Ozone Therapy and Combined PRP Applications

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Aim

This presentation intends to expose the state of the art related to the clinical use of the combination of ozone and autologous plasma rich platelet (PRP) in different procedures which belong to the field of vascular medicine, traumatology and aesthetic medicine.

PRP: Historical Perspective

The enhancement of tissue healing by the placement of supraphysiologic concentration of autologous platelets. The application of PRP has been documented in many fields. First promoted by M. Ferrari 1987 as autologous transfusion component after an open heart operation to avoid homologous blood product transfusion. It was Dr Rita Levi Moltancini, who in 1948 discovers the Nervous Growth Factor that led her, along with Dr. Stanley Cohen, to obtain the Nobel Prize of Medicine in 1986, as well as the description of the epidermal growth factor by Stanley Cohen in 1962. The initial popularity of PRP grew from its promise as safe and natural alternative to surgery. Studies suggest that platelets contain an abundance of growth factors and cytokines that can affect inflammation, post operative blood loss, infection, osteogenesis, etc.

Platelets Rich Plasma

- Activated platelets release numerous proteins, among them adhesive glycoproteins and growth factors
- It shows that this therapy has been able to promote soft tissue regeneration with a decrease in infection rates, pain and inflammation. Clinical experiences show a clear improvement in wound healing after having used ozonized rich platelet plasma along with ozone therapy.

Research

Researches now show that platelets also release many bioactive proteins responsible for attracting macrophages, mesenchyme stem cells and osteoblasts that not only promote removal of degenerated and necrotic tissue, but also enhance tissue regeneration and healing.

Introduction

Activated platelets release:
- adhesive glycoproteins
- growth factors
Following subcutaneous injection, these proteins and GF interact with cells residing in the subcutaneous tissues, eg:
- skin fibroblasts
- endothelial cells
- osteoblasts
Upon binding to their cellular receptors, glycoproteins and growth factors activate intracellular signaling events, mediating:
- angiogenesis
- cell proliferation
- migration
survival
production of extracellular matrix proteins

What is Autologous Platelet rich plasma (A-PRP)

A-PRP is a concentration of human platelets in a small volume of plasma measured as 1,000,000 platelets per nm3 or 2-6 times the native concentration of whole blood at pH of 6.5-6.7. Also referred to as
- autologous platelet gel
- plasma-rich growth factors (PRGFs) or
- autologous platelet concentrate
- PRP is also a concentration of seven fundamental protein growth factors that have been proved to be actively secreted by platelets to initiate all wound healing
- PRP includes 3 proteins in blood known to act as cell adhesion molecules: fibrin, fibronectin and vitronectin
- soft tissue repair - face skin rejuvenation
- bone tissue repair – bone graft healing

By
- Collagen formation
- Extracellular matrix synthesis
- Fibroblasts proliferation
- Angiogenesis

The objective of the combination therapy

- To boost, to enhance the effect
- To demonstrate that the combination of both techniques, accelerates the lysate of the thrombocytes, especially if it has been anticoagulated with heparina (Bocci, 2003).

How does PRGF work?

1. PLT clotting – after 10 min – need anti coagulant.
2. De-granulation – release of content of PLT α-granules, that contain GF, by budding from PLT membrane and addition of side chains – Need Viable PLT!
3. GF bind to Receptors of the cells attracted to the wound.
4. Initiation of production of collagen and cell proliferation – Chemo attractant activity – attract Mesenchymal stem cells to the wound.
5. Formation Of EXTRA CELLULAR MATRIX. = Acceleration of normal wound healing process.

How are platelets activated?

Dermal collagen and exposed endothelial collagen
- Arachidonic acid (inflammation pathway)
- Thromboxane A2 (Inflamation)
- ADP
- Thrombin
- Vasopressin
- Adrenalin
- CaCl2
- Controlled heat (radio-frequency)
- Vibration
- Medial Ozone
**PRP activation**

PRP must be activated prior to injection and it can be activated exogenously by:

- Thrombin
- CaCl2
- Mechanical trauma
- Medical Ozone

Once PRP is activated, a fibrin network begins to form and creating a fibrin dot or membrane. If PRP is activated too strongly, the fibrin network will be bivalent, unstable network. If it is activated in a more physiologic manner, a tetramolecular stable network forms that enhances enmeshment of cells and GFs. Although this can be useful for surgical procedures, it is undesirable to have the PRP overly viscous when injecting into soft tissue! Activation results in rapid GF release, with 90% prefabricated factors release minutes.d in ten. Many GFs have short half-lives so greater effectiveness may result if they are activated at or just prior to injection. Most commercial PRP kits do not activate PRP. Some replace Calcium that was bound by ACD to create a more physiological state. Employing inactivated PRP is used in soft tissue, it does not need to be exogenously activated. To avoid unintentional activation of platelets most protocols use large needles (>22) to draw the blood and re-inject PRP.

Collagen is a natural activator of PRP thus when PRP is used in soft tissue, it does not need to be exogenously activated. Once activation has occurred at the injection site, release of growth factors initiates an inflammatory response that last approximately 3 days.

Fibroblast s accumulate at the site of injection, which marks the beginning of the proliferate phase of healing that last several weeks. Remodeling occurs to the collagen matrix that was laid down by the fibroblasts. This remodeling phase that leads to the formation of mature tissue lasts about 6 months. It takes 3 phases for new tissue to form and provide long-term stability to tissue.

**Growth Factors**

Mitogenic Agents: The control and stimulate the cellular proliferation
Mitogenic Agents: They control and stimulate the cellular migration
Angiogenic Agents: They promote the creation of new blood vessels
Cito-protectors: They stimulate the cellular survival aggressions can turn out to be lethal to the cells.

Agents that induce the formation of matrix bone and the collagen synthesis.
Agents with antibacterial effects against Staphylococcus aureus and Echerichia coli.
Agents that even have anti-inflammatory properties come up as the blockade of the protein MCP-1 and generation of lipoxina A4.

<table>
<thead>
<tr>
<th>PDGF aa</th>
<th>Platelet derived GFs</th>
<th>Activated thrombocytes</th>
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<tbody>
<tr>
<td>PDGF bb</td>
<td></td>
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<td>TGF-alpha</td>
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<td>EGF</td>
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<td>Epidermal GFs</td>
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<td>VEGF</td>
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<td>Vascular endothelial GFs</td>
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<td></td>
<td>Leucocytes and endothelial cells</td>
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<tr>
<td>Growth Factor</td>
<td>Source</td>
<td>Function</td>
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<tr>
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<tr>
<td>PDGF (Platelet Derived GFs)</td>
<td>- Chemo-attractive to Mesenchymal Scs and endothelial cells</td>
<td>- Differentiation for fibroblasts</td>
</tr>
<tr>
<td></td>
<td>- Promote the synthesis of extracellular matrix</td>
<td>- Stimulate DNA synthesis</td>
</tr>
<tr>
<td>TGF (Transforming GFs)</td>
<td>- Promotes cell mitosis</td>
<td>- Significantly increases the synthesis of collagen</td>
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<td></td>
<td>- Stimulate collagen synthesis</td>
<td>- Proliferate various types of cells</td>
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<tr>
<td>VEGF (Vascular Endothelial GFs)</td>
<td>Stimulate angiogenesis</td>
<td></td>
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<tr>
<td>EGF (Epidermal GFs)</td>
<td>Regulate cell growth, proliferation and differentiation</td>
<td></td>
</tr>
<tr>
<td>IGF 1 and 2 (Insulin-like GFs)</td>
<td>Stimulation, proliferation and differentiation or different cell types</td>
<td></td>
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<tr>
<th>Growth Factor</th>
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<th>Function</th>
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<tbody>
<tr>
<td>Transforming Growth Factor-beta, TGF-β</td>
<td>Platelets, extracellular matrix</td>
<td>Stimulates undifferentiated mesenchymal cell proliferation;</td>
</tr>
<tr>
<td></td>
<td>of bone, cartilage matrix, activated TH₁ cells natural killer cells,</td>
<td>stimulates endothelial, fibroblastic and osteoblastic</td>
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<td></td>
<td>macrophages/monocytes and neutrophils</td>
<td>mitogenesis; regulates collagen synthesis and collagenase</td>
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<td></td>
<td></td>
<td>secretion; regulates mitogenic effects of other growth</td>
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<tr>
<td></td>
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<td>factors; stimulates endothelial chemotaxis and angiogenesis;</td>
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<tr>
<td></td>
<td></td>
<td>inhibits macrophage and lymphocyte proliferation</td>
</tr>
<tr>
<td>Basic Fibroblast Growth Factor, bFGF</td>
<td>Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts</td>
<td>Promotes growth and differentiation of chondrocytes and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>osteoblasts; mitogenic for mesenchymal cells;</td>
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<td></td>
<td></td>
<td>chondrocytes and osteoblasts</td>
</tr>
<tr>
<td>Platelet Derived Growth Factor, PDGFα-b</td>
<td>Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells</td>
<td>Mitogenic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/ta/ smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis</td>
</tr>
<tr>
<td>Epidermal Growth Factor, EGF</td>
<td>Platelets, macrophages, monocytes</td>
<td>Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis</td>
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<td>Vascular endothelial growth factor, VEGF</td>
<td>Platelets, endothelial cells</td>
<td>Increases angiogenesis and vessel permeability; stimulates mitigation for endothelial cells</td>
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<tr>
<td>Connective tissue growth factor, CTGF</td>
<td>Platelets through endocytosis from extracellular environment in bone marrow</td>
<td>Promotes angiogenesis, cartilage regeneration, fibrosis and platelet adhesion</td>
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</tbody>
</table>

**What is Ozone?**

Three atomic Oxygen – Trioxgen
Molecular formula is O₃
Has very light blue colour in gas form
Second the strongest Oxidant – Electron donor
Can kill all known bacterias, viruses and molds by rate 99.9%
Harmful to all living organisms over some limits
Dose concentration and effect time are parameters on damage

**Medical Ozone**

Therapeutic Medical Ozone is a combination of pure oxygen and ozone in microgram doses 0.05% O₃ – 5% O₃.
Figure 1: Growth factors acting on ‘healing cascade’

<table>
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<th>Factor</th>
<th>Name</th>
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<td>Mitogenes of mesenchymal stem cells promote the synthesis of the extracellular matrix</td>
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<td>Stimulates DNA synthesis and proliferation of various types of cells. Favours the synthesis of collagen</td>
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<td>Vascular endothelial growth factor</td>
<td>Leucocytes and endothelial cells</td>
<td>Stimulates angiogenesis and chemo-attraction of osteoblasts</td>
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<td>TGF-beta</td>
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<td>Stimulates proliferation and differentiation of epidermis cells, co-stimulating angiogenesis</td>
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In addition, the activated thrombocytes have on their surface a multitude of signalization molecules, for example: CD9, CD-W17, CD31, CD41, CD42a-d, CD51, CD-W60, CD61, CD62P, CD63.

99.95% O2 — 95% O2
1 µg/ml – 100 µg/ml O3

The Ozonized PRP apparently contributes to

Advantages of the combined therapy Ozone+PRP
Effective in a high 95-98% of cases
It is possible to apply to all range of aging groups.
It avoids the surgery in high amount of cases
It does not invalidate the surgery if it is necessary.
It is ambulatory, it avoids internments, anesthesia, post-operative, special care and recovery times.

Advantages

It is a conservative, minimally invasive treatment that respects the anatomy and physiopathology of the organism. It is correct to the cause, the imbalance of the organism and does not silence the symptom of alarm that in this case is the pain. Avoid the use of medicines along with its side effects.

Ozonized PRP in wound healing

In wound healing platelets, it plays an essential role since they are rich in platelet derived growth factor (PDGF), transforming growth factor-b (TGF-b); vascular endothelial growth factor (VEGF). Mustoe et al. demonstrated in experimental model, that only one PRP dose was increasing the volume of granulation of the tissues in 200% after 7 days. Some of the proteins liberated by the thrombocytes are absent in chronic wounds which contribute to the abnormal repairing tissue process. This is an evidence of the important role of these substances in the repair tissue process.