Biologic Treatment in Tendon and Muscle Injuries

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42.1 Introduction

In the last decades, we have observed a great increase in the number of people practicing sports around the world. Much of this increase is due to widespread media coverage of the health benefits of regular physical exercise, such as improved quality of life and reduced risk of various diseases [1].

However, these beneficial effects must be balanced with injuries that are to some extent unavoidable [2]. The estimated number of sports-related injuries annually in the UK is 10 million [3]. In Sweden the incidence of sports injuries is approximately 22.5 per 1000 inhabitants per year [4].

These numbers show not only the enormous medical but also the social and economic importance of the problem. Most of these injuries are not serious but are usually painful and lead to a temporary withdrawal from work and sports activities [5].

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42.2 Muscle Injury

In this context, muscle injuries are among the most common, accounting for approximately 10–55% of all sports injuries. Muscle injuries can be caused by contusion, stretching, lacerations, and other mechanisms [6]. These injuries correspond to approximately 40% of all soccer injuries and 25% of all injury-time withdrawals. And 15–20% of these muscular injuries are recurrences of previous injuries [7].

Woods et al. have described that 12% of injuries in professional soccer players affect hamstring muscles, and the financial burden of these injuries is estimated at 74.7 million pounds sterling [8].

Despite the enormous advances in medicine in recent decades, the treatment of muscle injuries has changed little and still consists of the use of the PRICE protocol (protection, rest, ice, compression, and elevation of the affected limb), medications, and physiotherapy for analgesia, muscular stretching, and strengthening [9]. In the case of mild muscle injuries, these treatment modalities are satisfactory. However, moderate and severe muscle injuries usually lead to prolonged absence and tend to form large areas of fibrotic tissue at the site of the injury that can lead to loss of strength and function as well as increase chances of a new injury [10].

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Another key factor is that none of these treatments addresses the major problems of muscle injury that are cell loss and scar tissue formation. In addition, routine treatments do not improve the number, proliferation, and differentiation of satellite cells [11]. Therefore, the efforts to develop new treatments that promote a faster and more complete recovery after muscle injuries with improved function and lower incidence of reinjuries are fundamental.

In recent years, some biological treatments have been studied with promising initial results: platelet-rich plasma (PRP), mesenchymal cells, and losartan and gene therapy.

42.2.1 Platelet-Rich Plasma

Platelet-rich plasma (PRP) can be defined as a blood derivative with higher platelet concentration than blood [12]. PRP is prepared from an initial volume of blood of the patient that is processed and centrifuged to separate the various blood components [13]. This type of treatment has been investigated due to promising initial results, low costs, and minimally invasive form of application. The concept of the use of this technique in the treatment of muscular lesions is that after the injection of PRP in the injured muscle, the local development of the platelet-rich fibrin structure provides hemostasis and allows the slow delivery of growth factors and cytokines from platelets and plasma, with anti-inflammatory and regenerative effects. PRP was effective in stimulating the proliferation and migration of mesenchymal cells in response to the release of some specific growth factors by platelets. Another possible action of PRP is the stimulation of proliferation of fibroblasts in the muscle [14, 15]. However, excessive deposition of type 1 collagen by fibroblasts can lead to the formation of large areas of fibrosis. The control of this process is done by TGF-β1, which may be present in great concentration in the platelet α granules, and we know that the formation of large areas of fibrosis in the muscle can lead to lesion recurrence as well as a decrease in functional capacity.

There are many protocols for preparation of platelet-rich plasma (open and closed systems, number of different centrifugation processes), so the products obtained differ in terms of cellular and molecular compositions. Distinct clinical findings are attributed both to variability in PRP formulations and to variability in application protocols. The perception that PRP was not a unique and similar product led some authors to classify the PRPs. The initial classification is still the easiest and most intuitive and divides PRPS into pure PRP (pPRP) and PRP with leukocytes (L-PRP) [16]. However, there are other widely used classifications such as PAW which is based on absolute number of platelets (P), platelet activation (A), and the presence or absence of leukocytes (W) [17].

Currently PRP is used in the definitive or coadjuvant treatment of many musculoskeletal and tendinous disorders. However, the results and outcomes in the treatment of muscle injuries are still controversial. Numerous doubts exist regarding the best preparation, ideal concentration of platelets and specific growth factors, and a better time to start treatment with PRP after a muscle injury.

Delos et al., in a study with rats, performed PRP applications acutely (2 h) and late (1 and 3 days) after gastrocnemius injury. The authors did not find functional and histological differences independent of the moment of application (early or late) [18]. A randomized clinical trial, however, showed improvement in pain and less time to return to sports after a single application of PRP (3 mL) after acute grade 2 hamstring injury (<7 days). In this study, patients treated with PRP associated with rehabilitation returned to sports on average 26.7 days after injury, whereas the control group treated only with physical therapy took, on average, 42.5 days [19].

On the other hand, a similar study did not find differences in time for complete recovery of patients with gastrocnemius or rectus femoris injury after PRP application (4–8 mL) with drainage of the hematoma when compared to the control group (isolated drainage). The authors also found no differences in pain improvement [20].

Some systematic reviews on the subject have concluded that despite the promising concept, animal and clinical studies with good results, effective treatment of muscle injuries with PRP has not yet been confirmed by recent randomized clinical trials. Therefore, there is still insufficient support in the literature for any benefit in terms of pain, function, return to sports, and recurrence of injuries using PRP applications in the treatment of muscle injuries [21, 22]. One way forward would be the customization and individualization of PRP formulations according to the patient and type of lesion. In this way, we would guarantee the beneficial effects of PRP in certain types of tissues avoiding their potential deleterious effects.

42.2.2 Mesenchymal cells

Muscle injuries and their reinjuries are a great challenge for sports medicine, as they cause great problems in sports, economic, and social areas. Despite the great capacity of healing and regeneration of muscles, a fully injured muscle regains only part of its function and around 50% of its strength [23]. The main treatments used today do not address the main problem of muscle injuries that is the cellular loss. Mesenchymal cell transplantation meets this requirement and has been tested in the treatment of muscle damage.

Mesenchymal cells are found in large numbers in adipose tissue and bone marrow. The Mesenchymal Stem Cell Committee of the International Society for Cell Therapy proposed three criteria for defining mesenchymal cells: (1) they should be adherent when maintained in standard culture; (2) must express CD105, CD73, and CD90 and exhibit poor expression of CD34, CD45, CD14 or CD11b, CD79a or CD19, and HLA-DR in culture; and (3) must have the potential to differentiate into osteoblasts, adipocytes, and chondrocytes in vitro [24].

Mesenchymal cells have multiple effects on the body that include anti-inflammatory and immunomodulatory action. Previous studies have reported that mesenchymal cells contain several vascular and multipotent cells. These cells would be responsible for the secretion of cytokines and growth factors, such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) [25, 26]. Andrade et al. tested in mice the use of mesenchymal cells in the treatment of muscle injuries and reinjuries. After 14 and 28 days of application, the authors observed a faster recovery and improved muscle function. However, they didn't observe any improvement in the scar formation tissue (fibrosis) in the injured area [11].

The application of mesenchymal cells derived from adipose tissue accelerates muscle repair and improves the function of the injured muscle with the promotion of angiogenesis and myogenesis and the prevention of fibrosis formation through the secretion of growth factors. The authors also believe that this mechanism of action is more important in faster muscle regeneration than the proliferation and differentiation of mesenchymal cells injected into the tissue. Recent studies have shown that direct cell differentiation is not always essential, as it is unlikely that these cells differentiate over such a short time in the setting of an acute muscle injury.

Despite the great prospect and hope in the success of mesenchymal cells in the treatment of muscular injuries, more studies are required, with larger samples and longer follow-up in order to have more safety and confidence in this treatment modality [26].

42.2.3 Losartan

Losartan is classically an antihypertensive drug that acts by blocking the angiotensin II receptor and is used in the treatment of patients with systemic arterial hypertension, congestive heart failure, and sequelae of these diseases [27, 28]. The use of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in patients with renal, hepatic, and pulmonary diseases caused a decrease in fibrotic tissue formation and an improvement in the function of these organs [29, 30].

The development of fibrosis after muscle injuries is a major concern of physicians involved in

the treatment of athletes due to the increased risk of reinjury and functional loss caused by scar formation in the injured muscle. The promising results in previous research incentivated new lines of research.

Bedair et al. [31] demonstrated a reduction in fibrosis area and increase in the number of fibers in the gastrocnemius muscle of rats after acute muscle injury and administration of losartan. The authors concluded that the use of losartan is safe and can aid not only in the treatment of sports-related injuries but also in muscular dystrophies, trauma, and postoperative injuries.

However, the optimal timing for administration of the drug is still controversial. Kobayashi et al. [32] investigated the dose and the best time to initiate treatment with losartan after acute muscle injury. The authors concluded that the standard dose of 10 mg/kg/day, used for hypertensive patients, started 3 or 7 days after the injury, led to a significant increase in muscle regeneration, a decrease in local fibrosis, and improvement of function.

Other studies have sought to evaluate the association of losartan with other substances such as platelet-rich plasma and mesenchymal cells. The concept is to take advantage of the strengths of PRP therapy and to use losartan to inhibit TGFβ and consequently the formation of fibrosis. Combination therapy of PRP and losartan improved muscle healing, increasing angiogenesis and follistatin expression and reducing Smad2/3 expression and fibrosis development. These results suggest that blocking TGFβ expression with losartan improves the effect of PRP therapy on muscle healing [33].

A similar study was conducted in rats comparing the isolated use of mesenchymal cells and combined with losartan in the treatment of muscle injuries. The simultaneous treatment of muscle contusions with mesenchymal cells and losartan significantly reduced fibrotic scar formation, increased fiber numbers, and improved muscle functional recovery. These effects would have been caused, at least in part, by the regulation of Smad7 and MyoD with the inhibition of TGFβ [34].

However, despite promising results, quality studies in humans are still needed to assess the safety and efficacy of this medication in the musculoskeletal system. However, these studies are fundamental for the development of biological treatments that aim to accelerate and improve muscle healing after injury.

42.2.4 Gene Therapy

Another promising treatment modality for muscle injuries is gene therapy. The principle of treatment is based on the transfer of genes to provide genetic products at the site where the tissue damage occurred [35].

The transfer of the genetic material is performed by a vector that transports the genes of interest to the host cells. Viruses are widely used as vectors because of their inherent ability to efficiently translocate their own genetic material. In order to create a vector for gene therapy, viral genome sequences that contribute to virulence and disease are usually removed and replaced with genes of interest. However, this method still has some safety and cost-benefit concerns [36].

Some alternatives have been tested in an attempt to improve and accelerate the process of muscle healing after injury. Schertzer et al. [37] carried out the transfer of IGF-1 gene using adenovirus as vector to improve angiogenesis and muscle regeneration. The authors concluded that gene transfer was superior to systemic administration of IGF-1 but that both methods were effective in the treatment of muscle injuries. Other authors promoted gene transfer of decorin in order to decrease the expression of TGFβ and formation of fibrotic tissue [38].

Currently, there is extensive literature supporting the concept of the use of gene therapy in the repair and regeneration of lesions of the musculoskeletal system; however the first clinical trials in humans are still ongoing [39]. Therefore, we must await the results of the studies already in progress and develop new quality studies in humans in order to confirm the safety, economic viability, and efficiency of this treatment modality.

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42.3 Tendon Injuries

Acute and chronic tendon injuries affect millions of people in both occupational and athletic settings each year. With the increasing popularity of sports activities, the frequency of sports-related lesions, such as tendon injuries, is rapidly increasing [40]. Tendon problems are one of the main causes of musculoskeletal morbidity, and it has been reported that 30–50% of all sports lesions are painful tendon injuries [41]. Tendon lesions may be caused by traumatic events or chronic degenerative changes, making tendon weaker and more prone to ruptures.

Healing of acute injuries results in the formation of scar tissue in tendons, which have inferior mechanical strength that makes them susceptible to reinjury. On the other hand, the current treatment of chronic tendon injury (or tendinopathy) is largely palliative because of the incomplete understanding of the tendon disorder [41]. The tendon healing process is slow, and multiple phases are well-defined, from inflammatory cytokine recruitment to growth factors and reparative cell involvement [42].

In recent years, new treatment option involving biologic has been used in orthopedic surgery and sports medicine to treat tendon injuries [43]. Nowadays, the aim of the treatment is the clinical application of biologically derived materials to stimulate the repair or regeneration of musculoskeletal tissues. The orthobiologic approaches include the administration of hyaluronic acid or biological factors, such as platelet-rich plasma (PRP) or growth factors. With the advent of those, several options exist for increasing the strength and ability of the repair, as well as decreasing the length of the recovery period [42].

Regenerative medicine approaches may be indicated in the case of tissue healing significantly impaired due to aging, disease, or the presence of an extended lesion [44].

42.3.1 Platelet-Rich Plasma (PRP)

PRPs can be used in the management of tendinopathy if we improve our understanding of pathophysiology and to integrate molecular knowledge about PRP participation in healing mechanisms [42].

Development of PRP treatments is challenging because a typical group of patients with tendinopathy does not exist, as it affects multiple segments of the population. Moreover, the pathophysiology and origin of pain are not elucidated yet. Although some degree of success has been achieved, PRP is not considered standard medical treatment, and it is largely not paid nor reimbursed by insurance companies. However, the arguments for using PRP in tendinopathy are increasing, and its potential to rebalance inflammation merits further research. Moreover, PRP contains tendoinductive factors that can drive the fate of stem cells. Tailoring PRPs to the specific needs of the host tendon has not been possible to date, because unanswered questions remain about the characteristics of tendinopathy within the different stages of progression [45].

There is still no consensus as to whether PRP confers a beneficial effect, as not all trials have failed to demonstrate a positive benefit. Six systematic reviews published between 2010 and 2014 assess the effectiveness of PRP in tendinopathy [46–51]. Despite analyzing the same data, they reported contrasting conclusions, from concluding that PRP is efficacious to finding that

there is "strong evidence against platelet-rich plasma" [46–49]. The majority of comments stated that there is great difficulty reaching a conclusion because of the variance of the type of PRP produced. In a Cochrane review of PRP in soft tissue injuries, Moraes et al. [51] indicated that "there is need for standardization of PRP preparation methods." However most of the authors state that "it would be better to break out the results by specific study design and PRP type" [49].

One critical component that affects PRP preparations is the presence or absence of white blood cells (WBCs) or leukocytes (neutrophils, monocytes, macrophages, and lymphocytes), which can be beneficial because they stimulate the immune response against infections; promote chemotaxis, proliferation, and differentiation of cells; and induce extracellular matrix production and angiogenesis. Owing to these properties, PRP-containing leukocytes (L-PRP) are often used to treat traumatic injuries [40].

Thus, a meta-analysis was performed and published in 2016 to assess the comparative effectiveness of PRP types in tendinopathy [42]. A total of 18 studies (1066 participants) were included, and all treatments consisted of intratendinous injections with a prior administration of 1–2 mL of local anesthetic (7 studies with autologous blood injection, 10 studies with leukocyte-rich PRP produced from the buffy coat layer, and 1 with leukocyte-poor PRP) [42]. The meta-analysis showed that the outcome of PRP is different depending on the method of preparation of PRP and the injection technique; for that reason, both informations should always be included to evaluate the study results. Nevertheless, this meta-analysis found strong evidence that leukocyte-rich PRP can improve outcome in tendinopathy [42].

A study conducted with tenocytes isolated from patellar tendons of rabbits indicates that the use of L-PRP to treat injured tendons may lead to scar formation in healing tendons [40]. Moreover, L-PRP induces extensive catabolic responses in differentiated tenocytes, which may delay the repair of acutely damaged tendon matrix and new matrix formation, thus slowing the healing of injured tendons. Last, because L-PRP induces inflammatory responses in tenocytes, its use to treat the already-inflamed tendinopathic tendons may only exacerbate the tendon disorder by prolonging the inflammatory phase, thus impairing the healing process and leading to increased pain in patients. Caution should therefore be exercised when using PRP.

Based on the data from this study, the authors suggest the use of pure PRP to augment the repair of tendinopathic tendons because of its anabolic properties and low inflammatory effects [40]. On the other hand, it is plausible that the strong anabolic effects of pure PRP may cause fibrosis/scar tissue formation in acutely injured tendons simply because tenocytes differentiated from stem cells after pure PRP treatment produce too much collagen in the wound areas. Therefore, they suggest that whether to use L-PRP or pure PRP depends on the type of tendon injury (acute vs. chronic) and treatment phase (early- or late-stage healing) in clinical settings [40].

42.3.2 Mesenchymal Stem Cells (MSC)

A growing field of research has explored tendon, bone, and cartilage regeneration using mesenchymal stem cells (MSCs), because of their multipotency and because they are relatively easy to harvest. Great expectations arose from the use of MSCs in regenerative medicine in the last decade, although both the potential and the drawbacks of this method remain under reflection [52].

Stem cells are cells with the capacity to differentiate into multiple types of tissues and able to self-renew. They are able to establish daughter-cell lines for tissue generation [53]. They have three main characteristics: multipotency, capacity to adhere to plastic, and the presence of stem cell-specific antigens on their surface with the absence of *negative markers* that are used to identify other cell lineages, such as hematopoietic endothelial cells (e.g., CD 14, 31, 34, and 35) [54, 55]. A common source of MSCs is the bone marrow, especially from the iliac crest. The cells harvested from the bone marrow are called

BMDSCs. Another common source of MSCs is the adipose tissue. In that case, they are commonly called ADSCs. They have an advantage: they are more readily accessible than BMDSCs. The poor regenerative capacity of tendons has greatly encouraged the research in finding new ways to aid in their repair after a tear. The good results found in animal models are encouraging, but there is lack of clinical studies supporting the use of stem cells in clinical practice. So, a recommendation for the routine use of stem cells cannot be made as yet [55].

Further research is needed to determine whether MSCs are an effective treatment option in augmentation of tendon healing. Also, the long-term safety of these cells and the best scaffold for their seeding and growth have to be demonstrated with larger animal model studies and randomized clinical trials with a longer follow-up period [56].

Nowadays there are only a few orthopedic studies that investigate the use of MSCs in the clinical practice. Some studies showed good results in terms of outcome scores, ultrasound appearance, pain, and mechanical performances in the treatment of lateral epicondylitis of the elbow [57, 58]. A study was performed even regarding patellar tendinopathy on a population on 60 patients that were treated alternative with skin-derived tenocyte-like cells (N = 33) or plasma (N = 27) [59]. There was an improvement in clinical scores in the group treated with the stem cells (VISA score) with a concomitant significant reduction of the thickness of the tendon. Ultrasonography demonstrated improvements in tendon hypoechogenicity and tear size in both groups.

The progress achieved with the rapid development of biomaterial-based strategies for tendon regeneration has not yielded broad benefits to clinical patients. In addition to the interplay between stem cells and biomaterials, the innate immune response to biomaterials also plays a determinant role in tissue regeneration. One of the principles for biomaterial development in tendon regeneration is to stimulate tenogenic differentiation of stem cells. However, recent progress indicated that innate immune cells, especially macrophages, can also respond to the material cues and undergo phenotypical changes, which will either facilitate or hinder tissue regeneration. This process has been, to some extent, neglected by traditional strategies and may partially explain the unsatisfactory outcomes of previous studies; thus, more researchers have turned their focus on developing immunoregenerative biomaterials to enhance tendon regeneration [60].

42.3.3 Growth Factors

The use of growth factors for healing of musculoskeletal injuries is relatively recent. Recombinant growth factors were first considered and proposed, but the high costs gradually reduced their use, in favor of autologous blood products. Several growth factors are expressed as tendons heal, but it remains unknown whether their combined application enhances the healing process. In an animal study, the authors concluded that the implantation of a GF-loaded collagen sponge at the time of surgery could provide a promising treatment, especially in high-performance athletes and revision cases prone to re-rupture. For conservative treatment, tiered percutaneous GF application could be an option for improving clinical outcome [61].

42.3.4 Prolotherapy

Prolotherapy, also called proliferation therapy, is an injection-based treatment used in chronic musculoskeletal conditions. It has been characterized as an alternative medicine practice [62]. It consists by rehabilitation of an incompetent structure, such as ligament or tendon, by the induced proliferation of new cells. Prolotherapy is differentiated from other regenerative injection therapies, such as platelet-rich plasma (PRP) and stem cell injection by the absence of a biologic agent. The most commonly used prolotherapy solution reported in current literature is hypertonic dextrose, a simple

monosaccharide synonymous with glucose [62]. The mechanism of action hypothesized of dextrose prolotherapy is that injection of dextrose causes a small amount of cell irritation or necrosis that results from osmotic shock [63]. This method of intentional small-scale cell trauma at the injection site initiates the body's wound healing cascade of inflammation, granulation tissue formation, and matrix formation and remodeling, promoting local healing of chronically injured tissues [63].

The use of prolotherapy injections for chronic, painful tendon and fascia overuse conditions has been predominantly guided by anecdotal clinical success [64]. Randomized controlled trial studies have demonstrated Level I–III evidence for injection of 10–25% dextrose in areas of damaged tendon to manage Achilles tendinosis, plantar fasciitis rotator cuff tendinopathy, and lateral epicondylitis [65–69].

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Take-Home Message

Muscle and tendon injuries are very common, and to better approach those injuries, more medical research are warranted to advance in the treatment and to enhance tissue healing. New therapies are emerging with promising results, but further investigations are required to better define indications techniques, modalities, safety, and cost-effectiveness.

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