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Leukocyte-rich PRP for knee osteoarthritis: Current concepts

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ABSTRACT

Knee osteoarthritis is a major painful and debilitating orthopaedic disease affecting a large number of adult individuals on a global scale. Over the years, this severe condition has been widely studied and while many alternatives have been utilized, platelet-rich plasma (PRP) remains one of the most popular solutions among researchers and clinicians alike. While there are different formulations and techniques involved in the preparation of PRP, produced either manually or via the use of commercial kits, the presence of leukocytes in a PRP mixture is a factor that raises concern due to their well-known pro-inflammatory activity. Although it is reasonable to worry about this, it should be taken into consideration that in order for the healing process to occur, the inflammatory phase is necessary. Leukocytes present in the inflammatory phase release both pro and anti-inflammatory molecules and, when combined with activated platelets, their potential increases. Additionally, due to the macrophage's plasticity to switch from the subtype 1 to subtype 2, it is suggested that the inclusion of the components from the buffy coat layer in a PRP mixture, classifying it as leukocyte-rich platelet-rich plasma or L-PRP, may provide benefits instead of detriments, from a standpoint of the regenerative potential of PRP.

1. Introduction

Osteoarthritis (OA), the most common progressive joint disease involving cartilage and surrounding tissues[1] is generally characterized by joint inflammation and a reparative bone response. It is one of the top five most disabling conditions, affecting more than one-third of the elderly population above 65 years of age, with global estimates reaching a number greater than 100 million individuals affected by this disease.[2] Knee osteoarthritis (KOA), in particular, is commonly attributed to aging and obesity and has doubled in prevalence since the mid-20th century.[3] This disease is typically defined by progressive loss of articular cartilage, thickening of the subchondral bone, formation of osteophytes, significant inflammation of the synovium as well as degeneration of ligaments and menisci of the knee and hypertrophy of the joint capsule.[4] Risk factors for OA encompass joint injury, obesity, aging and even genetic predisposition. Since the OA microenvironment becomes increasingly catabolic and destructive, continuous research with the rising popularity of platelet-rich plasma (PRP) therapy revealed that platelet alpha-granules which contain and release numerous growth factors such as hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and transforming growth factor-b (TGF-β), as examples, can be beneficial in modulating the status of the disease.[5] On top of that, investigations regarding leukocyte content in PRP formulations and the potential effects on osteoarthritis treatment have caused some controversy in the literature due to the fact the these cells, especially neutrophils, are known to cause inflammation by driving the inflammatory phase of wound healing. Although preoccupation still exists regarding the applications of leukocyte-rich platelet-rich plasma (L-PRP), some studies point out that apart from an anti-infectious property, leukocytes produce large amounts of VEGF, to illustrate a few of the multiple benefits attributed to this cell type.[6] This review provides some insights on the possible cellular mechanisms whereby L-PRP may act to manage the deteriorated microenvironment generated by osteoarthritis, particularly knee osteoarthritis (KOA), and the potential benefits of their involvement.

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1.1. Biological perspective of osteoarthritis

In the past, osteoarthritis was thought to be a disease of articular cartilage however recent research indicates that the condition encompasses the entire joint.[7] It has been previously proposed that degeneration of cartilage in OA occurs in two phases: a biosynthetic phase, where the chondrocytes, the resident cells found in cartilage, attempt to repair the damaged extracellular matrix (ECM); and the degradative phase, which is characterized by catabolic enzyme activity resulting in matrix digestion and subsequent matrix synthesis inhibition.[8] These biological events culminate in the erosion of cartilage, aggravating physical pain and debilitation. It has also been thought that loss of articular cartilage is the primary change. However, in addition to that, there is a combination of cellular alterations and biomechanical stresses which are responsible for numerous secondary modifications including: remodelling of the subchondral bone, formation of osteophytes, bone marrow lesions as well as alterations in the synovium, joint capsule, ligaments and periarticular muscles, and meniscal tears and extrusion[9].

1.2. Cellular and molecular alterations in the OA microenvironment

Synoviocytes and osteoarthritic chondrocytes are capable of producing high amounts of matrix metaloproteinases (MMPs), including MMP-1, MMP-3, MMP-9 and MMP-13.[10] Synoviocytes secrete proteolytic enzymes as well as proinflammatory cytokines IL-1β, IL-6 and TNF-α, molecules which seem to mediate the progression and pain related to OA.[11] Other cytokines, such as resistin, from the adipokine class, and osteopontin, whose increased expression is associated with disease severity, are molecules which are expressed in high quantities by the osteoarthritic synovial tissue.[12–14] Additionally, the synovium has also been reported to produce some of the chemokines and metalloproteinases which cause the degeneration of cartilage despite the fact that cartilage itself also produces most of the catabolic molecules via autocrine and paracrine mechanisms.[15] As a result, the final products derived from cartilage breakdown, due to mechanical or enzymatic destruction, can trigger the release of collagenase and other hydrolytic enzymes from synovial cells, leading to vascular hyperplasia in osteoarthritic synovial membranes.[16]

The normal articular cartilage of adults is mainly comprised of ECM (which is made up of water, collagen, proteoglycans and a small fraction of calcium salt) and chondrocytes.[17] The rate of collagen turnover is slow whilst that of the proteoglycan is relatively faster in comparison.[9] This process is regulated by chondrocytes, which synthesise the molecular components as well as the proteolytic enzymes responsible for their breakdown.[9] Chondrocytes are also influenced by various factors, including polypeptide growth factors and cytokines, structural and physical stimuli and even components of the ECM itself.[9]

Osteoarthritis arises when chondrocytes fail to maintain homeostasis between synthesis and degradation of the ECM components, even though the exact cause for the initiation of this imbalance is not entirely understood.[18] Physical trauma and microfractures or inflammation can cause a slight increase in enzymatic activity, resulting in the formation of “wear” particles attributed to the so-called “wear-and-tear” process.[19] Even though macrophages are able to engulf and eliminate microparticles and cellular debris, eventually, the overproduction of these particles overwhelms the system, making it harder to dispose of them, where they ultimately become mediators of inflammation, stimulating chondrocytes to release degradative enzymes.[19] Molecules derived from breakdown of collagen and proteoglycan are also taken up by synovial macrophages but cause the expression of proinflammatory cytokines TNFα, IL-1 and IL-6, which in turn bind to chondrocyte receptors, leading to further release of metalloproteinases and inhibition of collagen type 2 synthesis, thereby aggravating cartilage degeneration and favouring a more predominant destructive microenvironment.[20] Overall, perturbation in homeostasis results in increased water content and decreased proteoglycan content of the ECM, which weakens the collagen network since there is reduced synthesis of type 2 collagen and increased breakdown of pre-existing collagen. Lastly, there is also an increase in the rate of apoptosis in chondrocytes.[21]

1.3. Platelets and leukocytes combined: the mechanism of action

Platelet-rich plasma (PRP) has become a popular topic in the realm of medical research due to continuous study since the 1970s by haematologists.[22] It has grown ever since and been applied to the fields of orthopaedics, dermatology, plastic surgery, odontology and even veterinary medicine.[23] This biological product derived from autologous blood centrifugation contains a mixture of a variety of cells with a primary focus on concentrated platelets above baseline[24,25] and is obtained via the use of commercial kits or *in house* techniques, resulting in different PRP products and, therefore, different terminologies.[23] The platelets present in PRP contain granules with a broad range of active biomolecules which, upon activation, release these biomolecules, therefore stimulating the natural healing cascade.[26,27]

There are different ways to prepare PRP, and this is typically defined by the cell type and concentration of cells within the PRP product, which can include erythrocytes, leukocytes and also a small fraction of stem cells.[28] The presence of white blood cells, particularly neutrophils, in a PRP mixture, is cause for concern and controversy in the literature, since neutrophils release inflammatory cytokines and metalloproteinases which can escalate the early inflammatory response to tissue injury.[29] Although there is a certain level of preoccupation regarding the aggressive role of neutrophils in inflammatory processes, recent data indicate that the interaction between neutrophils and activated platelets can release anti-inflammatory molecules, and PRP products rich in leukocyte content are then termed leukocyte-rich PRP (L-PRP).

A study published by Parrish & Roides in 2017 reveals the anti-inflammatory potential of the interaction of platelets and neutrophils. The mechanism behind this process occurs firstly by the release of arachidonic acid by activated platelets, which is then picked up by neutrophils and converted into leukotriene and prostaglandins, both inflammatory molecules.[30] Platelets in association with neutrophils, however, can pick up leukotrienes and convert it into lipoxin, a potent anti-inflammatory protein capable of limiting neutrophil activation and preventing diapedesis, thereby promoting the resolution phase of the healing cascade.[30] Knowing that the production of lipoxin is only possible via the prior synthesis of leukotriene by neutrophils and that the subsequent shift in pro-inflammatory to anti-inflammatory molecules prevents the recruitment of neutrophils and inflammatory activation, this association seems therefore more beneficial to the resolution process of the healing cascade from a standpoint of the regenerative potential of L-PRP. In the case of knee osteoarthritis, this process could be of great interest as it is important to shift and maintain the knee microenvironment under an anti-inflammatory state and prevent it from progressing to prolonged inflammation and increased degeneration.

An investigation led by Kazemi & Fakhrjou, in 2015, comparing L-PRP and Leukocyte-Platelet Rich Fibrin (L-PRF) for articular cartilage repair of the knee in adult dogs suggests that both L-PRP and L-PRF could be used to effectively promote the healing of articular cartilage defects of the knee. The authors concluded that using L-PRF for the treatment of acute full thickness articular

cartilage defects of the knee produced a repair tissue similar to L-PRP treated defects both macroscopically and microscopically and better than the untreated defects.[31]

A level IV study conducted in 2013 by Filardo and colleagues aimed to describe the clinical results obtained after intra-articular injection of LP-PRP preparation to treat KOA. The authors recruited 45 patients, who were divided into either early-to-moderate OA or severe OA, and treated them with a cycle of three weekly injections of autologous conditioned plasma. It was concluded that overall, the clinical outcome for the treatment of knee OA with LP-PRP was positive and proved to be safe. The intra-articular injections were capable of reducing pain and improving knee functional status at short-term follow-up. The patients with a lower degree of joint degeneration responded best to the treatment whereas the patients suffering from severe knee OA experienced a less favourable outcome.[32]

Despite different observations and opinions, it is important to point out that the inflammatory phase is necessary for the progression of the healing process, especially in order to reach the final stages of this biological event, such as remodelling and tissue contraction. The next section shines light on another cell population which is just as relevant to the inflammatory phase.

1.4. The role of mononuclear cells in regeneration

Peripheral blood mononuclear cells (PBMCs) include T and B lymphocytes, natural killer (NK) cells and monocytes.[33] The monocyte's abilities to differentiate into macrophages, switch phenotype and display different functions due to microenvironmental stimuli are features that make this cell a key component in PRP therapy.[34] Peripheral macrophages play a crucial role by engaging in phagocytosis of cells undergoing apoptosis and protect the host through innate immunity. Monocytes from peripheral blood can differentiate into tissue macrophages once tissue migration occurs. The macrophage expresses two major phenotypes, either M1 or M2, which depends on how the activation is given. M1 is induced by microbial agents, therefore assuming a more pro-inflammatory role, whereas the M2 phenotype, conversely, is produced by a type 2 response and takes on an anti-inflammatory property, typically characterized by an increase in IL-4, IL-5, IL-9 and IL-13. The type 2 response, known to be directly involved in regeneration after injury and tissue repair, is mostly occurring in cells such as eosinophils, mast cells, basophils and Th2 cells.[35] Macrophages convey a protective immunological function and also promote angiogenesis via the release of angiogenic factors and cytokines.[36] Comparing M1 and M2 further, it has also been found that M2 triggers cell proliferation and repair through polyamine and collagen synthesis in addition to other tissue remodelling functions, releasing IL-10 and IL-4. The M1 type, on the contrary, displays microbicidal activity and inhibition of cell proliferation through nitric oxide mechanisms, releasing inflammatory cytokines IL-6 and TNF-α.[37]

The inflammatory process that takes place in injured areas is carried out by activated macrophages set out to eliminate potential microbes, remove pathogens and clear cellular debris, all during the inflammatory phase of wound healing. Once macrophages are through with this process they become deactivated and unresponsive to inflammatory stimuli, giving way to the promotion of angiogenesis, cell proliferation and extracellular matrix deposition for the remodelling phase.[38]

In the case of KOA, for example, synovial neovascularization may be largely driven by synovitis as the inflammatory cells, such as macrophages, which can secrete pro-angiogenic factors as well as other factors that stimulate other cells like endothelial cells and fibroblasts, which in turn produce VEGF, basic fibroblast growth factor (bFGF) among others which can further promote angiogenesis.[16,39]

1.5. Macrophage polarization

Although this process is not fully comprehended and still debatable, there are some hypotheses proposing that macrophages can polarize into different subtypes in response to different signals. It has been thought that M1 and M2 macrophages are two distinct cell populations acting on different phases of the inflammatory process.[40,41] A different hypothesis, on the other hand, suggests that M1 and M2 macrophages are the same cells but capable of altering their functional phenotype in response to microenvironmental stimuli.[42,43] The first hypothesis proposes that Ly6C + monocytes become the M1 macrophage in tissue with inflammatory functions whereas the Ly6C- monocytes or tissue-resident macrophages become M2 macrophages with reparative roles. The second hypothesis suggests that macrophages can polarize to different subtypes in response to the signals and stimuli received from the microenvironment, M1 in the early phase of healing, and M2 in the late phase.[42] In 2014, Italiani et al. observed that monocytes that polarized to M1 matured into M2 in a culture system that had induced sequential changes in the microenvironment. Taking that into consideration, it would appear that the various cytokines, signals and stimuli relayed in the cellular microenvironment are capable of shifting the macrophage subtypes.[44] With that said, in cases of knee osteoarthritis where there is an increased production of several inflammatory cytokines, it seems plausible that administration of white blood cells, more specifically macrophages, could be advantageous when combined with platelets in order to treat the severe osteoarthritic conditions.

2. Conclusion

Summarizing the concepts suggested in this review, leukocytes have displayed considerable importance and some positive effects towards the regenerative phase in inflammatory processes. The α-granules from platelets carry many important growth factors and active biomolecules. The leukocytes involved in the inflammatory phase can release both pro and anti-inflammatory molecules. The combination of neutrophils and activated platelets seems to elicit a more positive rather than detrimental effect on healing. Here, the macrophages have been extensively referred to in appreciation of their intrinsic fundamental roles in the body, especially during inflammation. Taking into consideration the macrophage's plasticity in order to switch from M1 to M2 phenotype, this feature is one more reason to suggest that the preparation of PRP products with the collection of the buffy coat, generating the leukocyte-rich PRP (L-PRP), may actually contribute to the promotion of a regenerative environment instead of bearing deleterious side effects. Currently, the literature remains divided regarding the overall effectiveness of L-PRP for the treatment of orthopaedic diseases, namely OA. There is still no general consensus on this subject since leukocyte-rich PRP has shown both positive and negative results according to different animal studies. There are no clinical studies specifically evaluating the use of L-PRP alone for knee OA. In light of this, more investigations analysing the effects of L-PRP for varying degrees of knee OA should be conducted in attempts to further outline and comprehend the mechanisms involved in the communication between leukocytes and other cellular and molecular agents and how they may cooperate to effectively treat different musculoskeletal disorders.

Conflict of interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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