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Review

Nitric oxide and repair of skeletal muscle injury ☆☆☆

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ABSTRACT

The muscle wound healing occurs in three overlapping phases: (1) degeneration and inflammation, (2) muscle regeneration, and (3) fibrosis. Simultaneously to injury cellular infiltration by neutrophils and macrophages occur, as well as cellular 'respiratory burst' via activation of the enzyme NADPH oxidase. When skeletal muscle is stretched or injured, myogenic satellite cells are activated to enter the cell cycle, divide, differentiate and fuse with muscle fibers to repair damaged regions and to enhance hypertrophy of muscle fibers. This process depends on nitric oxide (NO) production, metalloproteinase (MMP) activation and release of hepatocyte growth factor (HGF) from the extracellular matrix. Generation of a fibrotic scar tissue, with partial loss of function, can also occur, and seems to be dependent, at least in part, on local TGF- β expression, which can be downregulated by NO. Hence, regeneration the muscle depends on the type and severity of the injury, the appropriate inflammatory response and on the balance of the processes of remodeling and fibrosis. It appears that in all these phases NO exerts a significant role. Better comprehension of this role, as well as of the participation of other important mediators, may lead to development of new treatment strategies trying to tip the balance in favor of greater regeneration over fibrosis, resulting in better functional recovery.

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Normal structure of muscle

Traumatic muscle injuries, including crush, contusion, laceration or freezing is relatively frequent. However, when they do occur, can have dramatic and prolonged effects on muscle functional capacity [1]. Often, the injured muscle heals slowly and improperly, leading to an incomplete functional recovery, a tendency for recurrent injuries and/or scar tissue formation [2]. Hence, the use of therapeutic interventions aiming at enhancement of muscle regeneration and prevention of muscle fibrosis would be very useful in order to preserve function. However, the development of these strategies will require a better understanding of how these processes occur and what are the major factors involved.

Skeletal muscle is essentially composed of two main components: the *myofibers* and their innervating nerves, which are responsible for the contractile function of the muscle, and the *connective tissue*, which provides the framework to transform contraction force

into movement. Myofibers and connective tissue have an intimate relation, which is essential for effective, coordinated function. The individual myofibers are bound together by a connective tissue structure composed of three levels of sheaths called the endomysium, perimysium, and epimysium [3]. Moreover, each myofiber is attached at both ends to the connective tissue of tendon or tendon-like fascia at the so-called myotendinous junctions (MTJs) [4].

To facilitate the study, healing of muscle injury can be divided into three distinct, but overlapping phases. The *destruction phase* is characterized by the rupture and ensuing necrosis of the myofibers, the formation of a hematoma, and the inflammatory cell reaction. The *repair phase*, consisting of the phagocytosis of the necrotized tissue, the regeneration of the myofibers, production of a connective tissue scar; and the *remodeling phase*, a period throughout which the maturation of the regenerated myofibers, the contraction and reorganization of the scar tissue and the recovery of the functional capacity of the muscle occur. Remodeling phase is constituted of two processes, remodeling and fibrosis, the balance of which defines the degree of function recovery and scar tissue amount (Fig. 1) [2,5]. Therefore, understanding the mechanisms and factors involved in this balance could have a significant impact on therapeutic strategies for muscle injuries.

The nitric oxide (NO) is a very small molecule, freely diffusible through cell membranes, which regulates an ample variety of cellular functions [6,7]. It is produced by several cells, such as

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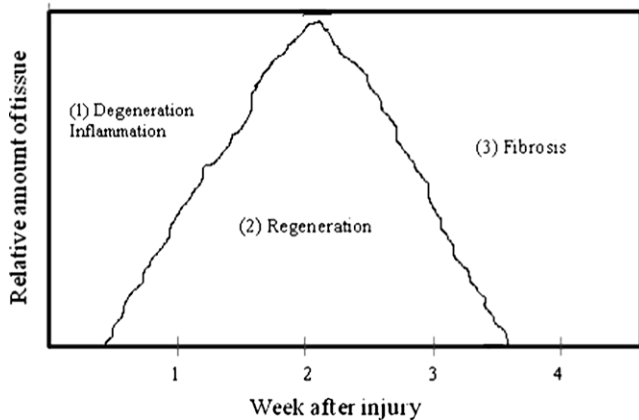


Fig. 1. The different stages of muscle healing after muscle injury. The first event is muscle degeneration and inflammation, which occurs within the first minutes and continues for up to 1–2 weeks after injury. Muscle regeneration begins in the first week post-injury and peaks at about 14 days post-injury. Fibrosis usually occurs at 2 weeks post-injury and increases over time for up to 4 weeks post-injury.

skeletal and cardiac muscle cells, epithelial cells, endothelial cells, macrophages, fibroblasts and hepatocytes [8], via a reaction catalyzed by the nitric oxide synthase (NOS) [9,10]. Three NOS isoforms have been characterized, each encoded by different chromosomes. Two enzyme isoforms are constitutively expressed (endothelial -eNOS- and neuronal -nNOS), whereas one isoform is an inducible enzyme (iNOS), initially found in macrophages [9].

NO can act as a signal molecule that activates guanylate cyclase and as a cytostatic/cytotoxic molecule that inhibits mitochondrial iron containing cytochromes and aconitase, as well as inhibiting ribonucleotide reductase. NO may also regulate gene expression by activating or inhibiting transcription factor binding, by reacting with the thiol binding site of the transcription factor NF- κ B [11,12].

NO has been demonstrated to be a modulator of skeletal muscle function, and a likely mediator of injury and disease [13]. NOS activity in rat skeletal muscles has been correlated with muscle fiber density [14], force development [15], regulation of blood vessel diameter and blood flow [16], modulation of the vascular tone, as well as muscle contractile properties. However, there are very few published studies investigating the role of NO in post-injury muscle inflammation and repair.

We will subsequently summarize the biological and pathological processes that occur in skeletal muscle post-injury and the possible roles of NO in these processes. Finally, we will discuss current trends in therapy research, trying to enhance regeneration and the inhibit fibrosis in injured skeletal muscle.

Destruction phase of muscle injury

When muscle is injured, overused, or mechanically stretched, tissue necrosis occurs, followed by cellular infiltration through several stages, generally characterized by early neutrophils invasion and sequential increase of macrophages [17]. Macrophages can lyse target muscle cells by a NO-dependent, superoxide independent mechanism and their cytolytic capacity is increased by the presence of neutrophils. [18] Additionally, the presence of muscle cells increases NO production by macrophages, suggesting that there may be a positive-feedback mechanism promoting lysis, in which initial muscle damage promotes increased NO-mediated toxicity by macrophages [19]. Therefore, the macrophages play a decisive role in the removal of necrotic tissue, and together with the fibroblasts, produce complementary chemotactic signals (cytokines, growth factors, and chemokines) for attraction of circulating inflammatory cells [5,17,19]. Pro-inflammatory cytokines, such as interleukin-1beta (IL-1 β),

IL-6, IL-8, tumor necrosis factor-alpha (TNF- α), are important to modulate chemotaxis to injured muscle [17].

These cytokines can also stimulate pathways that contribute to activation of the enzyme NADPH oxidase, which generates a 'respiratory burst' and the subsequent release of reactive oxygen species (ROS) [19–22]. ROS are produced during normal aerobic cell metabolism, have important physiological roles in maintaining cell redox status and are required for normal cellular functions, including cell proliferation, aggregation, chemotaxis and apoptosis, as well as regulation of intracellular signaling pathways and the activity of transcription factors, such as nuclear factor(NF)- κ B, activator protein 1 (AP-1) and hypoxia-inducible factor-1alpha (HIF-1 α) [23–25].

It seems likely that ROS generated by either PMN or macrophages during muscle injury cause oxidative modification of muscle proteins, such as creatine kinase (CK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Nevertheless, the extent of these potentially significant biomolecular transformations of specific proteins is largely unknown [26,27].

On the other hand, ROS may also have a positive role in the wound healing. They can enhance the affinity of the growth factors (FGF-2) to its receptor and also induce its expression. Hydrogen peroxide (H₂O₂), a specific ROS, induces collagen type I, III and IV synthesis and their subsequent cross linking. ROS also mediate conversion of fibroblasts to myofibroblasts, thus aiding in wound contraction [28,29].

Different cells have the capability to produce NO in the skeletal muscle. In human muscle nNOS is constitutively expressed in both type I and II fibers [14,30]. nNOS is located in specialized structures at the surface membrane, by binding to α 1-syntrophin, a dystrophin-associated protein [30]. The absence of dystrophin, due to a mutation in the gene for the protein, causes a secondary loss of nNOS from the sarcolemma, leading to muscle inflammation, muscle membrane lysis, muscle wasting, and death. This occurs in patients with Duchenne muscular dystrophy and an animal model of this disease (mdx mice), and demonstrates an important protective effect of NO.

The isoform eNOS is expressed in endothelial cells [8]. Although immunoblot analysis shows that rat extensor digitorum longus and soleus muscles express eNOS, its low level of expression has made it more difficult to localize and study [30]. Finally, iNOS activity in skeletal muscle depends on the disease state and species investigated. iNOS mRNA is absent or present at very low levels in normal skeletal muscles of rats [31] and human [32], however its levels are markedly increased in inflammatory conditions, such as autoimmune inflammatory myopathies [33]. The cytokines IL-1, TNF- α and interferon-gamma have been shown to stimulate NO production in skeletal muscle cells via induction of the macrophage-type iNOS gene [34].

During the inflammatory phase there is a great increase in local NO production. Several studies confirmed this data. Rubinstein et al. [31] in an experimental model of muscle crush injury observed the expression of all three NOS isoforms both in normal and crushed untreated limbs. However, induction of iNOS was observed only in the crushed limb, which was maximal at 6 h after the injury. Darmani et al. [35] investigated iNOS activity in crush-injured digital flexor tendon and observed a greatly increased expression of iNOS and TGF- β at 3 days subsequent to injury, which gradually returned to normal. Zhang et al. [36] investigated the change of local NO level in a rat experimental model of muscle crushing. In this study, the expressions of eNOS and iNOS were upregulated, and NOS activity and NO level in local muscles and serum were significantly increased in crush group compared with the sham group. The likely source of NO in this process is primarily iNOS expressed by macrophages and/or muscle fibers [13].

Using an experimental model of muscle crush injury, we have also observed an increase of the iNOS mRNA at 6 h after trauma. However, when the animals were exposed to L-NAME, they showed a great increase in the iNOS mRNA, probably attempting to compensate the inhibited NO synthesis. This demonstrates that NO increased levels post-injury can be strongly regulated at the transcriptional level. We also observed increased transcription of the pro-inflammatory cytokines, imbalance of redox status and increased local MPO expression.

Muscle-derived NO appears to be a particularly important regulator of muscle inflammation and muscle damage by invading inflammatory cells [18]. *In vitro* and *in vivo* studies have shown that muscle-derived NO reduces of damage by inflammatory cells by increasing their apoptosis and inhibiting the expression of adhesion molecules, such as intracellular adhesion molecules (ICAM), E-selectin and P-selectin. Muscle-derived NO can reduce neutrophil-mediated lysis of muscle cells and decrease superoxide concentration, forming less reactive intermediates, such as ONOO⁻ [30].

In skin, it is well known that the NO is implicated as a regulator of all phases of wound healing [37]. The beneficial effects of NO on wound repair may be attributed to its functional influences on angiogenesis, inflammation, cell proliferation, matrix deposition and remodeling [38]. Experimental studies demonstrated that inhibitors of NOS have been reported to delay wound healing [39,40] and the administration of NO donors has beneficial effects on wound repair, on both inflammatory and proliferative phases, improves wound contraction and, principally, accelerate reepithelialization [37,41]. Whether similar effects of NO occurs during muscle injury and repair processes is not known.

An essential process in the regeneration of an injured muscle is the vascularization. Angiogenesis is a prerequisite for the subsequent morphological and functional healing of the injured muscle. It leads to rebuilding of the damaged vessels, restoration of the blood flow and restoration of the oxygen supply to the tissue. NO plays a key role because it can act as a vasodilator and can promote activation of several growth factors involved, such as vascular endothelial growth factor and fibroblast growth factor [42].

Rubinstein et al. [31], in an experimental model, observed that muscle crush injury caused a threefold increase in the capillary mean cross-sectional area and a three- to fourfold increase in the femoral blood flow and capillary blood flow. Administration of N ω -nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor,

largely decreased the hyperperfusion that follows the muscle injury. Therefore, this data clearly suggest that the vasodilatation in the injured skeletal muscle is promoted by local NO production.

Repair and remodeling phase of muscle injury

Compared with inflammatory phase, the repair process is somewhat delayed, beginning about 7 days after injury.

The key cell involved in regeneration of skeletal muscle is the satellite cell (SC). These cells are mononuclear progenitor cells found in mature muscle between the basal lamina and sarcolemma. In mature skeletal muscles, they are normally quiescent, but can be activated in response muscle injury [43,44] (see Fig. 2). On activation, they enter the cell cycle, divide, differentiate and fuse with muscle fibers to repair damaged regions and to enhance hypertrophy of muscle fibers [45].

Satellite cell activation is limited to areas where there is necrosis of myofibers and can continue for 9–10 days, depending on the severity of the injury [2,46]. Apparently mechanical changes in muscle can lead to SC activation, although the mechanisms involved have not been clarified. There are several postulated mechanisms trying to explain the activation of muscle satellite cells after trauma. Some researchers have postulated that disruption of the integrity of the sarcolemma and basal lamina activates satellite cells [47]. Others sustain that cytokines released by infiltrating inflammatory cells result in satellite cell activation [2] Most likely there are a complex combination of these and other events, and NO seems to have a critical role.

During a crushing injury of the muscle fibers, macrophages invade the area of injury, phagocytose the necrotic tissue and produce several growth factors that are mitogenic for muscle precursor cells, such as FGF, insulin-like growth factor (IGF), TGF- β , hepatocyte growth factor (HGF), and IL-6 [5,29,43,48] (see Table 1). Interestingly, IL-6 seems to be involved in protein degradation and muscle degeneration [49], but can also induce proliferation of satellite cells and muscle regeneration [50], raising the possibility that this cytokine has a role in a transition phase from acute inflammation to repair [51].

Hepatocyte growth factor/scatter factor (HGF) has been recently well studied as an activator of satellite cells [52]. HGF is an α - β heterodimer produced by proteolytic cleavage of a single-chain inactive precursor of 728 amino acids. It is localized in the extracel-

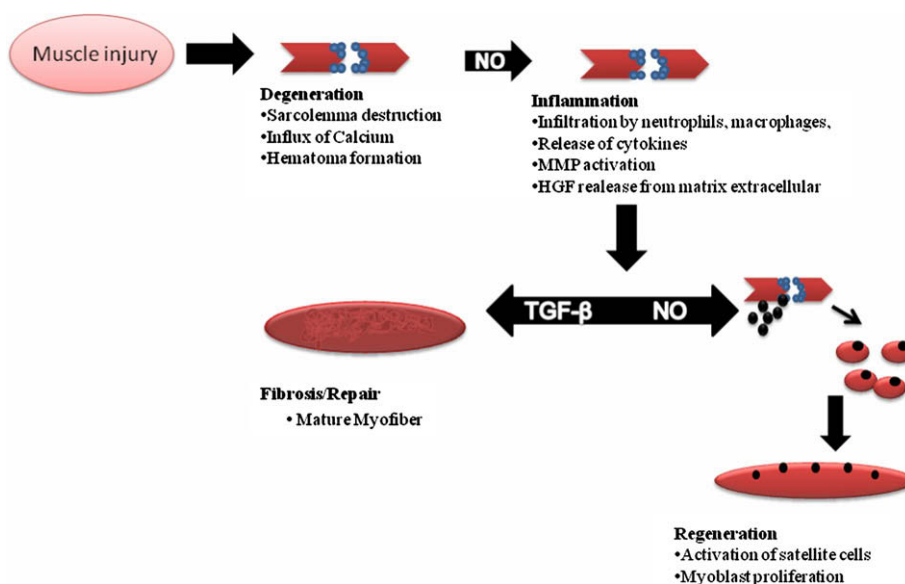


Fig. 2. Regeneration phase of muscle.

Table 1
Effect of growth factors on myoblasts *in vitro* and *in vivo*.

Growth factor	Cell proliferation	Cell differentiation	Ref.
Hepatocyte growth factor (HGF)	↑	↑	[82]
Fibroblast growth factor 2 (FGF-2)	↑	↓	[83]
Insulin-like growth factor-1 (IGF-1)	↑	↑	[84]
Interleukin-6 (IL-6)	↑	↑	[85]
Epidermal growth factor (EGF)	↓	↓	[5]
Transforming growth factor-beta (TGF-β1)	↓	↓	[86]

lular domain of uninjured skeletal muscle fibers [53–55]. Allen et al. [53], Tatsumi et al. [52], Sheehan et al. [56] have demonstrated that HGF can be released from muscle matrix upon injury, and has the ability to activate early division of adult satellite cells in culture and in muscle tissue. These authors have also demonstrated that HGF mRNA is expressed in adult SC and can act in autocrine fashion. Thus, regulation of HGF function in muscle can occur both in a transcriptional level and by its high-affinity binding to heparan sulfate proteoglycans of the tissue matrix [57].

HGF role in muscle regeneration is essential during the early phase of the repair process as demonstrated by the decrease in HGF immunostaining with time after injury and the inability of exogenous HGF injection to affect muscle regeneration when performed at later stages of muscle regeneration [58].

Tatsumi et al. [59], based in an *in vitro* experiments, proposed that mechanical stretch stimuli triggers an intracellular cascade of events in the muscle fibers, which is pH-dependent and involves NOS activation, eventually leading to HGF release from the extracellular compartment and subsequent SC activation. These authors studied whether the mechanism of release involves a proteolytic activation of pro-HGF, and observed that the active form of HGF is present in the extracellular compartment of uninjured skeletal muscle. Therefore, the mechanism of HGF release in response to stretch does not require proteolytic activation of pro-HGF [60].

The role of NO in the release of HGF from the extracellular matrix was investigated by administering L-NAME, an inhibitor of NOS function, or D-NAME, an inactive stereoisomer, before stretch treatment. *In vivo* activation of SC in stretched muscle was inhibited by L-NAME, but not by D-NAME, indicating the stretching of muscle fibers induces liberation of HGF in a NO-dependent manner [61].

The NO-dependent HGF-matrix release could be mediated by matrix metalloproteinases (MMP), a large family of zinc-dependent endopeptidases that are capable of degrading one or several extracellular matrix proteins, such as collagens, elastin, fibronectin, laminin, and proteoglycans [62]. Numerous MMPs, including MMP-2, -3, -7, and -9, are found in skeletal muscle [45,63], possibly playing fundamental roles in muscle fiber growth and repair by regulating the integrity and composition of extracellular matrix in skeletal muscle [63].

Yamada et al. [64] demonstrated that MMPs are involved in the NO-dependent release of HGF. When mechanically stretched rat SC were treated with NO donors in the presence of recombinant tissue inhibitor-1 of MMPs (TIMP-1), the activation response was inhibited, providing strong evidence that MMPs mediate HGF release from the matrix, and this process can be regulated by the presence of TIMPs.

Yamada et al. [45] demonstrated that MMP-2 mediates stretch-induced activation of skeletal muscle SC in a nitric oxide-dependent manner. In these experiments, the SC muscle isolated from 9-month-old male Sprague–Dawley rats of gastronomies were treated with sodium nitroprusside, a NO donor; mechanical cyclic stretch or L-NAME. MMP-2 was identified in both stretch-simulated and NO donor-treated SC culture, but not in L-NAME-treated, and HGF expression was detected in MMP-2, stretch and NO donor-treated

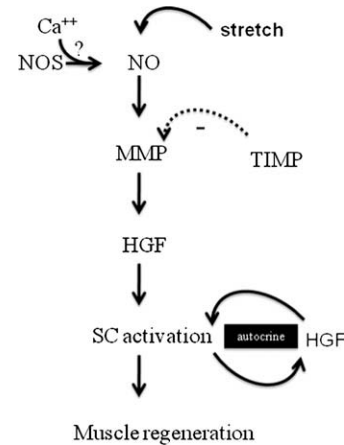


Fig. 3. Activation skeletal muscle SC NO-dependent. See the text.

cultures. Thus, results from this study provide evidence that NO-activated MMP2 may cause release of HGF from the extracellular matrix of SC and contribute to their activation *in vitro*.

More recently, Tatsumi et al. [65] demonstrated that calcium-calmodulin is also involved in the SC activation cascade *in vitro*. Cultures of SC that were treated with a calcium ionophore for 2 h induced production of HGF and activation of these cells, similarly to the effect of mechanical stretch or NO donor treatments. The response was abolished by addition of calmodulin inhibitors. Therefore, results from these experiments provide an additional insight that calcium-calmodulin mediates HGF release from the matrix and that this step in the activation pathway is upstream from NO synthesis.

Based in this series of experiments, a NO-dependent pathway of SC activation has been proposed as summarized in Fig. 3. Since HGF plays a central role in SC activation, it is likely that direct administration of HGF into damaged muscle may represent a potentially useful approach for stimulating muscle repair. However, although this is the best studied, it does not exclude other different pathways where NO could or could not be involved.

Fibrosis of muscle injury

The fibrotic scar tissue formation may lead to inadequate healing and a deficient muscle function. The muscle fibrosis is an overproliferation of components of the extracellular matrix beginning approximately 2 weeks after injury and accelerating thereafter for as long as 4 weeks.

Skeletal muscle healing following trauma can be understood as a balance between fibrosis and regeneration. Factors influencing this balance consist of inflammation, the growth factors and cytokines present in site of injury, and the interaction between infiltrating inflammatory cells and native myogenic cells [66].

During this process the predominant cell is the fibroblast and proteins, such as collagen (predominantly types I and III), glycoproteins, and proteoglycans. During tissue repair, fibroblasts continue to secrete their own pro-inflammatory cytokines, such as IL-6 and IL-1, as well as to recruit additional neutrophils through the production of IL-8. Ultimately, fibroblasts can perpetuate the chronic inflammatory response through their release of PGE2 in response to cell stress and strain. Although SC proliferation has been shown to be enhanced in the presence of macrophages, the release of excessive quantities of TGF-β1 inhibits SC differentiation, compromising fiber regeneration [19].

Some growth factors, such as EGF, myostatin, and TGF-β1, are released from inflammatory cells in initial phase and have been shown

to stimulate proliferation of the ECM and inhibit skeletal muscle regeneration both *in vitro* and *in vivo* as early as 72 h after injury [66].

TGF- β is believed to be responsible for the scar formation during the wound/skeletal muscle repair [5]. Two mechanisms through which TGF- β 1 promotes fibrosis have been postulated. TGF- β 1 stimulates the production of ECM proteins and simultaneously blocks their degradation and also promotes myogenic cells differentiation into myofibroblasts that produce collagen type I [67–69].

In both skeletal muscle disease and injury, TGF- β appears to be a major determinant for connective tissue proliferation and fibrosis. In muscle diseases characterized by fibrosis, such as muscular dystrophy and inflammatory myopathy, TGF- β has been localized to the extracellular matrix between myofibers and areas of inflammatory cell infiltration. [70].

Smith et al. [70] determined whether TGF- β protein is present and active 48 h following skeletal muscle strain injury in rats. In this study, the TGF- β expression and synthesis were evaluated by immunohistochemistry, RT-PCR and immunoblot analysis. TGF- β 1 was detected in areas of myofiber injury and, by RT-PCR analysis, there was increased expression of TGF- β 1 and TGF- β 2 precursors. Although there was no correlation between the extent of cellular injury and TGF- β transcript and protein amounts, elevated levels of TGF- β 1 and TGF- β 2 precursor proteins were present in strain-injured skeletal muscles 48 h after injury.

NO and TGF- β may have antagonist effects in muscle injury repair. Darmani et al. [35] in their study examined the expression of iNOS and TGF- β in macrophage infiltrates within crush-injured digital flexor tendon and synovium of control and L-NAME-treatment rats. The results showed that during normal tendon healing the levels of TGF- β are high at first and gradually decrease after 3 weeks of injury to slightly above control uninjured levels. However, inhibition of NOS by L-NAME-treatment at the time of injury leads to a chronic overexpression of TGF- β *in vivo* at 5 weeks after the injury, with no evidence of reduction.

We have also observed an increased TGF- β expression in an experimental model of muscle crush injury caused by a single impact blunt. Administration of L-NAME 2 h after injury leads to an overexpression of TGF- β at 7th day after injury, implicating an inhibitory effect of NO. This expression was large in fibroblasts and cells of skeletal muscle (unpublished data).

It is well established that chronic elevation of TGF- β levels in the healing wound will lead to increased deposition of collagen by stimulated fibroblasts and this chronic accumulation of collagen in the healing tendon and synovium is critical in the progressive loss of function in tendon healing [35].

Thus, based on these initial studies it can be hypothesized that the regulation of the regeneration/repair and fibrosis is dependent of the balance between NO and TGF- β local levels, with NO stimulating regeneration by induction of SC activation, and TGF- β inducing collagen deposition and fibrosis.

Therapeutic strategies

Studies designed for therapeutic strategies are extremely limited, especially considering the clinical importance of effective strategies to improve regeneration/repair of muscle after injury. Therapeutic interventions to improve outcomes following muscle injuries revolve around three concepts: regulating inflammation, enhancing regeneration and blocking excessive fibrosis.

The knowledge about the role of NO in the inflammatory process has significant potential to provide an understanding of the mechanisms of action of current therapies and, especially, for developing new therapeutic strategies to handle the repair process.

As previously mentioned, the arginine is substrate of NOS resulting in the production of citrulline. Arginine is a precursor

for three pathways, the products of which are involved in tissue injury and repair: (1) nitric oxide; (2) polyamines, which are required for DNA synthesis and cell growth; and (3) proline, which serve as substrate for collagen synthesis [71,72].

Additionally, the L-arginine might be metabolized via arginase, which is present in high concentrations in healing wounds due to macrophage production [73]. Through the action of arginase, ornithine is formed which is a precursor for proline and polyamine generation [74]. Therefore, arginine supplementation could have an impact in muscle regenerative processes.

Barbul et al. [75] and Williams et al. [76] established that dietary L-arginine intake can improve collagen deposition and wound strength in both animals and humans. Nevertheless, the finding that L-arginine intake does not improve collagen deposition in iNOS-deficient mice to the same extent as in wild-type littermates implicates that part of L-arginine effect involves NO directly [77].

Bettors et al. [78] investigated supplementation of the L-arginine or diethylenetriamine (DETA) to muscle cell cultures from mice in different stages of life. Single intact myofibers were isolated from the gastrocnemius muscles of young (2 mo), adult (10 mo), and aged (22 mo) mice. They reported that L-arginine bioavailability and NO production can enhance SC activity in old myofibers, and the decline in SC activity in early senescence can be partially extended.

Buchman et al. [79] investigated the effect of arginine or glycine supplementation on gastrointestinal function, muscle injury, serum amino acid concentrations and performance during a marathon run in a randomized controlled trial. The extremity pain scores and fluid intake was similar between both groups; CPK increased significantly and similarly in both groups immediately post-race, and even more dramatically after 48 h. The authors concluded that skeletal muscle injury was unaffected by arginine or glycine supplementation.

Zhang et al. [80] investigated the effect of L-arginine supplementation on protein metabolism in skin wound and muscle in anesthetized rabbits. L-[ring-(13)C(6)]phenylalanine was infused as a tracer on day 7 after ear injury, and the scalded ear and uninjured hindlimb were used as arteriovenous units to reflect protein kinetics in these two tissues. One group received amino acid mixture (10% Travasol) with supplemental L-arginine, and the other, L-NAME. The arginine supplementation increased net protein balance in skin wound and muscle, indicating an anabolic effect. In group 2, L-NAME infusion markedly reduced the blood flow rate in the scalded ear and increased net protein balance in skin wound and in muscle. Thus, arginine supplementation increased net protein balance in skin wound and muscle by a mechanism which was independent of nitric oxide production.

Besides arginine, few studies have proposed the use of exogenous MMP to improve repair. Bedair et al. [81] have showed that the introduction of exogenous MMP-1 into the zone of injury following skeletal muscle laceration can decrease the amount of residual fibrosis and, in turn, result in more regenerating myofibers in the area of injury. These results suggest that the direct injection of MMP-1 into the zone of injury during fibrosis can enhance muscle regeneration by increasing the number of myofibers and decreasing the amount of fibrous tissue.

Wang et al. [82] propose that MMP-1 could enhance muscle regeneration by improving myoblast migration and differentiation, which is a critical step in the sequence of muscle regeneration. By biomolecular analyses of *in vitro* wound healing assays (C2C12 cells culture), they demonstrated that MMP-1 enhances myoblast migration but is not chemoattractive. *In vivo*, myoblast transplantation was greatly improved after MMP-1 treatment within the dystrophic skeletal muscles of MDX mice. MMP-1 may therefore be able to improve muscle function recovery after injury or disease by increasing both the number of myofibers that are generated by

activated myoblasts and the size of myoblast coverage area by promoting migration.

Pharmacological blockade of pro-inflammatory cytokines, such as TNF- α , has been proposed for the treatment of dystrophic skeletal muscles [83]. Antibody depletion of host neutrophils resulted in a delayed and significantly reduced amount of skeletal muscle breakdown in young dystrophic mdx mice. A more striking and prolonged protective effect was seen after pharmacological blockade of TNF- α bioactivity using etanercept, suggesting that this important class of therapeutic agents that have been used for chronic arthropathies could also be useful for the management of muscle damage. However, a preliminary study of 12-week treatment with anti-TNF agents did not show any improvement in the fat-free mass in rheumatoid arthritis patients [84].

Physical therapies, besides immobilization, are commonly used for the treatment of muscle injuries, but their mechanism of action and effects at the molecular level have not been well studied. We have investigated the effect of low-level laser therapy (LLLT) of gallium arsenide (Ga-As) in our experimental model of gastrocnemius muscle crush injury. LLLT application 24 h post-trauma by 7 or 14 days markedly inhibited the oxidative stress, followed by inhibition of iNOS expression, NF- κ B activation and collagen deposition [85]. None of these effects could be seen when LLLT was applied to uninjured muscle.

An additional therapeutic strategy used for the treatment of muscle injuries is the ultrasound (US), although the scientific evidence on its effectiveness is somewhat unclear. Rantanen et al. [86] investigated the regeneration of contusion injury to the rat gastrocnemius muscle during treatment with pulsed ultrasound. The speed of myoregeneration in ultrasound-treated animals was compared with that in control animals by immunohistochemical, morphometric, and scintigraphic analyses. This study concluded that pulsed ultrasound-treatment does not seem to have significant effects on the overall morphological manifestations of muscle regeneration.

Early mobilization was first recommended for the acute treatment of muscle trauma [87]. This therapeutic strategy has been shown that induces more rapid and intensive capillary in growth into the injured area, better regeneration of muscle fibers, and more parallel orientation of the regenerating myofibers in comparison to immobilization, the earlier preferred treatment for injured muscle [5]. However, the nitric oxide role in this strategy not is clear.

Conclusion

In summary, muscle injury healing occurs in overlapping phases and this process is influenced by the type and severity of the injury and involves numerous growth factors and signaling molecules. The repair process depends on a delicate balance between muscle regeneration and fibrosis, which is certainly determined by the effectors of the early inflammatory response. As discussed, NO is intimately involved in all these phases, mediating many aspects of the inflammatory response and SC activation and probably modulating TGF- β mediated fibrosis. However, a better assessment of the importance of the NO role in muscle repair requires further studies.

The best treatment for muscle injuries has not clearly been defined yet. Further research is necessary to improve our attempt to understand the muscle healing process, in order to develop the necessary methodology to promote efficient muscle healing and also to achieve complete functional recovery, and perhaps to contribute for the development of innovative muscle diseases therapies.

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