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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 vitronection
- 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
- Vasoporessin
- 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
- CaC12
- 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 releaseminutes.d in ten. Many GFs have short half-lives so greatest 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
Motogenic 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.

PDGF aa PDGF bb PDGF ab	Platelet derived GFs	Activated thrombocytes
TGF-alpha TGF-beta	Transforming GFs	Activated Thrombocytes
IGF-I IGF-II	Insulin-like GFs	Activated thrombocytes
EGF	Epidermal GFS	Activated thrombocytes
VEGF	Vascular endothelial GFs	Leucocytes and endothelial cells

PDGF (Platelet Derived GFs)	**- Chemo-attractive to Mesenchymal Scsand endothelial cells** **- Differentiation for fibroblasts** **- Promote the synthesis of extracellular matrix**
TGF (Transforming GFs)	- Promotes cell mitosis - Significally increases the synthesis of collagen - Stimulate DNA synthesis - Proliferate various types of cells
VEGF (Vascular Endothelial GFs)	Stimulate angiogenesis
EGF (Epidermal GFs)	Regulate cell growth, proliferation and differentiation
IGF 1 and 2 (Insulin-like GFs)	Stimulation, proliferation and differentiation or different cell types

Growth Factor	Source	Function
Transforming Growth Factor-beta, TGF-ß	Platelets, extracellular matrix of bone, cartilage matrix, activated TH_1 cells natural killer cells, macrophages/ monocytes and neutrophils	Stimulates undifferentiated mesenchymal cell proliferation; regulates endothelial, fibroblastic and osteoblastic mitogenesis; regulates collagen synthesis and collagenase secretion; regulates mitogenic effects of other growth factors; stimulates endothelial chemotaxis and angiogenesis; inhibits macrophage and lymphocyte prolifeation
Basic Fibroblast Growth Factor, bFGF	Platelets, macrophages, mesenchymal cells, chondrocytes, osteoblasts	Promotes growth and differentiation of chondrocytes and osteoblasts; mitogenetic for mesenchymal cells; chondrocytes and osteoblasts
Platelet Derived Growth Factor, PDGFa-b	Platelets, osteoblasts, endothelial cells, macrophages, monocytes, smooth muscle cells	Mitogenetic for mesenchymal cells and osteoblasts; stimulates chemotaxis and mitogenesis in fibroblast/glial/smooth muscle cells; regulates collagenase secretion and collagen synthesis; stimulates macrophage and neutrophil chemotaxis
Epidermal Growth Factor, EGF	Platelets, macrophages, monocytes	Stimulates endothelial chemotaxis/angiogenesis; regulates collagenase secretion; stimulates epithelial/mesenchymal mitogenesis
Vascular endothelial growth factor, VEGF	Platelets, endothelial cells	Increases angiogenesis and vessel permeability, stimulates mitogenesis for endothelial cells
Connective tissue growth factor, CTGF	Platelets through endocytosis from extracellular environment in bone marrow	Promotes angiogenesis, cartiage regeneration, fibrosis and platelet adhesion

What is Ozone?

Three atomic Oxygen – Trioxygen
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'

Factor	Name	Principal source	Effects
PDGF aa PDGF bb PDGF ab	Platelet derived growth factors	Activated thrombocytes Activated thrombocytes	Mitogenes of mesenchymal stern cells promote the synthesis oft he extracellular matrix
TGF-alpha TGF-beta	Transforming growth factors	Activated thrombocytes	Stimulation of DNA synthesis proliferation of various types of cells. Favours the synthesis of collagen
IGF-I IGF-II	Insulin-like growth factors	Activated thrombocytes	Stimulates proliteration and differentiation of osteoblasts
EGF	Epidermal growth factor	Activated thrombocytes	Stimulates proliferation and differentiation of epidermis cells, co-stimulating angiogenesis
VEGF	Vascular endothelial growth factor	Leucosytes and endothelial cells	Stimulates angiogenesis and chemo-attraction of osteoblasts
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 µ/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 to. 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.