

Focus

Reference 1.2.3.1

Knee joint - cartilage

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Intra-articular injection of platelet-rich plasma for cartilage repair

A case study

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Summary

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A potential strategy for treating traumatic and degenerative cartilage injury is to stimulate cartilage anabolism in the affected joint. In vitro and in vivo studies have confirmed the anabolic effect of endogenous growth and differentiation factors on chondral tissue.

Platelet-rich plasma contains a high concentration of these growth factors.

69 patients with arthroscopically confirmed slight to moderate knee joint cartilage injury were treated via intra-articular administration of autologous platelet-rich plasma obtained using a double syringe.

The WOMAC score revealed significant improvements in the parameters "pain," "stiffness," and "physical activity." There were no side effects or complications.

Introduction

In the past, arthrosis was considered a natural consequence of aging. Currently, however, it is understood to be an expression of complex, active disease processes (1, 2, 11). This knowledge has made it necessary to develop new therapeutic approaches.

The pathogenesis of this for the most part chronic disease is characterized by numerous biomechanical, biochemical, molecular biological and genetic processes.

Arthrosis is characterized by a progressive loss of hyaline joint cartilage accompanied by a fibrotic transformation of the synovial membrane. Radiographs show shrinkage of the joint gap, sclerosis of the subchondral bone and the formation of osteophytes.

One peculiarity of cartilage is that it consists of only a single cell type, that is, chondrocytes. Chondrocytes are responsible for maintaining the extracellular matrix, and regulate the construction and breakdown of the primary components: type II collagen and proteoglycan. Numerous findings have shown that special proteins influence specific processes related to the progression of arthrosis and control cellular differentiation patterns (3, 4, 5, 6, 11).

Biochemically, the onset of arthrosis is first and foremost characterized by the loss of proteoglycan in the superficial layers of the cartilage matrix (7). The metabolism of local chondrocytes is disturbed, and they do not react sufficiently to the elevated catabolism. However, it is unclear whether it is the cells that change first, thereby impairing the supply to the matrix, or whether outside factors alter the matrix and thereby prevent cells from effectively making repairs. Consequently, the collagen network loosens, becoming softer and less able to handle mechanical stress. The release of soluble fragments of the matrix molecules, such as collagen or fibronectin, causes inflammatory reactions. Activated lymphocytes, macrophages, chondrocytes and synovial fibroblasts subsequently secrete more inflammation mediators such as interleukin-1, -6, -17, -18 (IL-1, IL-6, IL 17, IL-18) and tumor necrosis factor alpha (TNF- α). Interleukin-1 promotes the breakdown of the matrix by inducing the synthesis of matrix-decomposing enzymes such as collagenases and aggrecanases (8).

However, desirable repair mechanisms such as chondrogenesis are insufficiently active in adult joints. This may be due to the lack or insufficiency of certain morphogenetic factors. One obvious step therefore would be to stimulate cellular differentiation using such endogenous factors. These growth and differentiation factors are found in the blood, especially in platelets (9).

The research of different working groups has demonstrated the anabolic and differentiating effect of growth and differentiation factors on chondrogenesis (10, 11, 12, 13). The endogenous anabolic factors cited in Table I therefore appear to be suited for repairing cartilage injury primarily based on their known modes of action and biological effects. Animal experiments by *Simank* et al. (6) on rabbits, and experiments by *Milano* et al. (14) on the sheep knee have confirmed these considerations in in-vivo experiments.

These findings, in addition to the positive results of autologous serum use by various working groups for muscle injury in animal experiments (15), the practical use of platelet concentrates for sports injuries (16), oral and maxillofacial surgery (17) and plastic surgery (18), all confirm the anabolic effect of platelet-rich plasma fractions and suggest that it may be useful for treating cartilage damage. The work of *Milano* et al. (14) clearly indicates that the repair of iatrogenic cartilage injury in sheep is closely correlated with the applied concentration of growth factors, whereas IL-1Ra does not manifest any definite effect. These results led us to use platelet-rich or growth-factor-rich plasma for treating cartilage injury in the joint.

Materials and methods

The approach described here was employed at two centers in Bonn and Hamburg. 69 knee joints were treated that manifested arthroscopically confirmed grade II-III cartilage injury per Outerbridge classification with a maximum focal injury up to grade IV. Large areas of grade IV injury and axial deviations greater than 5 degrees were excluded.

A "Double Syringe[®]" manufactured by Arthrex was used to produce the autologous conditioned plasma (ACP). The procedure for creating the autologous conditioned plasma (ACP) is as follows:

Materials:

Centrifuge with special inserts for holding the double syringe, anticoagulant (ACD-A), injection cannulae, butterfly cannulae, tourniquet, a sterile cover, sterile gloves, sterile disposable smock, an assistant.

- 1) The puncture site for drawing the blood is disinfected and covered with a sterile disposable cloth. Approximately 1 mL ACD-A is drawn into the double syringe under sterile conditions.
- 2) Approximately 9 mL venous blood is drawn into the sterile double syringe under sterile conditions. The double syringe is sealed with a sterile cap.
- 3) The blood and ACD-A are mixed well with each other by rotating the syringes.
- 4) The double syringe is placed in a sterile beaker of the centrifuge, and a second syringe inserted as a counterweight.
- 5) The double syringe is centrifuged at 1,500 RPM for 5 minutes and then carefully removed.
- 6) After being centrifuged, the middle part of the double syringe is removed while simultaneously pulling the inner part of the double system to cause a retrograde flow of the plasma to transfer it into the inner syringe of the double syringe system.
- 7) The inner, smaller syringe containing the ACP is then unscrewed out of the plunger of the larger syringe. The sterile ACP is then ready for intra-articular injection.
- 8) The ACP is then injected in the joint under the usual conditions.

To ensure the highest possible platelet concentration and to prevent decomposition caused by freezing, the ACP is prepared fresh and then injected under sterile conditions into the knee joint within 30 minutes after the blood is drawn. There is cause for concern regarding platelet deformation or destabilization caused by the centrifuging, since the obtained ACP is applied immediately afterward.

Six separate injections were given, one week apart. The patients were surveyed using the Womac-D arthritis index 4 to 6 months after treatment.

69 patients were observed, yielding 69 Womac surveys (47 women and 22 men). The age of the patients ranged from 21 to 74 years (with an average of 56.2 years).

Results

The analysis of the autologous condition plasma (ACP) yielded the results shown in Table II.

The quantitative analysis of the autologous conditioned plasma (ACP) yielded the concentrations shown in Table III.

The concentration of growth factors in the ACP is significantly elevated in comparison to whole blood and plasma ($p < 0.02$). A T test was used for statistical evaluation of the associated samples. For PDGF-AB, the increase was 25%. The concentration of EGF, VEGF and PDGF-BB was 5 to 11 times greater. The concentration of IGF-I, TGF-b1 and TGF-b2 increased by a factor of less than 5.

A significant release of growth factors was seen in the ACP almost immediately. The ACP triglyceride levels did not differ from whole blood or plasma.

The Wilcoxon pair difference test was used for statistical evaluation. Subjective symptoms of "pain," "stiffness" and physical activity" improved significantly. With $p < 0.0001$, this improvement was statistically highly significant.

Tab. I: Growth factors (Gelse 2007).

| Factor | Name | Formation location | Effects |
|------------------------|------------------------------------|---------------------------------|---|
| IGF-I | insulin-like growth factors | Activated platelets | Stimulates cell proliferation and matrix synthesis, differentiation of osteoblasts |
| EGF | epidermal growth factor | Activated platelets | Stimulates the proliferation and differentiation of epidermal cells, a co-stimulant of angiogenesis |
| VEGF | vascular endothelial growth factor | Leukocytes Endothelial cells | Stimulates angiogenesis, chemoattractant for osteoblasts |
| PDGF aa PDGF bb | platelet-derived growth factors | Activated platelets | Mitogens for mesenchymal stem cells, promotes the production of the extracellular matrix |
| TGF-beta1 TGF-beta2 | transforming growth factors | Activated platelets | Stimulates DNA synthesis and the proliferation and differentiation of different types of cells. Promotes collagen synthesis, the induction of chondrogenesis, stimulation of matrix synthesis |

Tab. II: Analysis of autologous conditioned plasma (ACP).

| Factor | Name | Formation location | Effects |
|---------------------|------------------------------------|---------------------------------|---|
| IGF-I | insulin-like growth factors | Activated platelets | Stimulates the proliferation and differentiation of osteoblasts |
| EGF | epidermal growth factor | Activated platelets | Stimulates the proliferation and differentiation of epidermal cells, a co-stimulant of angiogenesis |
| VEGF | vascular endothelial growth factor | Leukocytes Endothelial cells | Stimulates angiogenesis, chemoattractant for osteoblasts |
| PDGF aa PDGF bb | platelet-derived growth factors | Activated platelets | Mitogens for mesenchymal stem cells, promotes the production of the extracellular matrix |
| TGF-beta1 TGF-beta2 | transforming growth factors | Activated platelets | Stimulates DNA synthesis and the proliferation and differentiation of different types of cells. Promotes collagen synthesis |

Table IV and Fig. 1 summarize the results of monitoring using the Womac-D arthritis index. The results were totaled for each category and patient before the average and standard deviation were calculated.

Discussion

A major question regarding cartilage injury is, are there endogenous repair mechanisms, and if so, how can they be positively influenced so they can be used in therapy?

In principle, complete chondrogenesis is possible even in the adult organism, as illustrated by the example of osteophytes. Osteophytes manifest all the characteristics of hyaline cartilage, at least in phases.

It is also known that bone marrow, periosteum, perichondrium, subcutaneous connective tissue, fatty tissue, muscle and synovial tissue contain mesenchymal precursor cells that undergo chondrocytic differentiation (11, 19, 20, 21). The investigations of *Gebhard et al.* (22) as well as other analyses of gene expression have shown that chondrocytes in arthritic cartilage manifest an elevated protein synthesis rate at certain locations and produce an increased amount

of cartilage matrix. The chondrocytes attempt to restore the disturbed equilibrium by shifting from matrix decomposition to matrix creation or, in other words, these constitute indications of an early endogenous attempt at repair (4, 22). In superficial fibrillated zones and in advanced stages of arthrosis, investigations have revealed a reduced synthesis of cartilage-specific matrix molecules (5). The insufficient metabolic function of the chondrocytes at certain locations and especially in the late stages of arthrosis is ascribed to oxidative, cumulative DNA damage or mitochondrial degeneration from cell aging processes with telomere shortening (23).

Our clinical data also reveals a very limited intrinsic healing ability of purely chondral defects, since there is no invasion of repair cells in comparison to what occurs with osteochondral defects. The goal of the endogenous anabolic therapy approach for treating cartilage injury therefore needs to be the continuous stimulation of the endogenous regeneration capacity.

What options are available for increasing endogenous anabolism?

It is known that TGF- β , "insulin-like growth factor" (IGF) and "platelet derived growth factor" (PDGF) regulate cartilage growth metabolism as anabolic growth factors that are synthesized by the chondrocytes themselves (1). It has already been reliably demonstrated in vitro that differentiation factors such as bone morphogenetic protein-2 (BMP-2), bone morphogenetic protein-7 (BMP-7) or cartilage derived morphogenetic protein-1 (CDMP-1) and growth factors such as insulin-like growth factor-1 (IGF-1) induce the synthesis of type-II collagen and aggrecan (2, 24, 25). Gene therapy studies have also demonstrated that the adenoviral gene transfer of BMP-2, TGF β or IGF-1 into chondrocytes causes them to synthesize a significantly greater amount of aggrecan, type-II collagen and other matrix proteins in cell cultures. IGF-1 appears to be the factor with the fewest negative side effects for stimulating matrix synthesis (27). Differentiation and growth factors such as bone morphogenetic proteins -2, -7, (BMP-2, BMP-7) transforming growth factor-beta (TGF β), CDMP-1 and CDMP-2 can induce chondrocytic differentiation of mesenchymal cells in vitro (10, 26). *Borzini* and *Mazzucco* (13) have also confirmed the regenerative effect of platelet preparations for various types of tissue.

Tab. III: Quantitative analysis of autologous conditioned plasma (ACP).

| Growth factors | | Plasma from whole blood | ACP without T | T released | Total GF in ACP | Literature/ PRP |
|----------------------|------|-------------------------|---------------|------------|-----------------|------------------------|
| IGF-I [ng/ml] | Mean | 121 | 122 | 6 | 128 | 84 |
| | SD | 50 | 51 | 0 | | |
| EGF [pg/ml] | Mean | 113 | 396 | 146 | 543 | 24 |
| | SD | 116 | 112 | 32 | | |
| VEGF [pg/ml] | Mean | 32 | 130 | 208 | 338 | |
| | SD | 40 | 124 | 189 | | |
| PDGF-AB [pg/ml] | Mean | 2467 | 30979 | 29952 | 60931 | 117500 |
| | SD | 2210 | 8525 | 8350 | | |
| PDGF-BB [pg/ml] | Mean | 1760 | 4529 | 6298 | 10827 | 9900 |
| | SD | 2042 | 1458 | 1774 | | |
| TGF-b1 [pg/ml] | Mean | 36506 | 92824 | 56410 | 149234 | 169400 |
| | SD | 38380 | 21504 | 9168 | | |
| TGF-b2 [pg/ml] | Mean | 98 | 197 | 38 | 236 | 400 |
| | SD | 128 | 131 | 44 | | |
| Triglyceride [mg/dl] | Mean | 506 | 466 | | | Weibrich et al. (2002) |
| | SD | 126 | 171 | | | |

The bioavailability of insulin-like growth factor-1 (IGF-1) in arthrotic joints is reduced, despite increased production, due to an elevated level of IGF-binding proteins (IGFBPs) in the synovial fluid (27). Enrichment of IGF-1 in the knee joint of rabbits via adenoviral gene transfer demonstrably and significantly increases proteoglycan synthesis in the joint cartilage without any adverse effects in this model (28). In 2004, *Simank* et al. (6) published on the effect of the intra-articular application of growth and differentiation factor-5 (GDF-5) in rabbits with cartilage injuries. They were able to demonstrate the nearly complete repair of "full-thickness" injuries. Animal experiments by *Milano* et al. (14) on the sheep's knee revealed similar results. Platelet rich plasma (PRP) was injected following defined cartilage injuries.

The intra-articular use of platelet-rich plasma for cartilage damage in the human joint appears to us to be worthy of recommendation, based on the results of prior animal experiments and the scientific data underpinning this therapeutic approach.

Since there is no generally recognized definition of PRP, the plasma that was used in this case with the aforementioned production method was termed autologous conditioned plasma (ACP).

Conclusions

The intra-articular injection of ACP in knee joints suffering from light to moderate cartilage damage yielded significantly favorable results in regard to the parameters "pain," "stiffness," and "physical activity." There were no side effects or complications. The results presented here must of course be verified through further studies. The positive clinical results and absence of side effects, however, justify and encourage the use of ACP at the present time, when properly indicated. Numerous scientific works on the effects of growth and differentiation factors on chondrocytes and the histological results of animal experiments indicate that the positive effect may arise from structure-modifying properties.

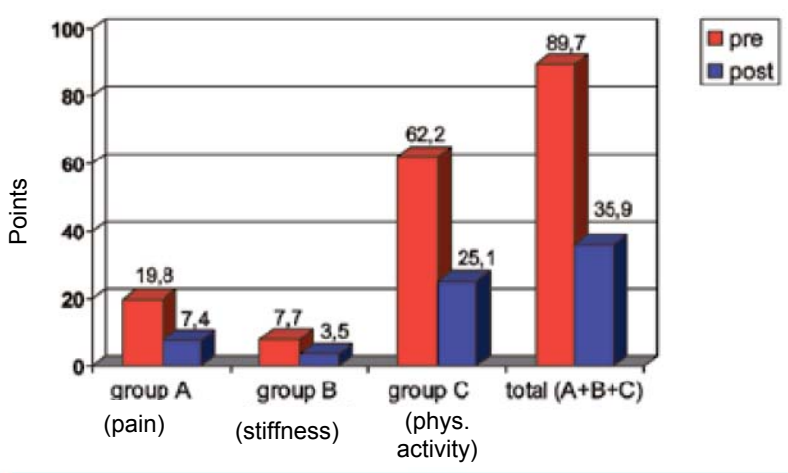


Fig. 1 Statistical results of monitoring.

Tab. IV: Statistical results of monitoring.

| | Pain | | Stiffness | | Physical activity | |
|------|--------|-------|-----------|-------|-------------------|-------|
| | Before | After | Before | After | Before | After |
| Mean | 19.8 | 7.4 | 7.7 | 3.5 | 62.2 | 25.1 |
| SD | 10.8 | 7.5 | 5.5 | 3.4 | 39.0 | 25.6 |

Literature

1. Müller-Hilke, B.: Zur Pathogenese der Arthrose – eine Momentaufnahme. [On the Pathogenesis of Arthrosis – A Snapshot] [in German] Z. Rheumatol 66 (2007) 703–705.

[all other literature entries in English]

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