin, urea and creatinine in the blood serum of rabbits were carried out on BA-88 A (Mindray, P.R. China).

The most well-known clinical tests are the determination of the activity of transaminases, which allow to assess the degree of impairment in the function of the liver, heart, skeletal muscles. An increase in the activity of enzymes in the blood serum is associated with their release from damaged organs and tissues. This makes it possible to assess the degree of damage using enzymes specific to a particular organ. The study of the activity of specific enzymes in the blood serum of rabbits after intramuscular administration of APRP for the treatment of femoral fractures is associated with the need to assess the state of a number of organs involved in the systemic response of the body to external influences.

The dynamics of changes in the enzymatic activity of AlaT, AsaT in the blood serum of rabbits on the 7, 14, 30, 60 and 90 days of experiments is presented in figure 1.

The level of transaminases in the blood serum of rabbits of group 2, starting from the 7th day of the experiments, exceeded the control values. On day 14, there was a statistically significant increase in AlaT up to 69.63 ± 1.02 U/L (control 48.80 ± 1.19, p < 0.05) and a significant activation of AsaT at the level of 72.00 ± 3.07 U/L (control 44.65 ± 1.40, p < 0.05), which was associated with the release of enzymes from the operated limb, which are contained in large quantities in skeletal muscles. The dynamics of a significant increase in the enzymatic activity of AlaT and AsaT in group 2 can be traced up to 90 days. On the 90th day, there is a slight decrease in the indicators, however, the AlaT values are 1.45 times, AsaT is 1.92 times higher than in the control and 1.25 and 1.66 times higher than the initial values, respectively (Figure 1).

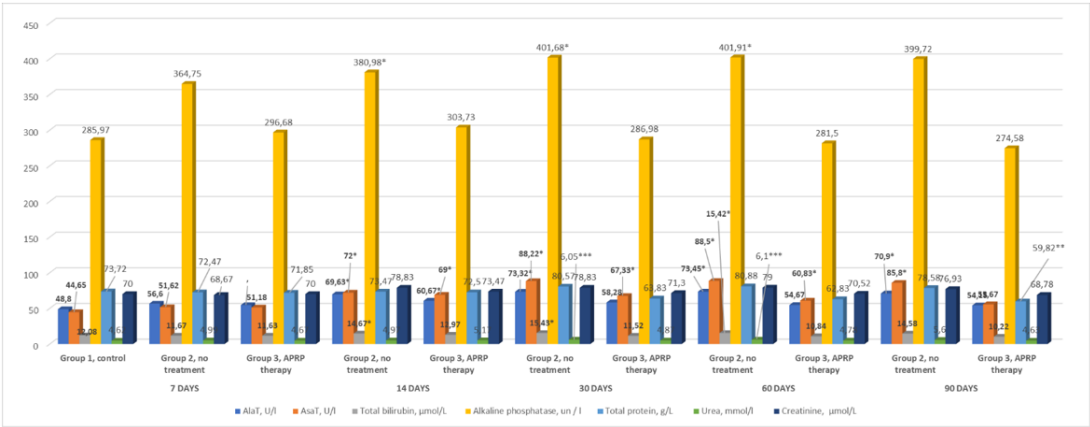

Figure 1: Dynamics of biochemical parameters of blood serum of rabbits on 7, 14, 30,60 and 90 days of experiments, M ± m (n + 60).

Note:

*: Significant differences (p < 0.05),

**: Significant differences (p < 0.01),

***: Significant differences (p < 0.001).

In group 3 animals subjected to APRP treatment, a similar tendency of a significant increase in the activity of AlaT and AsaT to 60.67 ± 0.47 and 69.00 ± 2.21 U/L, respectively, was established from the 14th day, corresponding to the maximum peak values for the entire period of the experiments. Starting from 30 days of APRP therapy, a decrease in the activity of indicators was found, which is possible in combination due to the activation of mechanisms for removing enzymes from the serum, increasing the processes of their inhibition and reducing their synthesis in the liver, aimed at preserving nitrogenous material for synthetic processes in other organs. Maximum peak values are set at 60 days. On the 90th day, they reach the initial levels

The dynamics of changes in the ratio of transaminases AsaT/AlaT (de Ritis coefficient) is of certain interest. The de Ritis coefficient is normally 1.33 ± 0.42 or 0.91-1.75. Changes in this indicator during the entire experiment were of a random, unreliable nature, indicating the absence of damage to organs and tissues rich in these enzymes (liver, myocardium, skeletal muscles) (Figure 2).

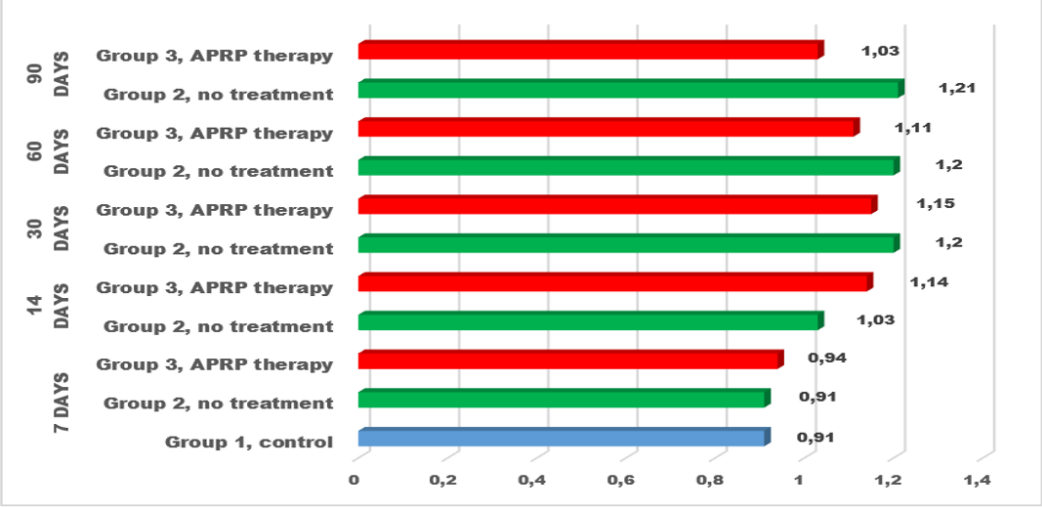

Figure 2: Dynamics of changes in the ratio of transaminases AsAT/AlAT (de Ritis coefficient).

Thus, the research results indicate that temporary hyperenzymemia is observed during APRP-treatment of experimental bone fractures.

Taking into account that alkaline phosphatase (ALP) is formed in bones in special cells - osteoblasts, which play an important role in the formation and renewal of bone tissue, we studied the concentration of alkaline phosphatase in the blood serum of experimental animals. The higher the activity of osteoblasts, the higher the ALP in bone fractures. Analysis of the concentration of alkaline phosphatase showed that in group 2, after 14 days, the activity of alkaline phosphatase increased sharply to 380.98 ± 15.42 U/l compared with the control 285.97 ± 22.99 ($P < 0.05$). After 60 days, ALP activity statistically significantly increased to its peak reaching 401.91 ± 3.87 ($P < 0.05$). On the 90th day of the experiment, the content of alkaline phosphatase reached to its initial and control values (Figure 1).

In group 3, against the background of APRP therapy, on the 14th day, there was a slight increase in the concentration of alkaline phosphatase up to 303.73 ± 2.57 U/L compared to the control 285.97 ± 22.99 and the initial values on the 7th day of 296.68 ± 10, 54. On the 30th day, the indicators returned to the level of control and baseline indicators - 286.98 ± 3.85, 285.97 ± 22.99 and 296.68 ± 10.54 U/L, respectively (Figure 1).

Therefore, against the background of treatment of bone fractures with autologous platelet-rich plasma, a decrease in the activity of alkaline phosphatase on day 30 indicates a slowdown in the destruction and softening of bone tissue, as well as a decrease in the activity of the inflammatory process in bone tissue.

Studies of the total protein (TP) content in rabbits of group 2 showed that on days 7 and 14, the indicators remained at the level of control values. On days 30 and 60, the TP content significantly increased. On the 90th day, there was a decrease to the control and initial value. A different trend was found in group 3 rabbits that received APRP treatment. So, on the 7th and 14th days, the TP content did not undergo significant fluctuations in comparison with the control and initial values. The minimum amount of TP was observed on the 90th day at the level of 59.82 ± 0.48 g/L ($p < 0,01$). Thus, in APRP therapy of femoral fractures, changes in the protein composition of blood serum are reversible.

For an indirect assessment of the intensity of the catabolic phase of protein metabolism in the blood serum of experimental animals, the content of the end products of nitrogen metabolism, urea and creatinine, was studied. In clinical and laboratory practice, the concentration of urea in the blood depends on extrarenal factors and indicates the early or late phases of the postoperative period.

The obtained results of these parameters from blood serum of rabbits, revealed that the level of urea in animals of group 2 on days 7 and 14 was within normal limits, but by days 30 and 60 the degree of variation of the indicator increased to 6.05 ± 0.13 and 6.10 ± 0.13 mmol/l, respectively, according to the timing of the experiments in comparison with the control values 4.62 ± 0.16 ($p < 0.05$). On the 90th day, the indicator decreased to 5.60 ± 0.16 mmol/L. In animals of group 3, which received APRP therapy at all times of the experiments, the urea content ranges from 4.63 ± 0.05 to 4.87 ± 0.09 and remains at the control level of 4.67 ± 0.22 mmol/L ($p < 0,001$).

Consequently, an increase in the concentration of urea in the blood serum of group 2 rabbits is associated with the severity of the traumatic factor and the lack of treatment for bone fractures. An increase in the level of urea in the serum in the long term of the experiments is obviously an indicator of the increased breakdown of proteins in the body as a whole. In rabbits of group 3, who received APRP therapy, there were no significant changes in the concentration of urea in the blood at all times of the experiments, which is obviously associated with the effectiveness of the treatment of bone fractures with platelet-rich plasma.

The study of the content of creatinine (C), which is involved in the energy metabolism of muscle and other tissues, showed that in group 2, at all periods of observation, the fluctuation of C concentrations was quite stable and the indicators were at the control level. In rabbits of group 3, a clear tendency of stabilization of the values of this end product of the creatine phosphate reaction was also observed at all periods of observation. The concentration of C varied from 70.99 ± 5.65 on the 7th day to 68.78 ± 1.10 on the 90th day, with the control values of 70.00 ± 5.35 μmol/L.

The interpretation of the obtained data on the study of serum creatinine in diaphyseal femoral fractures is somewhat difficult, since the level of C in the blood is quite constant. The concentration of creatinine can be increased with muscle damage and increased intensity of physical activity. The decrease may be due to a decrease in the synthesis of the original compound - creatine in the liver and its slow decay in muscle tissue. Therefore, the results of our studies together allow us to interpret the variation in creatinine content at the level of control values, balancing the processes of its synthesis and decay in diaphyseal femoral fractures.

The obtained results on the content of total bilirubin (TBil) in group 2 showed a gradual insignificant increase in indicators from 14 to 60 days of experiments. On the 90th day, the TBil content decreased to 14.58 ± 0.15 μmol/L compared to the control (12.08 ± 0.38). Maximum concentration on day 30 was 15.43 ± 0.22 μmol/L. In animals of group 3, the maximum value on the 14th day corresponds to 12.97 ± 1.05 μmol/L (in the control 11.63 ± 0.28). Starting from the 30th day, the indicator gradually decreases to the initial values.

Findings

- Normalization of the main parameters of the liver tissue (AsAT, AlAT, urea, creatinine and total bilirubin) can be considered as favorable functional characteristics that reflect the compensatory abilities of the body and the positive dynamics of the consolidation of diaphyseal fractures of the femur bones amid APRP therapy.

- The content of urea and creatinine in blood serum can be used to indirectly assess the intensity of the catabolic phase of protein metabolism in the dynamics of treatment of fractures of the diaphyseal bones of the femur.
- The activity of alkaline phosphatase in blood serum is an additional biochemical marker of the processes of reparative osteogenesis in fractures of the diaphyseal bones of the femur.
- A decrease in the activity of alkaline phosphatase during APRP treatment indicates a slowdown in the destruction and softening of bone tissue, as well as a decrease in the activity of the inflammatory process in bone tissue.
- It is advisable to use biochemical markers to assess the effectiveness of the treatment of fractures of long tubular bones.

Conclusion

Based on the study of the biochemical parameters of the blood of rabbits in the dynamics of treatment of experimental bone fractures using APRP, a tendency for a significant increase in the activity of AsAT and AlAT was established from the 14th day of the experiments. A decrease in the activity of indicators on the 30th day of APRP therapy is possible in combination due to the activation of mechanisms for removing enzymes from the serum, an increase in their inhibition processes and with a decrease in their synthesis in the liver, aimed at preserving nitrogenous material for synthetic processes in other organs. Against the background of treatment of bone fractures with autologous plasma enriched with platelets, a decrease in the activity of alkaline phosphatase on the 30th day indicates a slowdown in the destruction and softening of bone tissue, as well as a decrease in the activity of the inflammatory process in bone tissue.

For an indirect assessment of the intensity of the catabolic phase of protein metabolism in the blood serum of experimental animals, the content of the end products of nitrogen metabolism, urea and creatinine, was studied. It was found that an increase in the concentration of urea in the blood serum of group 2 rabbits is associated with the severity of the traumatic factor and the lack of treatment. An increase in the level of urea in the serum in the long term of the experiments (30, 60 and 90 days) indicates an increase in the breakdown of proteins in the body as a whole. In rabbits of group 3, who received APRP therapy, there were no significant changes in the concentration of urea in the blood at all times of the experiments, which is obviously associated with the effectiveness of treatment of bone fractures with platelet-rich plasma. Analysis of the results of the study of the content of creatinine and total bilirubin showed that these indicators are not sufficiently informative markers of reparative osteogenesis.

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