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Available methods to enhance regenerative potential of plastic materials for bone defects replacement in orthopedics. Part 2. Use of autologous human platelet lysate

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Abstract

In the previous article, we talked about the use of platelet-rich plasma. One of the promising ways to stimulate the processes of repair and regeneration in the tissues of the damaged organ in different types of pathology is the use of platelet-rich plasma lysate. This part of the literature review covers the mechanism of action of platelet-rich plasma lysate, indications and contraindications for its use, describes the results of treatment when platelet-rich plasma lysate is used to stimulate osteogenesis. The preparation technology provides for the removal of all cellular components from the plasma, so it becomes possible to store the obtained graft for a long time. The procedure for the preparation of platelet lysate allows the simultaneous isolation of all growth factors from the cells, since the platelet lysis occurs. Lysate of platelet

concentrates can be considered as a preparation that contains a complete set of stimulating growth factors. Under the influence of the lysate, the proliferation of latent osteoblasts is resumed, the signaling pathways of angiogenesis are activated, the secretion of the factors accelerating angiogenesis is stimulated, the differentiation of osteoblasts and the formation of bone tissue are triggered. The aim of this article is to summarize the results of treatment using autologous platelet lysate to improve bone regenerative potential in orthopaedics. In a final article, we shall look at the ways to use autologous red bone marrow.

Keywords: bone regeneration, pseudoarthrosis, platelet-rich plasma, growth factors, bone grafting

Conflict of interests Authors declare no conflict of interest

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PRP, platelet-rich plasma

MMSCs, multipotent mesenchymal stromal cells

Introduction

In the previous article “Available ways to increase the regenerative potential of plastic material in emergency traumatology. Part 1. The use of autologous platelet-rich plasma” we examined general issues, the mechanism of action of platelet-rich plasma, indications and contraindications for its use. Autologous platelet-rich plasma (PRP) is an

easy-to-obtain material that is successfully used as an activator of tissue reparative processes with the possibility of combining it with bioinert synthetic materials. It contains all the elements of blood except for the red blood cells: leukocytes, lymphocytes, platelets, fibrin filaments and adhesive molecules, a damaged platelet membrane.

One of the promising ways to stimulate the processes of repair and regeneration in the tissues of a damaged organ in various types of pathology is the use of lysate PRP. Thanks to the manufacturing technology, which provides for the removal of all cellular components from the plasma, it becomes possible to store the resulting individual graft for a long time. In a study by SC Notodihardjo et al., three options for preserving platelet lysate were evaluated. In group 1, it was stored in a freezer at a temperature of -80 degrees, in group 2 it was frozen at a temperature of -80 degrees, then lyophilized; in group 3, it was lyophilized and stored at a temperature of +4 degrees. All that took place over the course of 9 months. Based on the results of the study, it can be concluded that in all groups, the concentration of growth factors was determined as approximately the same. The optimal storage of platelet lysate was determined to be at a temperature of +4 degrees. Due to such storage conditions, there is no need for multiple blood sampling from the patient and it becomes possible to use platelet lysate at different times, there is no need to purchase an expensive freezer [1].

The purpose of the undertaken analysis of the special literature was to summarize the available information about current approaches to the ways of improving the regenerative potential in emergency trauma surgery.

Literature search strategy

The search for sources was made in open access electronic databases of scientific literature PubMed and eLibrary. Key words used for search were "bone healing stimulation", "autologous bone marrow", "bone graft, PRP, "lysate" and their corresponding terms in Russian. Search has included literature for the recent 20 years. In order to analyze and evaluate the literature data, we developed criteria for including sources in an analytical study.

The criterion for including sources in the study was the presence of the full text of the article or a structured abstract with specific quantitative data.

Exclusion criteria: clinical cases, abstracts, unpublished works, studies showing signs of duplication (similar study protocol, groups, number of patients, etc.). In case of detecting duplicate articles, a more recent source by the date of publication was chosen.

The use of platelet lysate in genetic engineering

Currently, in regenerative medicine, it is common to use platelet lysates obtained by destroying autologous platelets of a patient at low or ultra-low temperatures. In multipotent mesenchymal stromal cells (MMSCs) of bone marrow origin cultivated in a platelet lysate-containing medium, the proliferation process is faster. At the same time, normal osteogenic differentiation is preserved. In the few preclinical studies using MMSCs together with platelet lysate, good results of bone tissue regeneration were obtained on osteoconductive scaffolds in vivo. There is another approach to using platelet lysate on osteoconductive scaffolds, that is without MMSCs. The studies conducted in vivo showed improved bone formation and vascularization when compared to the scaffold untreated with platelet lysate.

D.T.B. Shish and T. Burnouf described the methods for preparing the platelet lysate to influence stromal cells and regeneration [2]. One of the benefits of platelet lysate is the relative ease of its preparation. However, if the platelet lysate is used to improve bone regeneration, a number of factors should be considered. The method of blood sampling, the stage of leukoreduction, the method of platelet concentration, the temperature of freezing and unfreezing, the addition of anticoagulant factors, each of these points can affect the concentration and level of adhesion factors in platelet lysate, which in turn affect the growth and differentiation of MMSCs [3].

Obtaining a platelet lysate

Platelet lysates can be prepared from samples with a very high platelet content, which allows preparations saturated with growth factors. Procedure for making a platelet lysate allows you to simultaneously isolate all growth factors from the cells, because of the occurring lysis of platelets.

After sampling, it is possible to remove leukocytes from the blood by leukoreduction. The process of leukoreduction is the intentional removal of white cells from donated blood in order to reduce the residual number of leukocytes to less than $1x10^6$ and reduce the risk of adverse reactions in humans. The removal of leukocytes is performed by using leukofiltering devices. The stage of leukoreduction is very important, because the leukocyte molecules can affect the proliferation of MMSCs [4]. Leukocyte lysis releases matrix metalloproteinases and free oxygen radicals, which have a direct damaging effect on cell membranes [5].

The next step is a centrifugation with the removal of the supernatant and resuspension (washing) of platelets with a phosphate-buffered saline solution (PBS) with pH of 7.3, or isotonic 0.9% sodium

chloride solution until the platelet conglomerates disappear. Initially centrifugation takes place at 300-500 g for 5-10 minutes to obtain a plasma fraction with platelets without admixture of leukocytes and erythrocytes, then at 700-1000 g for 5-20 minutes to precipitate platelets.

Freezing and unfreezing cycles are used to release growth factors from platelet concentrate [6-10]. The most often used temperatures are -80°C for freezing, +37°C for unfreezing. As the platelet lysate contains coagulation factors, anticoagulants must be added. Usually, heparin is added to the collection tube for this purpose. The heparin concentration should not exceed 0.61 IU/mL for unfractionated heparin or 0.024 mg/mL for low molecular weight heparins. A high concentration of heparin can negatively affect MMSC proliferation, colony formation, and differentiation [11].

When prepared by apheresis technique in a closed system using centrifugation to separate cells by specific gravity, or filtration to separate cells by size, or a combination of both, a total platelet count of $1x10^9$ platelets/mL was obtained. Such platelet concentrates have a large volume (200–300 ml), and the total platelet content is approximately 6–8 times higher than in a conventional donor whole blood platelet concentrate [2].

Platelet lysate advantage include the fact that it can be made from platelet concentrates that are not suitable for transfusion (at more than 5 days after collection). One study found that the lysate made from stale platelets had the same effect on the growth and osteogenic differentiation as a lysate made from fresh platelets [12]. Freezing followed by unfreezing is the most effective way of releasing growth factors, which occurs when platelets are destroyed. It should be noted that the released growth factors are unstable in plasma [3].

The reparative potential of the platelet lysate

A reparative effect of the PRP lysate depends both on the initial quality of platelets in its composition and on the content of platelet components in the final lysate. It should be taken into account that the total cytokine composition of PRP is capable of stimulating both regenerative and pathological processes. The presence of leukocytes in PRP increases the level of the pro- inflammatory cytokine IL8. Activation of PRP with calcium chloride significantly reduces the content of growth-stimulating factors. Platelet lysate prepared from non-activated PRP without leukocytes, has an optimal balance of growth factors (EGF, FGF, PDGF) with pro-inflammatory cytokines, which determines its high regenerative potential, and can be recommended for clinical trials [13]. It is important to take into account the content of biologically active substances in PRP, since it is known that human cells, including osteoblasts, reduce their viability in conditions of excess PDGF and other growth factors. Thus, it has been shown in osteoblast culture that 5 μg/ml of platelet exosomes per 200,000 cells in vitro increase the activity of bone matrix synthesis by cells, and 50 μg/ml completely suppresses synthesis [14]. Platelets play an important role not only in primary hemostasis, but also in tissue healing and regeneration. Significant knowledge about the biology and pathophysiology of platelets has been gained over the previous two centuries, starting from 1865-1882, when M.Schultze and G. Bizzozero described the third group of blood cells [15]. Since the study of J. Roskam in 1922, it is believed that substances in platelets trigger the coagulation cascade when the endothelium is damaged and are not involved in active synthesis processes [16]. The stimulatory and inhibitory effects of bioactive mediators cause the proliferation and migration of fibroblasts, smooth muscle cells, and endothelial cells, which leads to vascular remodeling and tissue repair

[17]. Composition and quantity of growth factors in platelet lysate are sufficient for endothelial cell survival and vascularization in vivo in the absence of pericytes [18].

Effect of platelet lysate on osteoblasts

It is necessary to know the sequence of reactions that occur with the participation of local osteoblasts in the process of bone repair. After exposure to platelet lysate, there is an abrupt acceleration in the proliferation of inactive or very slowly dividing osteoblasts. Meantime, when the platelet lysate is removed, there is a disappearance or a significant slowdown in the proliferation of osteoblasts. The stimulating effect of platelet lysate on osteoblasts, the activation of hypoxia-induced factor 1–alpha, as well as the signal transducer and activator of transcription 3 (STAT 3) involved in both pathways - angiogenesis and bone regeneration were revealed.

The dual action of platelet lysate on resting osteoblasts, i.e. the resumption of proliferation and activation of pathways promoting both the angiogenesis and bone formation provides a rationale for its use as a therapeutic agent in a post-traumatic bone repair.

The effect of platelet lysate on bone regeneration is currently being studied. Platelet lysate is known to have a combined effect on osteoblasts. There is a resumption of proliferation of latent osteoblasts, activation of angiogenesis signaling pathways, stimulation of the secretion of factors that accelerate angiogenesis. It is by the effect of platelet lysate that the osteoblast differentiation and bone formation are triggered. All these properties of platelet lysate allow the use of the preparation for fractures and their nonunions. In the in vitro studies at the molecular level, in conditions similar to those observed after a fracture (release of latent osteoblasts from bone tissue after injury, blood flow to the site of injury,

and temporary exposure of osteoblasts to platelet growth factors), the addition of platelet lysate promoted a rapid acceleration of the proliferation, growth and differentiation of osteoblasts, which continued for some time after the action of platelet lysate had terminated. Then the differentiation of blast cells stopped, while their active growth and proliferation continued. This mechanism is necessary for the preservation and deposition of osteoblasts in the resulting bone for subsequent bone formation [19].

Damage to any tissue leads to the destruction of blood vessels and disruption of local blood supply, resulting in local hypoxia, which requires a local response of cells to changed environmental conditions. Hypoxia-inducible transcription factors (HIFs), complex factors that regulate the cellular response to hypoxia, in particular the HIF-1 alpha subfactor present in the cellular cytoplasm and disrupting the ubiquitin-proteasome system, stabilize under hypoxic conditions and migrate to the nucleus to bind with HIF-1 beta and coactivators. Thus, a complex is formed that activates the transcription of many genes responsible for cell survival under hypoxic conditions and triggers metabolic processes. Although the activation of the HIF system usually occurs as a result of hypoxia; the formation of HIF complexes also occurs under normal conditions without hypoxia [20]. In a published study by V. Nguyen reported that under the effect of platelet lysate there is an increase in the concentration of HIF-1 alpha protein, its movement into the nucleus and binding to specific DNA elements in cartilage cells grown under conditions of normal oxygen content [21]. This study reports that under normal oxygen conditions, platelet lysate induces an increase in the expression of HIF-1 alpha, increased its translocation into the nucleus and binding to specific DNA-sensitive elements in osteoblasts. Similarly, STAT 3 activation and nuclear translocation, as well as increased

secretion of VEGF, were observed in cultured osteoblasts. These data suggest that osteoblasts stimulated by platelet lysate may be involved in bone healing. HIF-1 has also been reported to enhance bone formation by regulating osteoblast differentiation [22].

In fact, a marked decrease in bone volume was observed in mice lacking HIF-1 alpha in osteoblasts [23]. Decreased bone mass in mice deprived of HIF-1 in osteoblasts was confirmed by Shomento et al. [22]. They proposed that HIF-1 is critical in linking angiogenesis to osteogenesis during endochondral ossification. Other authors have reported that HIF-1 is a pro-osteogenic factor in bone formation after injury [24]. The role of HIF-1 in controlling the interaction between osteoblasts and osteoclasts has also been reported [25]. Undoubtedly, the HIF-1 alpha pathway is a key component in skeletal development, and activation of the HIF complex is critical for linking angiogenesis to osteogenesis during bone formation and for maintaining bone homeostasis [26]. In the transgenic mice overexpressing HIF-1 in mature osteoblasts, the processes of osteo- and angiogenesis, the main processes of bone regeneration, are enhanced. Indeed, VEGF plays an important role in both osteoblast differentiation and induction of angiogenesis [27-28]. Moreover, in a recent publication by A. Romaldini et al. the platelet lysate has been reported to have a direct effect on endothelial cells, inducing their proliferation while maintaining their differentiation potential [29]. STAT 3 is a universal transcription factor. Upon activation, STAT 3 is phosphorylated by tyrosine residues to form homo- and heterodimers, which are translocated to the nucleus, where they induce the transcription of genes that regulate cell proliferation, differentiation, lifespan, apoptosis, and cellular immunity [30-33]. In addition to other biological functions, STAT 3 plays an important role in the regulation at the level of transcription, including the regulation of

VEGF transcription activation in both normal and pathological conditions. In MMSCs, the peak of VEGF expression occurs after three days of hypoxia. Hypoxia also affects the expression and phosphorylation of STAT 3. Signal transducer and activator of transcription 3 is important and necessary for the proliferation and migration of endothelial cells and the formation of microvessels [34]. STAT 3 plays an important role in the bone formation and homeostasis. In humans, a STAT 3 mutation results in a decrease in bone mass and an increase in the incidence of fractures with minimal exposure [35, 36]. In transgenic mice, upon inactivation of STAT 3 in osteoblasts and osteocytes bone formation caused by mechanical stress reduces [37]. Cobalt chloride, used to simulate hypoxia, stimulates MMSC migration and activates osteogenic differentiation of MMSC, healing of a bone marrow defect through triggering STAT 3 phosphorylation [38]. Activation of STAT 3 phosphorylation is also observed in the differentiation of human osteoprogenitor cells [39]. According to the literature, the platelet lysate activates osteoblasts and induces the movement of STAT 3 into the cell nucleus under conditions of normal oxygen content in the tissues. These properties of the platelet lysate suggest its use to improve bone union.

Studies using the platelet lysate for a fracture of the proximal humerus

H. Jiang et al. were among the first to publish a study using platelet-derived lysate. The patient underwent osteosynthesis of the right tibia with a plate, and three administrations of platelet lysate into the fracture site in a volume of 5 ml with an interval of a week. The result was evaluated after two, four, and six months. The pain syndrome was insignificant, complete bone union had been achieved by 8 months [40].

On the base of N.V. Sklifosovsky Research Institute for Emergency Medicine, we conducted a study on the use of autologous platelet lysate in patients with a fracture of the surgical neck of the humerus. At 3-4 days after the injury, the patients of the main group were injected with 1.0-1.5 ml of platelet lysate in 2-4 most painful points in the soft tissues surrounding the fracture. Injections were repeated 3 times with an interval of 24 to 48 hours. The procedures contributed to pain relief, acceleration of hematoma resorption, edema resolution. After 3 injections, in almost all cases, there was a positive trend in the relief of soft tissue inflammation in the fracture area [41].

The efficacy of the platelet lysate use in the treatment of osteoarthritis, Achilles tendonitis, and burns has been proven [42]. There are published works using platelet lysate with MMSCs for the treatment of fracture nonunions [43, 44]. However, higher evidence-based studies in humans are needed to confirm the efficacy of platelet lysate and its stimulatory effect on bone union.

Conclusion

Given the available literature data, the platelet lysate can be considered as a preparation containing a complete set of stimulating growth factors. The use of PRP is one of the promising ways to stimulate the processes of repair and regeneration in the tissues of a damaged organ in various types of pathology. According to clinical studies, PRP lysate stimulates bone regeneration. The resulting agent is convenient for injection, can be combined with wound dressings, bioconstructs, various carriers and other substrates, which makes it the most promising for use in trauma surgery and orthopedics for the treatment of bone fractures and nonunions.

In the final article of our literature review, we will consider the use of autologous red bone marrow.

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