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REVIEW

Use of thrombocyte concentrates in treatment of bone defects

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Abstract: The use of plasma enriched with thrombocytes is a trend in surgical treatment of bone defects (Platelet-rich plasma, thrombocyte concentrate, hereinafter PRP). It contains a set of identified as well as unknown growth factors. It is nontoxic, has no immunity reaction, accelerates and improves the healing of wounds. The increased level of growth factors leads to improved formation of new bone matter, and at the same time speeds up healing of soft tissues surrounding the bones. In addition, the presence of various factors mutually modulates and influences their functions. These functions are specific and differentiate the growth factors from recombinant growth factors, which are simple and focus just on one regenerating operation. PRP and ABPG (autologous bone-platelet gel) are used for augmentations in implantology, alveolar injuries with loss, treatment of parodontopathies, cleavages, and for osteodistraction of atrophic mandibles *(Ref. 24)*.
Full Text in free PDF *www.bmj.sk*.
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Platelet concentrate is a source of several autogenous growth factors for bone graft. In 1980s, the main knowledge about wound healing was based on the role of tissue oxygenation (1, 2). Since oxygenation increases the phagocyte-like and bactericide ability of immunity cells, and supports the collagen together with other synthetic proteins it remains the fundamental need to be promoted by a surgeon (2–4). Growing knowledge on identifying and understanding the growth factors shifts the focus of research of wound healing exactly to this area (5–7). It has been established that oxygen in general, and oxygen gradients in particular act with the help of macrophages by stimulating certain angiogenous and other growth factors supporting wound healing and resistance against infection (4, 8). The use of PRP is one of recent strategies for modulating and improving the wound healing. The processing of PRP has an impact on sequestration and concentration of platelets together with their growth factors. Simply said, it is an accelerated and elevated effect of platelet-derived growth factors representing an all-purpose initiator for almost all types of wound healing. By taking the advantage of these natural processes of regeneration while using both, identified and unknown growth factors from non-toxic platelet concentrate we speed up healing processes free of immune reaction.

Reparation of PRP and its components

Platelet concentrate is gained by separation of cells from authologic blood in a separator. During blood centrifugation, three basic blood components are separated depending on their density, namely superficial platelet-poor layer (PPP – Platelet Poor Plasma), PRP, and the densest part with erythrocytes (RBC – red blood cells). The PPP component is composed of acellular plasma constituting some 50 % and at a major collection could be returned into the circulation. It is similar with the layer of red cells. The PRP fraction is a part with concentrated content of platelets and white cells. PRP and PPP are plasma fractions containing a lot of fibrinogen and coagulation factors. The formation of fibrin despite itself not being a growth factor creates a natural osteoconductive matrix necessary for bone regeneration.

Specific PRP trials have identified minimally three important growth factors in platelet alpha-granules, namely platelet-derived growth factor (PDGF), transforming growth factor β_1 (TGF- β_1) and transforming growth factor β_2 (TGF- β_2). Further trials prove the presence of insulin-like growth factor I (IGF-I) (9).

PDGF is connected with a dual role of platelets almost in all types of wound healing, namely as a reserve of growth factors and homeostatic factors. This double role is probably developed as a surviving mechanism. PDGF shows several positive wound healing effects, including mitogenesis (by multiplying the number of healing cells), angiogenesis (formation of new vessels) and up-regulation of other growth factors and cells (promotion of fibroblastic and osteoblastic functions of cell differentiation and acceleration effects of growth factors on other cells such as macrophages). PDGF is a primary platelet growth factor, being synthesized and excreted by means of other cells (macrophages, endothelial cells) (11). The presence of platelets in blood stopper is the first growth factor in the wound and promotes revascularization, collagen synthesis, and bone regeneration (10, 11) It exists as a heterodimer of two chains, namely A and B (11, 12). The human body recognizes also homodimers A-A and B-B with the same effect on bone remodeling (10, 11). PDGF factors

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are disseminated during degranulation of platelets. There is about 0.06 ng of PGDF in 1 million platelets (13). It corresponds to a value of $6x10^{-17}$ or 1200 molecules of PGDF per one platelet (13, 14). The effect of PDGF becomes evident after binding with membrane receptors. This link activates the internal cytoplasmatic signal protein together with high-energy phosphatase connection (15). This protein activates the gene of expression on specific acts of mitosis (by increase in healing cell population), angiogenesis (by initiating the endothelial mitosis, and its organization in functional capillaries), and activation of macrophages (by cleaning the wound, and launching the second phase for growth factors).

TGF-β is a term used for a group of growth and differentiation factors, where the members are bone morphogenic factors (BMPs) (16). TGF-β contained in PRP are proteins TGF-β_1 and TGF-β_2, known as most frequent components of TGF-β group, and are basic growth and differentiation factors connected to regeneration of connective tissue and bone (17, 18). With an exception of platelets, they are synthesized by macrophages, osteoblasts, and other cell types. In platelet degranulation or in active macrophage secretion, they act as a paracrinne growth factor (growth factor formed by one cell with an impact on the second connected cell), on fibroblasts, stem cells, and pre-osteoblasts. These cells form also their own TGF-β proteins with paracrinne as well as authocrinne functions (19). TGF-β acts not only in ignition of bone remodeling but also in remodeling and maturation of bone grafts. The most important function of TGF-β_1 and TGF-β_2 seems to be chemotaxia and mitogenesis of osteoblast precursors, as well as an ability to stimulate their deposits into collagen matrix of connective tissue, and bone formation (20). Moreover TGF-β inhibits the osteoclasts and absorption of bone tissue, thus the new formation takes over tissue drop out (5).

IGF-I and IGF-II are normally formed by secretion of osteoblasts during bone formation to increase the creation of osteoblasts and thus to accelerate the deposition of bone tissue (21). IGFs are also accumulated in bone matrix at their absorption to override these acts (5, 22). The presence of IGF in platelets is expected in osteoblast precursors (cells of osteoblast line not producing osteoid so far), and in endosteal osteoblasts, i.e. in cells producing the initial phase (phase I) of bone graft. IGFs represent a mitogenic to osteoblastic cell line, as well as stimulators of bone formation by existent differentiated osteoblasts.

Role of growth factors in bone regeneration and wound healing

Bone graft in majority of cases just fills the "dead" space created by the coagulum. This space is hypoxic (PO_2 from 5 to 10mm Hg), acidic (pH 4 to 6), and contains platelets, leukocytes, erythrocytes, and fibrins in a complex network of transferred osteocytes, endosteal osteoblasts, and stem cells (1, 4, 22). Under normal circumstances, the graft stem cells, i.e. the main regeneration cells, exist in very low numbers (1 in 250000 cells at age of 35) (23, 24). Outside the periostal surgical wound closure, the tissue is normoxic (PO_2 45 to 55 mm Hg), with physiologic pH (pH 7.42), and contains a population of structural cells, and stem cells able to heal the wound (also in very small quantities) and cut the capillaries with blood precipitates and naked endothelial cells (4). Nowadays the healing could be accelerated and improved by adding the growth factors in form of PRP. It has been proved that deployment of PRP and ABPG significantly improves the subjective postoperative status of patient (less swelling, less pain), accelerates the healing of soft tissues and formation of new bone tissue. On x-ray, the grafts are more mature than expected in relation to the real healing period. This enables early functionality and possibility of earlier pressure. It also stimulates the angiogenesis, supports the collagen synthesis, increases the firmness of regenerated tissue, staunches the blood, and accelerates the deposition of extracellular matrix, and closing of the wound.

Conclusion

PRP represents a remarkable advancement in the treatment of bone defects. A large target group is represented by patients with a traditionally weak level of bone remodeling, i.e. seniors, patients with osteoporosis, patients with diabetes mellitus, and patients after radiation treatment. It offers the clinicians a possibility to use growth factors by means of a simple and available technology. The mechanisms of cross-impact of growth factors in the target cell still need further research. The future is in rapid platelet separation with higher density and particularly their availability for all physicians.

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