Basic Principles of Muscle Healing

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Contents

Abstract

Skeletal muscle has the ability to regenerate following injury, and this response implicates a specific type of resident muscle stem cell, the satellite cell. Three main phases have been identified in the process of muscle regeneration, including (I) a destruction phase with the initial inflammatory response, (II) a repair phase with the activation of satellite cells, and (III) a remodeling phase with the maturation of the regenerated myofibers. Nevertheless, in severe muscle injuries, we also observed the formation of fibrosis that impairs muscle function. Various

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strategies, including the use of growth factors, transplantation of muscle stem cells, or antifibrotic therapies, may become therapeutic alternatives to improve functional recovery after severe muscle injuries.

2.1 Introduction

Human skeletal muscle is about 40 % of the body mass and is formed by bundle of contractile muscle fibers. Muscle fibers are multinucleated cells resulting from the fusion of myoblast, the muscle progenitor cells. Myofibers are surrounded by the sarcolemma, the plasma membrane of muscle fibers. Located between the plasma membrane and the basal lamina, we find satellite cells, i.e., the reserve adult muscle stem cells, which play a key role in the muscle regeneration process [19, 26]. After muscle injury, satellite cells are activated and form myoblasts, then fuse into myotubes, and mature into new myofibers that participate in the muscle regeneration process.

2.2 Muscle Injury

Muscle injuries can stem from a variety of events, including direct trauma (i.e., muscle lacerations, contusions, or strains) and indirect causes (i.e., ischemia or neurological dysfunction) [10, 15, 18, 23, 31]. Muscle injury is one of the most common injuries in professional and recreational sports. In fact, muscle injuries constitute between 10 and 55 % of all injuries sustained by athletes, depending on the type of sport [33]. Whereas relatively minor muscle injuries, such as strains, can heal completely without intervention, severe muscle injuries typically result in the formation of dense scar tissue that impairs muscle function and can lead to muscle contracture and chronic pain. Injured muscle undergoes a sequential cycle of healing phases. Three phases have been identified in this process (Fig. 2.1):

I. Destruction phase, including muscle degeneration/inflammation: Characterized by the rupture and then necrosis of the myofibers, formation of a hematoma, and an important inflammatory reaction.
II. Repair phase: In this phase, we observed phagocytosis of the damaged tissue, followed by regeneration of the myofibers, leading to activation of the satellite cells.
III. Remodeling phase: A period during which we observed maturation of the regenerated myofibers with recovery of the functional capacity of the muscle (III b) but also a period where we can observed fibrosis deposition (III a).

2.2.1 Muscle Degeneration and Inflammation

Active muscle degeneration and inflammation occur within the first few days after injury. In injured muscle, mechanical trauma destroys the integrity of the myofibers. The injured ends of the myofibers undergo rapid necrosis. Similar to cell necrosis, the inflammation starts with invasion of mononuclear cells, activated macrophages,

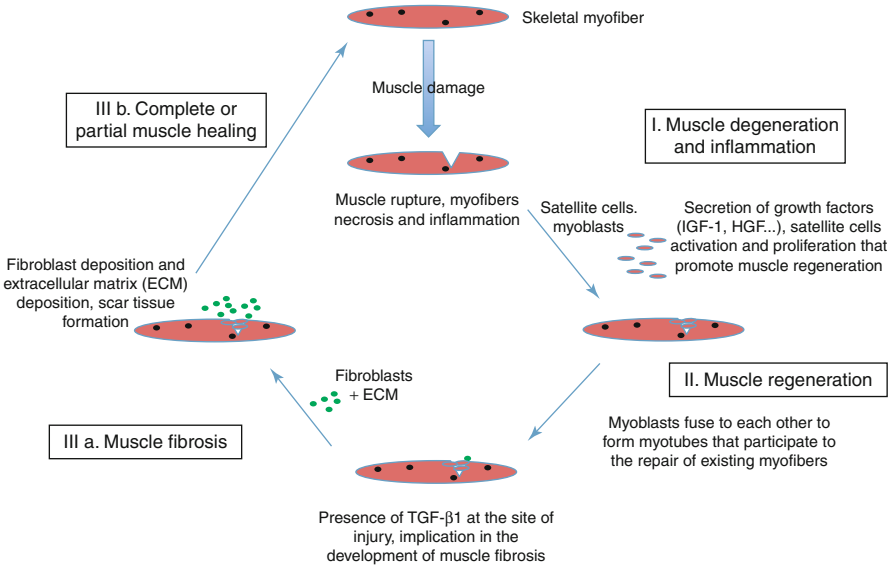

Fig. 2.1 Sequential cycle of muscle healing phases

and lymphocytes at the injury site [45]. Necrotic debris of the damaged myofibers are phagocytized by macrophages, which simultaneously secrete growth factors that enhance muscle regeneration by favoring satellite cells activation and proliferation [7].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are often prescribed to relieve pain after muscle injury. However, the effect of this group of drugs on the muscle healing process remains largely controversial. Human studies are lacking, but some studies have been performed in animal models. It appears that short-term use of different NSAIDs had no major adverse effect on muscle healing [28].

2.2.2 Muscle Regeneration

Muscle regeneration usually starts during the first week after injury, peaks at 2 weeks, and then gradually diminishes 3–4 weeks after injury. Regeneration is linked to the activation of the satellite cells. Satellite cells proliferate, form myoblasts, and fuse with each other to form new multinucleated myotubes that will participate in the muscle regeneration process.

2.2.3 Muscle Fibrosis

Despite the fact that the majority of skeletal muscle lesions heal without formation of an extensive scar tissue, we often observe formation of a dense scar tissue that can prevent the skeletal muscle regeneration process in severe muscle injuries or muscle

re-ruptures. Fibrosis usually starts between the second and third week after muscle injury. The amount of scar tissue increases in size over time due to excessive fibroblast proliferation and an increase in production of type I collagen [22].

2.3 Improving Muscle Healing

2.3.1 Growth Factors

Many reports have shown that growth factors play a variety of roles during muscle regeneration [16, 30]. Although hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) are of interest because of their capacity to stimulate satellite cells [1, 39, 47], insulin-like growth factor-1 (IGF-1) appears to be of particular importance for the muscle regeneration process notably because IGF-1 stimulates myoblasts proliferation and differentiation [13]. IGF-1 is implicated in the regulation of muscle growth [38]. In a mouse model, direct injections of human recombinant IGF-1 at 2, 5, and 7 days after injury have enhanced muscle healing in lacerated, contused, and strain-injured muscle [20, 30]. However, the efficacy of direct injection of recombinant proteins (growth factors) is limited by the high concentration of the factor typically required to elicit a measurable effect. This is mainly due to the bloodstream's rapid clearance of these molecules and their relatively short biological half-lives. Gene therapy may prove to be an effective method by which to deliver high, maintainable concentrations of growth factor to injured muscle [2, 3, 32]. Although we observed improved muscle healing, histology of the injected muscle revealed muscle fibrosis within the lacerated site, despite the production of a high level of IGF-1 [21]. Some studies suggest that the stimulatory action of IGF-1 on myofibroblast proliferation and the deposition of extracellular matrix (ECM-scar tissue) might interfere with the ability of this growth factor to improve muscle healing after injury, even at high concentrations [11].

2.3.2 Stem Cell Therapy

Transplantation of myogenic precursor cells represents a promising therapeutic strategy for treatment of extensive skeletal muscle destruction. Myogenic precursor cells can participate directly in the muscle regeneration process but also create a reservoir of secreting molecules that may impact the different stages of muscle healing. Despite encouraging results obtained in animal models [36], the subsequent clinical trials of myoblast transfer in human patients have been disappointing due to rapid death, limited spread of the injected cells, and rejection of transplanted myoblasts [17, 29, 40]. Although the use of myoblasts for cell therapy applications is prevalent, concerns associated with myoblast proliferation, cell migration, and the limited life span of these cells have brought the usage of stem cells to the forefront of such applications. Stem cells are defined as cells that can both self-renew and

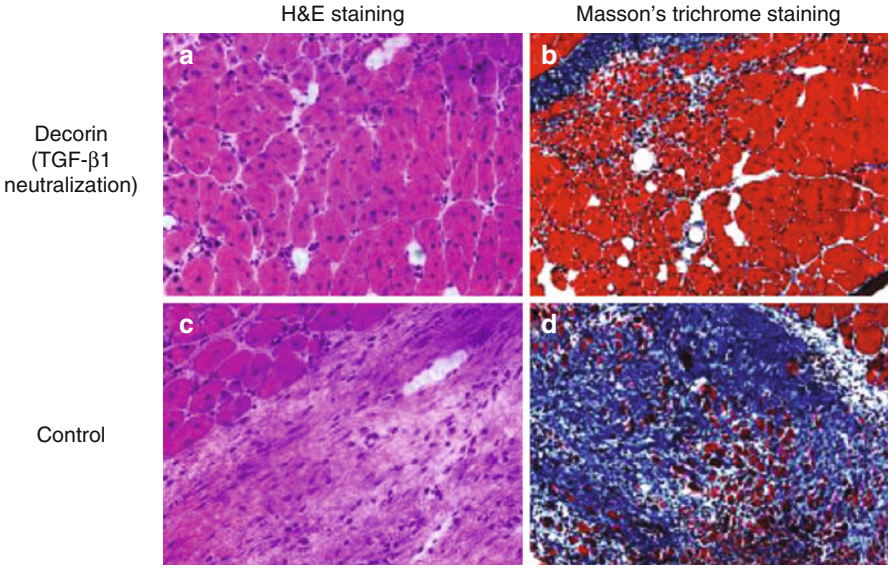

Fig. 2.2 Four weeks after injury, decorin-treated muscle (**a**, **b**) exhibits a greater number of regenerating myofibers (significantly higher numbers of centronucleated myofibers) and contained significantly less fibrosis (less collagen deposition, area in *blue*) than the control muscle (**c**, **d**) (Adapted from Li et al. [25])

give rise to clonal progeny with the ability to differentiate [46]. Isolation of muscle stem cells that can overcome these limitations would enhance the success of muscle cell transplantation significantly.

A population of murine muscle-derived stem cells (MDSC) displayed a high transplantation capacity in both skeletal and cardiac muscles [34, 37]. The MDSCs' ability to proliferate in vivo for an extended period of time combined with their capacity for long-term proliferation, strong capacity for self-renewal, resistance to stress, ability to undergo multilineage differentiation, and ability to induce neovascularization at least partially explains the high regenerative capacity of these cells in various musculoskeletal tissues including skeletal muscle [12, 34, 37]. Recently, it has been demonstrated that blood vessel progenitors (including myo-endothelial cells and pericytes) share a number of features with MDSC [9, 42]. In particular, they share cell-type marker profiles and have high myogenic potentials in vitro and in vivo. The use of such myogenic progenitors cells for improving muscle healing may become an interesting therapeutic alternative [8, 43, 44].

2.3.3 Antifibrotic Therapy

Some reports indicate that scar tissue formation precludes complete regeneration of muscle tissue. Although various studies have implicated TGF-β1 in the onset of fibrosis [24, 41], very few reports have examined the role of this cytokine in skeletal

muscle fibrosis. It has been demonstrated that TGF-β1 is expressed at high levels and is associated with fibrosis in the injured skeletal muscle [6, 24]. These results support the hypothesis that TGF-β1 expression in skeletal muscle plays an important role in the fibrotic cascade that occurs after the onset of muscle injury. Therefore, neutralization of TGF-β1 expression in injured muscle could inhibit the formation of scar tissue. Indeed, the use of antifibrotic agents (i.e., decorin, relaxin, antibody against TGF-β1) that inactivate TGF-β1 appears to reduce muscle fibrosis and, consequently, improves muscle healing, leading to a near-complete recovery of the lacerated muscle [14, 25] (Fig. 2.2). Losartan, an angiotensin II receptor antagonist, has recently been demonstrated to neutralize the effect of TGF-β1 and reduce fibrosis, making it the treatment of choice, since it already has FDA approval to be used clinically [4, 35].

2.4 Clinical Implementation After Muscle Injury: From the Bench to the Sport Field

Muscle injuries constitute one of the most frequent sports lesions. Prevention of muscle strain includes proper conditioning and warm-up and good management of fatigue. However, most muscle strains occur in sports competition requiring velocity and force. Muscle injuries are currently identified as mild, moderate, and severe injuries based on muscle impairment (from few muscle fibers contusion to the entire muscle with complete loss of muscle function). In clinical practice, treatment regimens have been designed based upon empiricism and experience.

The objective in the treatment of a muscle strain is to create the best mechanical and biological environment to allow rapid and complete healing and thereby prevent a re-tear.

Treatment must start within minutes after the injury, following the algorithm known as PRICE (Protection, Rest, Ice, Compression, Elevation) to prevent further damage and limit hematoma formation. Protection is a crucial step for the first 2–3 days (crutches or even immobilization) to prevent excessive scar formation and re-rupture at the injury site. In the coming years, the use of IGF-1 injection may improve and accelerate the healing process. Recently, a new treatment approach came from basic science research. From days 3 to 5, the athlete is advised to perform a light exercise for 20′ per day (Fig. 2.3). Berg and Bang [5] have demonstrated a 27 % increase of IGF-1 after 10′ moderate exercise (10–28 μg/l), favoring thus the environment of the initial healing. Moreover, such exercise may increase satellite cell numbers and, thus, appear as an efficient strategy to improve muscle function and repair after injury [27].

After this protective phase, which can extend up to 5 days in severe injuries, controlled isometric, isotonic, and isokinetic contractions of the injured muscle group are performed with increasing intensity. At the same time, one should begin general reconditioning of the athlete, either by activation of the upper extremity in the presence of a lesion of the lower extremity or by activation of the contralateral limb. Reconditioning of the injured muscle group is mandatory. Gentle, progressive,

Fig. 2.3 From days 3 to 5 post-injury, the athlete performs 15–20 min of light exercises (50 % VO_2 max) using the uninjured limbs to enhance circulating IGF-1

and pain-free sports-specific reprogramming is rapidly begun. The criteria for time to return to sports include: (a) the ability to stretch the injured muscle as much as the contralateral healthy muscle, (b) pain-free use of the injured muscle in sports-specific movements, (c) comparable strength between injured and healthy muscles, and (d) the recovery of the proprioceptive and coordination capacity in the injured segment as well as the reprogramming of the sports movement. There is an obvious lack of evidence in determining these criteria, and these guidelines are mostly empirical.

In patients with a true muscle rupture, surgical reinsertion and repair should be considered, particularly with lesions in the proximal hamstrings or distal pectoralis major. The surgical management of these injuries permits a reduction in the length and degree of functional disability. The means to reduce the length of disability in athletes with muscle strains are the following: (a) Take them off the sports field; do not even permit them to play; (b) apply the proper treatment immediately and protect the injured muscle; (c) start controlled motion and general reconditioning; (d) recondition the injured muscle and rapidly begin sports-specific reprogramming; (e) surgically reinsert and repair a muscle rupture (especially hamstrings proximally); and (f) consider the use of hyperthermia which appears to be a promising technique to reduce the length of disability.

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