A LETTER FROM

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Our experience with biological therapies in sports medicine

– Written by Eduardo Mauri, Montserrat Garcia-Balletbo and Ramon Cugat, Spain.

Biological therapies and the abundance of literature for and against their use, evoke both interest and controversy. In the 1940s Levi Montalcini discovered nerve growth factor and in the 1950s Stanley Cohen discovered epidermal growth factor. When we talk about Growth Factors (GFs) it is also important to mention the work of Prof Marshall R Uris, who in 1965 discovered a substance in the extracellular bone matrix which had the capacity to induce bone formation, subsequently called bone morphogenetic protein[1]. Another scientist who studied and isolated regenerative substances was Harry Antoniades, who in 1981 identified platelet derived growth factors I and II, obtained from platelets. In 1970, Matras used a fibrin preparation for re-implantation of rat skin and in 1994 Tayaponsak used autologous fibrin adhesive in mandibular reconstruction for the first time. Subsequently, Fung proposed the name 'tissue engineering' to describe the developing field, mixing biology and engineering[2]. In 1997, Whitman developed and used a platelet gel, an autologous alternative to fibrin gel and finally, in 1998, Marx was the first to use platelet rich plasma.

Since 1995, a multidisciplinary group lead by oral surgeon Eduardo Anitua, who founded the Biotechnology Institute, has made significant contributions to both the clinical and scientific understanding of platelet function and its therapeutic applications. Since then, many other authors have established research in this area[3-9].

REGENERATIVE BIOLOGY

The objective of tissue regeneration is to create restored tissue with properties indistinguishable from the original. The goal is not to repair but to regenerate, reconstruct the form and restore function by stimulating artificial or natural bio-substances, cell migration, proliferation and differentiation.

GROWTH FACTORS: FUNCTIONS AND TYPES

GFs are multi-functional; for example on one hand they stimulate proliferation of certain types of cells, on the other hand they inhibit the proliferation of others. GFs are found in and derived from the extracellular matrix, the platelets-megakaryocytes and plasma, among others.

Platelets have two functions: interrupting bleeding where there has been a vascular injury and stimulating cell proliferation and tissue scarring when they release GFs from their alpha-granules[8-12].

GFs include:

- platelet-derived growth factor,
- vascular endothelial growth factor,
- transforming growth factor-beta,
- acidic and basic fibroblastic growth factors,
- insulin-like growth factor I and II and
- epidermal growth factor.

The biological effects of GFs include:

- haemostasis,
- stimulation of angiogenesis,
- promotion of cell proliferation,

- acceleration of tissue remodelling,
- pain reduction and
- antimicrobial action.

GROWTH FACTOR THERAPY

The aim of GF therapy is to use platelets to mimic the physiological process when a tissue is damaged: platelets deliver GFs to the injured area[12]. Currently, various techniques are being used all over the world and as a result, it is not possible to compare between sites because each uses different techniques and applications, with different elements and/or substances being injected.

Our group has vast experience using Eduardo Anitua's Technique (Platelet Rich Growth Factor®-Endoret) a plasma enriched in platelets 2 to 2.5 times the peripheral blood platelet concentration plus 5% CaCl2. This technique does not use bovine thrombin to coagulate and does not include leukocytes in the preparation. Leukocytes reduce fibrin stability and they express matrix metalloproteinases that contribute to extra cellular matrix degradation[13]. Using this technique we can obtain a range of preparations including non-liquid-activated, liquid-activated, coagulant/clot, fibrin membrane and supernatant. PRGF®-Endoret can be applied to injuries of all types of connective tissue such as cartilage, bone, ligament, tendon, muscle and skin[14].

Cartilage: the aim of treating chondral injuries with PRGF®-Endoret is to re-fill defects with new chondral tissue. There are numerous research studies that support the treatment[15-18] and some publications about clinical applications[19-22].

Bone: in-vitro studies have demonstrated that platelet-derived growth factor stimulates the proliferation of human trabecular bone cells and osteoblast-like cells. Initial in-vivo studies were reported in the fields of oral-maxillofacial surgery and dentistry, focusing on the effects of isolated platelet rich plasma.

Sanchez et al published results obtained in non-union fractures treated with PRGF®[23].

Ligament: many studies have been published in recent years on the study and clinical use of the bone morphogenetic protein and GFs. The goal is to identify:

1. the optimum combination of these proteins,
2. the most effective therapeutic dosages and
3. the right ways to release them[24-35].

Animal studies have found that transforming growth factor-beta 1 and epidermal growth factor, as well as GFs obtained from autologous platelet concentrate can be used in anterior cruciate ligament (ACL) graft remodeling to:

- increase collagen synthesis,
- increase fibroblast synthesis,
- improve scarring speed,
- increase tension strength resistance and
- increase maturing speed[36-39].

The use of PRGF®- Endoret in ACL surgery has two goals: to prevent anterior knee pain and to achieve a quicker fixation and maturity of the graft. Sánchez et al reported that compared to a control group, 50 patients who underwent ACL repair surgery and were treated with PRGF®-Endoret had:

- minimised haematomas,
- reduced postoperative signs of inflammation,
- reduced pain,
- a reduced recovery period,
- accelerated integration of the graft and
- reduced the probability of laxity post-surgery.

Radice et al also reported the that the application of PRGF®-Endoret in ACL repair surgery in a group of 25 patients significantly reduced the biological maturing time of the graft (by at least 49%), compared with a control group[40,41].

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An animal study by Kuroda et al found that GF release in reconstructed ACL ligaments peaks between the 3rd and 6th week and almost completely ceases at 12 weeks[42].

Yoshikawa et al showed up-regulated expression of vascular endothelial growth factor is a potent stimulator of angiogenesis at 2 to 3 weeks post-reconstruction. Recent studies found that exogenous application of vascular endothelial growth factor enhanced cell infiltration and fibroblast expression during the proliferation phase of healing, but this also induced significant deterioration of the mechanical properties of the graft. These findings support the reports of numerous other studies that all found the mechanical properties to be at a minimum around the proliferation phase of healing at 6 to 8 weeks[43].

In the early phase, Kawamura et al and Kuroda et al reported that graft necrosis leads to a release source of interleukins that trigger a cascade of GF expression, resulting in cell migration and proliferation as well as extra cell matrix synthesis and revascularisation[44].

Scheffler, Unterhauser & Weiler share the same results with the studies of Kuroda Kawamura and Yoshikawa[45].

Tendon: the goal is to obtain new healthy tendon tissue[46]. Sanchez et al reported good results in Achilles tendon repair injuries[47].

Muscle: GFs shorten injury recovery time. However one must control the creation of fibrosis or, in extreme cases, calcification.

Skin: GFs facilitate healing with a regeneration-repair process.

THE FUTURE

The literature includes evidence for both positive, neutral, and negative results from the clinical utilisation of GFs. For the last 8 years the number of randomised controlled clinical trials has progressively increased. In 2006 there was just one trial considered as level 1 evidence, there are now 47 randomised controlled clinical trials with level 1 evidence. However, among these works there is a variety of collection methods, applied substances (different cellular and chemical characteristics) volumes, number of doses and clinical follow-up (some late, some early). These 'small details' directly influence a positive or negative result and must be considered when critically reviewing the evidence.

Furthermore, both ongoing basic studies and clinical trials are required, paying special attention to the 'small details'. A Consensus is required. Our group's experience began in 2002 and ranges from laboratory research, clinical practice both with animals and humans. The results are satisfactory if the methodology of obtaining the substance is respected and when the diagnosis indicates the treatment.

References

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