

HIGHLIGHTED TOPIC | *Role of Inflammation in Skeletal Muscle, Connective Tissue, and Exertional Injuries: To Block or Not to Block?*

Anti-inflammatory interventions and skeletal muscle injury: benefit or detriment?

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Urso ML. Anti-inflammatory interventions and skeletal muscle injury: benefit or detriment? *J Appl Physiol* 115: 920–928, 2013. First published March 28, 2013; doi:10.1152/jappphysiol.00036.2013.—Exercise, eccentric contractions, acute trauma, and disease are all causal mechanisms of skeletal muscle injury. After skeletal muscle is injured, it undergoes sequential phases of degeneration, inflammation, regeneration, and fibrosis. Events that occur in response to inflammation trigger regenerative processes. However, since inflammation causes pain, decreases skeletal muscle function, has a negative effect on performance, and contributes to fibrosis, which is one of the leading causes of delayed regeneration, the general practice has been to reduce inflammation. The problem with this approach is that preventing inflammation may hinder recovery. Current treatment options for inflammation are not necessarily effective and, in some cases, they may be unsafe. This review focuses on the question of whether the most beneficial course of treatment should be to block inflammation or if it is sensible to allow inflammatory processes to progress naturally. If blocking inflammation is perceived as a beneficial approach, it is not yet known at what time point during the inflammatory response it is most sensible to interfere. To address these issues, this review evaluates the effects of various anti-inflammatory agents on recovery processes in response to exercise-induced, traumatic, and disease-associated models of skeletal muscle injury. A collective analysis such as this should lay the foundation for future work that systematically manipulates the inflammatory response to most effectively promote regeneration and functional recovery in injured skeletal muscle, while reducing the negative effects of inflammatory processes such as pain and fibrosis.

inflammation; regeneration; eccentric injury; NSAIDs

EXERCISE, ECCENTRIC CONTRACTIONS, acute trauma, and disease are all sources of skeletal muscle injury. Despite the type of injury, the general injury and repair mechanism is similar. After skeletal muscle is injured, it typically undergoes stages of degeneration, inflammation, regeneration, and fibrosis (96), depending on the severity of the injury. After muscle injury, myofibers rupture and necrotize, which results in the infiltration of inflammatory cells due to damage to the matrix and surrounding vasculature. The first inflammatory cells to arrive to the site of injury are polymorphonuclear leukocytes, which are eventually replaced by monocytes within hours of the injury. Over the next 24–48 h these cells transform into macrophages that phagocytose and remove necrotic tissue (12). Macrophages, along with fibroblasts and the extracellular matrix, also produce growth factors, cytokines, and chemokines (59, 76, 99). It is these factors that activate regenerative mechanisms such as myogenic factors and satellite cells. Dur-

ing the repair phase, satellite cells proliferate and differentiate into myoblasts that fuse with injured myofibers (39, 54). In cases where the injury damages the basal lamina, a connective tissue scar is formed from fibrin and fibronectin (22, 40). Although this scar tissue strengthens the muscle during contractions as the muscle heals, if there is continued injury to the muscle and excessive proliferation of fibroblasts, a dense scar tissue may form that interferes with the repair process and contributes to incomplete functional recovery (89).

Inasmuch as inflammation contributes to fibrosis (1) and causes pain that may impair skeletal muscle function (89), the general practice has been to reduce inflammation. Much of the research on inflammation has focused on inhibiting it with drugs. The problem with this approach is that while inflammation causes further injury to muscle (19, 48, 96), preventing inflammation may hinder recovery (51, 61, 87). As a result, current treatment options for inflammation are not necessarily effective and, in some cases, they may be unsafe. Therefore, the question exists whether the most beneficial course of treatment should be to block inflammation or if it is sensible to

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allow inflammatory processes to progress naturally. Or perhaps, there is a benefit in blocking inflammation, but only for a specific time point and duration postinjury. Incidentally, given the time course over which pro- and anti-inflammatory molecules are recruited to the site of injury, it is not clear when it is appropriate to intercept the inflammatory response. It is also imperative to identify the magnitude of inflammation that provides the most benefit to injured tissue without inducing further injury. To improve the use of existing interventions and to develop new interventions to treat inflammation it is necessary to better understand these issues.

The overarching goal of this mini review is to synthesize the literature to categorize the state of the science in regards to interventions designed to manipulate the inflammatory response to promote healing after skeletal muscle injury. Because of the number of reports on this topic, this review will not attempt to provide an exhaustive overview of the existing literature on the subject. Instead, this review will examine several experimental models of skeletal muscle injury and those interventions that provide exemplary insight into effective or potentially harmful management of the inflammatory response. A greater emphasis is placed on those interventions examined within the past decade. A collective analysis such as this should lay the foundation for future work that systematically manipulates the inflammatory response to most effectively promote regeneration and functional recovery in injured skeletal muscle.

ANTI-INFLAMMATORY TREATMENTS AND EXERCISE-INDUCED MUSCLE INJURY

Eccentric contractions are typically responsible for exercise-induced injury to skeletal muscle. These contractions produce more injury than isometric or concentric contractions according to histological evidence of ultrastructural disruptions and functional analysis demonstrating losses in muscle strength (23, 24, 48, 60). Negative outcomes that follow a bout of eccentric exercise include delayed onset muscle soreness (DOMS), swelling, decreased range of motion of the affected limb, and losses in function (9, 19, 74). However, eccentric exercise does not always result in inflammation or necrosis and is not a contributor to DOMS (55, 109). Because of the common misconception that inflammation causes DOMS, the treatments used most frequently following exercise-induced muscle injury target inflammatory pathways. These include nonsteroidal anti-inflammatory drugs (NSAIDs) and natural compounds with anti-inflammatory properties.

NSAIDs. An estimated 70 million prescriptions for NSAIDs are written annually and 30 billion purchases are made for over-the-counter NSAIDs (21). The reason why NSAIDs have become so widely used is related to their selective inhibition of the cyclooxygenase (COX) enzymes (28). In response to injury, the COX enzymes (COX-1 and COX-2) produce prostaglandins that promote inflammation and pain, thus inhibition of these enzymes suggests a reduction in inflammation (if present) and pain. However, few studies have reported increases in COX-2 mRNA and protein in hours after muscle injury in humans and animals (3, 7, 108), whereas others have shown no change in the COX-1 or COX-2 enzyme after muscle injury (62, 100). With this in mind, a liberal review of the literature

provides opposing views on the efficacy of NSAIDs in reducing the negative consequences of exercise-induced muscle injury as a result of COX inhibition.

Ingestion of ibuprofen was unable to decrease pain perception and soreness after a single bout of leg exercise or during a 6-wk resistance training program of the biceps (42, 44). Several others found no effect of ibuprofen on attenuating soreness after exercise-induced muscle injury, despite the muscle under investigation (80, 90, 97, 102). Alternatively, in two instances when COX inhibitors were administered prophylactically before the bout of exercise and continued during the hours postexercise, soreness was attenuated (33, 75). It should be noted that in one of these cases, a specific COX-2 inhibitor (celecoxib) was used (75), as opposed to ibuprofen, which was used in the previous studies and nonspecifically targets COX-1 and COX-2.

The use of COX inhibitors to attenuate losses in range of motion or skeletal muscle strength has not proven to be beneficial after eccentric, resistance, or endurance exercise (8, 18, 44, 80, 90). Only two investigations reported decreased strength loss and improved range of motion in subjects taking ibuprofen (33, 78). Of concern are those reports that have documented increased strength loss and serum creatine kinase after eccentric exercise while taking ibuprofen (18, 81). Increased exercise-induced levels of serum creatine kinase, especially when combined with NSAID use, may contribute to rhabdomyolysis (6, 20). Additionally, two investigations documented decreased satellite cell activity in humans treated with NSAIDs in the days postexercise, the time period when regenerative processes take place (51, 61). Although studies such as this indicate that NSAIDs may be detrimental to muscle cell repair and adaptation because satellite cells are necessary for skeletal muscle regeneration, it is important to note that the long-term effects of satellite cell inhibition on regeneration have not been explored in human studies.

Paradoxically, although most people use NSAIDs in an effort to reduce pain after exercise so that they may continue to engage in physical activity, one potential consequence of NSAID use is the impact on skeletal muscle protein synthesis. Trappe et al. (102) reported that skeletal muscle fractional synthesis rate (FSR) was increased ~75% in young subjects taking a placebo, whereas those taking oral doses of ibuprofen had no increase in FSR 24 h postexercise, and the normal increase in prostaglandin synthesis was suppressed (101). A subsequent investigation by this group concluded that the COX-1 enzyme is the isoform responsible for the COX-mediated increase in protein synthesis, stressing the importance of this enzyme in human muscle and the potential negative effect of COX-1 specific inhibition (100).

Exercise is also a known contributor to skeletal muscle mitochondrial biogenesis (35). NSAIDs may affect mitochondrial adaptations to exercise. Analysis of isolated mitochondria from rodent cells treated with doses of NSAIDs comparable to therapeutic doses in humans revealed increased proton leak and decreased rates of ATP synthesis (43), uncoupled oxidative phosphorylation (64), and inhibition of mitochondrial respiration (71). Endurance exercise-dependent adaptations in skeletal muscle were also blocked during 4 wk of wheel running in rodents administered ibuprofen (50).

Conversely, in older adults, chronic administration of ibuprofen presented unique findings. Older adults (>65 yr) par-

icipating in 12 wk of resistance training and taking NSAIDs experienced an 11% increase in muscle volume, whereas subjects taking a placebo only increased muscle volume 8.5% (100). In addition, older adults taking NSAIDs increased quadriceps strength ~20% more than the placebo group during the 12 wk. The authors suggest that in older adults, COX-1 inhibition suppresses protein degradation to a greater extent than protein synthesis, ultimately resulting in an increased net protein balance, more protein accretion, and more muscle mass (100). An investigation in aged rodents also reported an increase in muscle mass after 5 mo of ibuprofen administration (82). Results were thought to be a consequence of the reduction in the low-grade inflammation that often occurs with aging.

Collectively, these data do not provide overwhelming reason to avoid the occasional use of NSAIDs postexercise. However, the efficacy of NSAIDs in resolving muscle pain, losses in muscle function, and changes in blood markers of muscle injury is not validated. Thus the use of NSAIDs to alleviate moderate pain after acute or exercise-induced muscle injury remains questionable. Careful analysis of the various experimental designs suggests that occasional use of NSAIDs with progressive resistance training is not problematic. In unique cases where chronic inflammation is negatively impacting protein turnover such as in the elderly or in individuals with pathologies that are associated with muscle wasting, NSAID use may be beneficial in restoring the balance between protein degradation and synthesis.

Natural interventions. Sources of flavonoids such as phenols, quercetin, and anthocyanins, have been investigated because of their high levels of antioxidant and anti-inflammatory activity. In fact, an inquiry on PubMed at the time of this publication returned 9,496 hits with the search terms “antioxidant and muscle injury.” An exhaustive review of the research is not possible, but there seems to be an abundance of investigations that report little to no benefit of the common antioxidants such as vitamins C, E, and quercetin (72, 91, 95). In general, the data are overwhelmingly equivocal. Most research indicates that inhibition of reactive oxygen species (ROS) may impair sport performance and hinder training adaptations (5, 77, 88, 94).

The natural compounds that have reported benefits as a result of reducing inflammatory mediators appear to be anthocyanins. Anthocyanins are considered anti-inflammatory agents because they function to inhibit COX activity, nitric oxide (NO) production, and modulate tumor necrosis factor (TNF)- α secretion (47, 92, 93). In various paradigms of exercise-induced skeletal muscle injury, foods or supplements containing anthocyanins have been shown to reduce circulating markers of inflammation, oxidant activity, and muscle soreness (4, 14, 36, 45, 49, 58, 79). Importantly, despite the mechanism of exercise-induced muscle injury, several studies reported faster recovery of muscle strength in groups ingesting anthocyanin supplements (cherry juice) compared with those ingesting a placebo (4, 14, 36, 45). These observations have considerable implications on the use of anthocyanins before and after exercise that causes muscle injury.

One natural compound lends clues to the importance of a specific phase of the inflammatory response. In response to inflammation, the conversion of prothrombin to thrombin initiates the conversion of fibrinogen to fibrin (105). Because fibrin activates proinflammatory prostaglandins, clotting factors, and edema, interventions directed at blocking fibrin activity or upstream

mediators may be an effective strategy. These interventions can alter the pro-to-anti-inflammatory ratio, and although not directly opposing the inflammatory response, they may shift the balance to a more anti-inflammatory environment. The proteolytic enzyme Bromelain, which is derived from pineapples, is one such compound. Rodents ingesting Bromelain daily after lengthening injury to the extensor digitorum longus (EDL) had less muscle fiber disruption and faster recovery of maximal force 72 h postinjury (105). In humans, Bromelain reduces muscle soreness in the 72 h posteccentric exercise, although it has no impact on muscle function and circulating cytokines (63, 103).

Figure 1 provides a model to summarize the results of the selected examples discussed above. There appears to be some benefit of natural interventions on systematically manipulating the inflammatory response. A mild reduction of circulating oxidants and proinflammatory molecules may portend less secondary muscle injury, reduced soreness, and faster time to recovery. Long-term investigations are warranted to verify that these acute changes postinjury do not affect skeletal muscle adaptation and repair, because no studies have been conducted to measure the effects of these agents on protein kinetics in skeletal muscle.

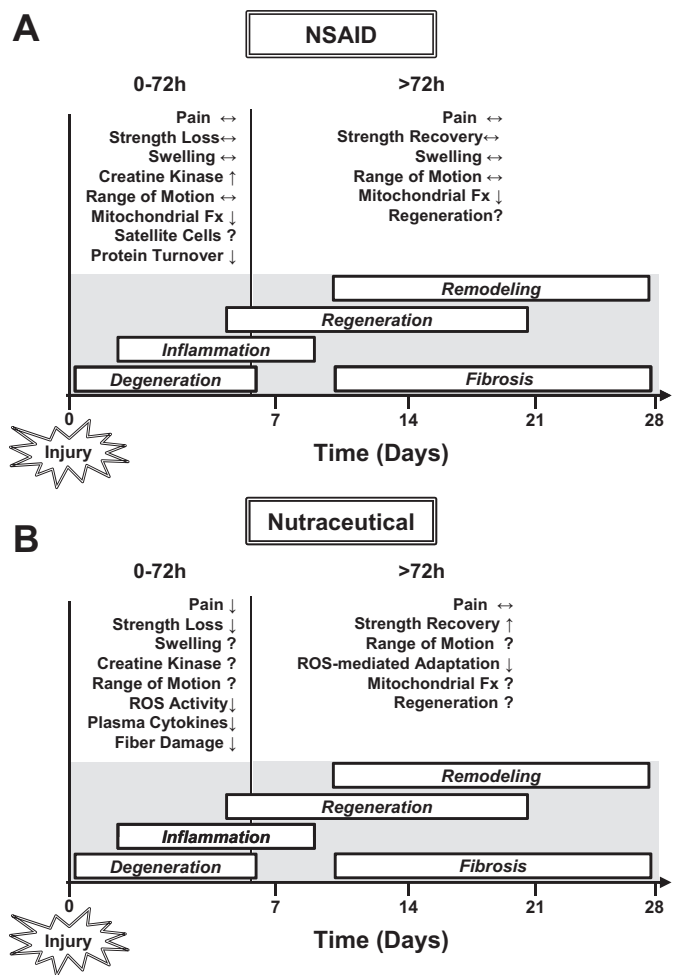


Fig. 1. Model summarizing the effects of non-steroidal anti-inflammatory drugs (NSAIDs; A) or nutraceuticals (B) on cellular and systemic processes after exercise-induced muscle injury. ↑, Increase; ↓, decrease; ↔, equivocal data; ?, unknown, insufficient research.

EFFECTS OF ANTI-INFLAMMATORY AGENTS AFTER ACUTE MUSCLE TRAUMA

Although various experimental models have been used to induce skeletal muscle injury, the universal outcome is myofiber necrosis and inflammation followed by the infiltration of neutrophils and macrophages. It is not yet established whether the role of neutrophils and macrophages is beneficial or detrimental to the muscle tissue. Although neutrophils liberate cytokines and free radicals that are responsible for exacerbating skeletal muscle injury, macrophages phagocytose damaged and necrotic tissue while releasing cytokines and growth factors in the injured area (96). Key signaling molecules hypothesized to regulate pro- and anti-inflammatory actions have been the targets of pharmacological and gene manipulation experiments after acute muscle injury. Unfortunately, the information to date is mainly based on animal studies, and the data may not be transferable to humans.

Pretreatment with anti-inflammatory agents. Experimental models of ischemia-reperfusion injury have been used to understand inflammation and potential countermeasures to attenuate inflammation because ischemia-reperfusion injury is clinically relevant. Ischemia-reperfusion generates ROS and reactive nitrogen species (RONS) and activates inflammatory cells (25, 30, 37). The resultant oxidative stress increases the expression of pro-inflammatory mediators including cytokines, chemokines, and adhesion molecules. Consequently, neutrophils and macrophages infiltrate the tissue and contribute to a repeated production of RONS, cytokines, chemokines, and cytotoxic proteases, intensifying the inflammatory response. The ischemia-reperfusion model is also ideal because activated macrophages produce transforming growth factor (TGF)- β 1, which is responsible for the induction of fibrosis (25).

Adenosine and its receptors have been implicated in protecting skeletal muscle against injury. Stimulation of the adenosine A3 receptor exerts anti-inflammatory and cytoprotective effects in skeletal muscle via alterations in phospholipase C (PLC)- β 2/ β 3 and protein kinase C (PKC) signaling. Specifically, stimulation of the A3 receptor reduces macrophage activity, immune cell function, calcium influx and overload, and ROS formation (27, 29, 110). Therefore, activation of the A3 receptor in skeletal muscle is thought to promote a systemic anti-inflammatory response. In one study that supports this hypothesis, activation of the adenosine A3 receptor in mice decreased the activation of pro-inflammatory metalloproteases and the number of injured muscle cells when delivered prior to traumatic muscle injury (104). The reduction in injured muscle cells points to a mechanism of action via attenuation of the secondary inflammatory response.

Cyclosporine A has also been investigated as a potential anti-inflammatory agent. Cyclosporine A exerts its anti-inflammatory effects by inhibiting calcineurin, a Ca^{2+} -dependent phosphatase that activates nuclear factor of activated T cells (NFAT) (16). Inhibition of calcineurin also suppresses transcription of the interleukins, decreasing proinflammatory cytokine production (16). Pretreatment with cyclosporine A was effective in improving muscle viability and decreasing edema after ischemia-reperfusion (69). Similarly, treatment with cyclosporine A before crotoxin injections reduced skeletal muscle necrosis (66).

These data suggest that inhibition of calcineurin and the coupled reductions in ROS, cytokines, metalloproteases, and

proteolytic enzymes may be a more effective means of skeletal muscle protection in the early stages postinjury than blocking the COX pathway. The aforementioned interventions resulted in decreased circulating markers of inflammation and, most importantly, less tissue injury. Although the former may be an effect of pretreatment preventing the “normal” initial inflammatory response, possibly because of a reduction in the available proinflammatory molecules, the latter may be attributable to a consequential reduction in secondary muscle injury. Therefore, although pretreatment modalities lend some clues to effective anti-inflammatory treatment strategies, several questions remain unanswered. It is not clear if these same agents will be effective if only delivered postinjury. In most cases of skeletal muscle injury, pretreatment is not practicable. This is a therapeutic obstacle. For example, treatment with cyclosporine A postinjury resulted in extensive inflammation, increased fiber atrophy, and calcification in regenerating muscle (85). A second limitation of the experimental approach of these investigations is the lack of long-term data that capture regenerative potential of the injured and treated muscle. None of the reports provide data to allow for conclusive evidence regarding the efficacy in regeneration and functional recovery.

Posttreatment with anti-inflammatory agents. Corticosteroids have been used as anti-inflammatory therapeutics because they function to inhibit the infiltration of monocytes and neutrophils to sites of inflammation (31). These drugs also block T-cell activation through inhibition of cytokine release. The result is decreased tissue levels of interleukins and TNF- α . To date, results indicate that corticosteroids may be beneficial in the short term, but they cause injury to healing muscle with chronic use, including disruptions in fiber integrity, incomplete healing, and reductions in force generating capacity (2, 31, 73).

Work-related musculoskeletal disorders, although a less severe form of muscle injury, are common and result in a substantial impact on the manual labor workforce. Pathophysiological changes that lead to these overuse injuries are not completely understood, but interventions have been incorporated to try and minimize the incidence and severity. One model in rodents explored the efficacy of anti-TNF treatments in reducing fibrosis and decreases in grip strength that occurred after 4 wk of performing a repetitive contraction task (1). Two weeks of anti-TNF treatment increased overall grip strength and decreased fibrosis. This intervention points to the importance of TNF and fibrosis in functional deficits with chronic inflammation.

Manipulation of various genes postinjury has identified several potential targets for pharmacological intervention. Fn14, the receptor for TNF-like weak inducer of apoptosis (TWEAK), serves a key role in balancing inflammatory and regenerative processes. Mice deficient in the Fn14 receptor had an attenuated inflammatory response and delayed muscle fiber regeneration compared with wild-type mice after cardiotoxin-induced injury (26). However, ablation of TWEAK improved myofiber regeneration (65).

Toll-like receptors (TLRs) have inflammatory and myogenic roles in skeletal muscle. TLRs are membrane bound receptors that are activated by growth factors and cytokines. Upon activation, TLRs stimulate a complex immune signaling network that makes them difficult targets for modulating inflammation. In one example, cardiotoxin injury in TLR3-deficient mice reduced expression of the pro-inflammatory cytokines

IL-6, IL-1 β , and TNF- α but also decreased myosin heavy chain protein expression, likely a consequence of impaired myogenesis (57). These examples provide insight into the important balance between inflammation and myogenesis, as well as the challenges associated with pharmacological intervention of complex signaling pathways.

Some chemotactic factors have been identified in injured skeletal muscle as promising targets. Chemotactic factors are produced by infiltrating macrophages and leukocytes and are induced by inflammatory cytokines such as TNF- α . Monocyte chemoattractant protein-1 (MCP-1) is one such factor. MCP-1 is responsible for initiating signals for tissue healing, although uncontrolled production of MCP-1 stimulates the recruitment of additional inflammatory cells, which may lead to additional tissue injury (96). Knockout of Ccl2, the gene that encodes MCP-1, reduces the number of macrophages in injured muscle and the outcome is impaired regeneration and incomplete functional recovery (15, 87, 107). This is likely attributable to the inability to launch a “normal” inflammatory response. More studies are encouraged to identify the most effective approach to manipulating this molecule; specifically, understanding the level of expression that is most effective in producing an inflammatory response that optimizes regenerative potential.

Taking these data into consideration, an elegant experiment by Hunt and colleagues (38) illustrates the importance of inflammatory mediators at single time points postinjury, while at the same time highlighting the complexity of inflammatory signaling and an organism’s innate ability to induce negative feedback mechanisms that prevent an overwhelming inflammatory response. In this example, the investigators manipulated leukemia inhibitor factor (LIF), a cytokine that signals cell survival, inhibits caspase-dependent apoptosis, and stimulates regeneration through gp130 and LIF receptor subunits. After noxoin injection, a LIF antagonist (MH35-BD) was administered at two time points postinjury when LIF upregulation was highest. In this experimental model, the first phase of LIF induction immediately postinjury coincided with increased proinflammatory cytokine expression, whereas the second phase of LIF induction coincided with myogenic differentiation and de novo myotube formation. When the LIF antagonist was administered during the acute inflammatory phase, there was increased gene expression for the proinflammatory cytokines IL-6, IL-1 β , and TNF- α ; an increased inflammatory response; and inhibition of myotube formation. Conversely, when the LIF antagonist was administered during the second phase of LIF induction, there were no effects on inflammation, myogenic differentiation, or regeneration. These data seem to indicate that the primary inflammatory phase is essential for the induction of signals that control tissue regeneration. However, attenuating the secondary inflammatory phase and the subsequent secondary muscle injury may be an effective treatment approach.

Indeed, posttreatment of skeletal muscle injury is a more practicable approach, whether the injury is acute or slow to appear such as with repetitive use injuries. Overall, there is a need for interventions that can be used that will not affect recovery or promote a catabolic environment, such as what has been shown with corticosteroid treatment. From the aforementioned examples from the literature there is compelling evidence that the release of proinflammatory molecules in the first

hours postinjury is necessary for appropriate activation of regenerative processes. Molecules with dual inflammatory and regenerative functions in skeletal muscle seem to have the most promise as effective targets for therapeutics after traumatic muscle injury. Future research must focus on filling the gap in the scientific literature regarding the critical pro- to anti-inflammatory switch that enhances regeneration. Also imperative is identifying how to obtain the same benefits with posttreatment as those observed after pretreatment of anti-inflammatory agents (Fig. 2). Furthermore, without scientific approaches that provide data in regard to functional recovery and morphological evidence of complete regeneration, this undertaking will not be complete.

ANTI-INFLAMMATORY DRUGS AND DISEASE

Duchenne muscular dystrophy (DMD) is an ideal experimental model of muscle injury because the lack of dystrophin leads to severe muscle degeneration, inflammation, fibrosis, and muscle cell death. Membrane deficits and mechanical injury promote dystrophic pathology that is made worse by alterations in intracellular signaling cascades that regulate inflammatory processes and contribute to the degenerative

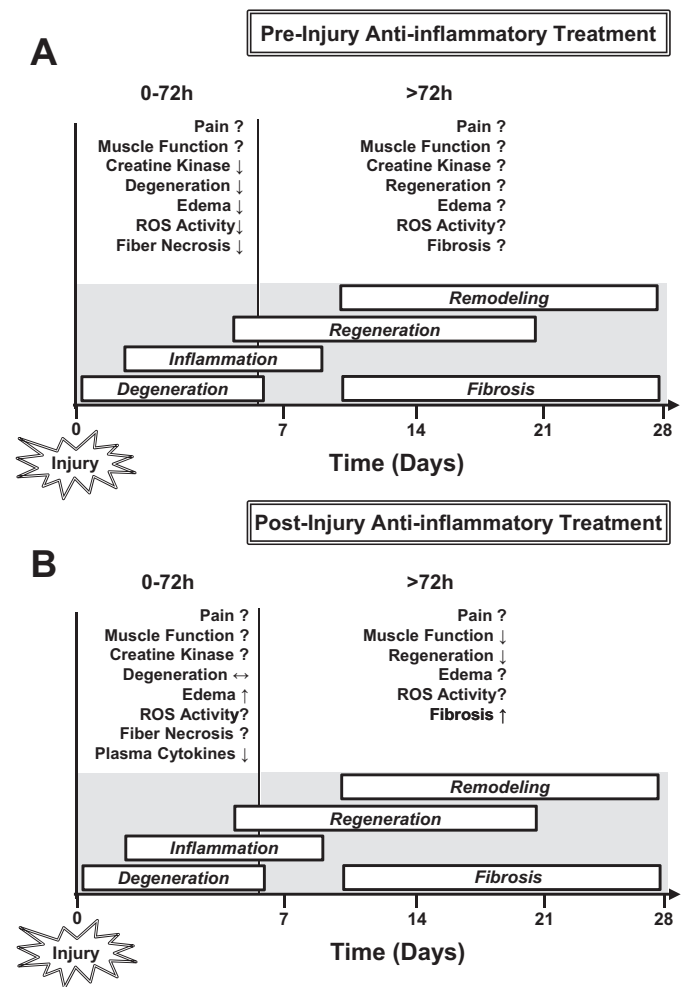


Fig. 2. Model summarizing the effects of preinjury anti-inflammatory treatment (A) or postinjury anti-inflammatory treatment (B) on cellular and systemic processes after traumatic injury. This model is predominantly based on data from animal models.

process. Because inflammatory gene expression is upregulated and immune cell infiltrates are prevalent in dystrophic muscle, resulting in impaired regeneration functional recovery, the identification of specific targets for anti-inflammatory therapies is of primary importance.

As discussed with acute trauma to skeletal muscle, glucocorticoids have also been explored as potential therapies for DMD. *Mdx* mice subjected to 8 wk of downhill walking were treated with cyclosporine A daily for the duration of the intervention. Compared with *mdx* mice treated with a placebo, cyclosporine A prevented the decline in forelimb strength that is typically induced by exercise. Concomitantly, increases in serum creatine kinase and markers of fibrosis were blunted and there were more uninjured fibers (17). Similarly encouraging was an investigation in young men with DMD who received injections of cyclosporine A daily for 8 wk, resulting in a 25% increase in tetanic force and a 15% increase in maximal voluntary contraction (86). This report was published almost two decades ago and, unfortunately, because of increased reports of potentially fatal side effects, the use of glucocorticoids has been limited.

Matrix metalloproteases-2 and -9 (MMP-2, MMP-9) are extracellular proteases. MMPs influence the inflammatory response via processing of chemokines and cytokines. MMP induction becomes problematic when MMPs are disproportionately increased compared with their natural inhibitors, the tissue inhibitors of metalloproteases (TIMPs) (13, 41, 83, 111). Gene ablation of MMP-2 and MMP-9 has been explored in *mdx* and healthy mice. In an *mdx* model, deletion of the MMP-9 gene reduced inflammation, fiber necrosis, and improved muscle structure and function, likely a consequence of a reduction in overall injury (46). Interestingly, although MMP-2 also functions to promote myogenesis and extracellular matrix remodeling during muscle regeneration and fiber growth, MMP-2 ablation in *mdx* mice increased gene expression of chemokines and cytokines and impaired growth of regenerating muscle fibers (67). However, adenosine A3 receptor agonist treatment, which has been shown to manipulate MMP signaling and reduce injured cells after traumatic muscle injury (104), has also been shown to lower serum creatine kinase levels and decrease tissue injury in dystrophic mice subjected to downhill running (106).

Although multiple natural-derived interventions have been explored to reduce the consequences of inflammation in skeletal muscle, melatonin is one of the few that has generated encouraging treatment outcomes for muscle pathologies (10, 11). Treatment with melatonin improved muscle function in *mdx* mice (34), and, in DMD patients, 3 mo of melatonin ingestion reduced circulating markers of oxidant activity and inflammation (10, 11). Although these data are encouraging, systemic markers are a weak indicator of skeletal muscle morphology and functional ability (70).

The role of protein kinase C (PKC) manipulation has also been explored in skeletal muscle. The different isoforms of PKC promote inflammatory responses via T-cell activation and adhesion. PKC θ is predominantly expressed in skeletal muscle and strongly upregulated after injury to skeletal muscle, supporting its role in skeletal muscle growth and remodeling (52, 68, 98). However, in DMD PKC may contribute to impairments in calcium homeostasis, resulting in an influx of calcium into muscle cells (84). This is supported by recent work demonstrating that PKC inhibition restores normal calcium

levels in DMD (32). In *mdx* mice lacking the gene for PKC θ , proinflammatory pathway activation is attenuated (53). In contrast to other molecules that have been explored in healthy models of skeletal muscle injury, the blunted inflammatory response resulting from the elimination of PKC θ in *mdx* mice decreased tissue injury, reduced muscle degeneration, and preserved exercise performance (53). Because of the consistent success with manipulation of PKC θ , inhibitors are already in clinical trials for immune disorders; however, additional research is necessary in models of skeletal muscle injury as few studies have been published to date.

The severity of DMD introduces an urgent need for treatment options that help to reduce chronic inflammation and cellular injury. Investigators have been aggressive in moving potential therapeutics toward clinical trials. However, certain factors have not yet been elucidated and the potential exists for unsuccessful clinical trials. Most importantly, despite extensive research efforts to understand the critical role for PKC θ in inflammatory responses, PKC isoforms are induced by multiple stimuli and downstream PKC signaling is rather complex. The long-term efficacy of these interventions has not yet been scrutinized.

CONCLUSIONS

Considering the number of published reports in the past two decades alone, one would presume that scientists have identified a viable intervention to mitigate inflammation and promote tissue regeneration and functional recovery after injury to skeletal muscle, whether chronic or acute. Instead, although many of these interventions have effectively minimized inflammation and/or markers of inflammation, muscle injuries continue to heal with a functional deficit. In addition, there seems to be a lack of uniformity across studies. As a consequence, the scientific community is faced with great diversity in treatment options and conflicting results. To date, there is no clear message with regards to the effect and mode of action of anti-inflammatory interventions and how they can best promote muscle healing and functional recovery.

Attempts at reducing eccentric contraction-induced skeletal muscle injury have shown variable levels of efficacy. It is remarkable that despite the number of studies addressing contraction-induced skeletal muscle injury, these studies have failed to provide a clear strategy to effectively diminish inflammation at critical time points to promote healing. Evidence of the repeated bout effect, where muscle adapts after injury from eccentric exercise, is also associated with reduced inflammatory responses (56). This raises the possibility of clinical applications of mild eccentric exercise to protect muscle against subsequent injury.

Still, effective strategies to heal skeletal muscle after this form of injury are needed. The opinion here is that the therapy should not be to obliterate the inflammatory response, but instead to restore the normal regulation of inflammatory processes.

A more urgent focus for future research is to identify suitable interventions for acute traumatic injury to skeletal muscle as well as interventions for disease conditions that are marked by chronic inflammation. The data thus far suggest that during the initial stage of an acute injury, interventions to modulate inflammation may facilitate healing, but the timing of these interventions appears to be of paramount importance. The precise reason for the difference in healing properties between “normal” inflammation

and “abnormal” inflammation are not yet understood. Future research should first focus on identifying key cellular mediators involved in each condition, specifically those that seem to function as a pro- to anti-inflammatory switch. These targets will affect both inflammatory and regenerative processes. In most examples discussed in this review, the use of existing therapies to block inflammation appears to have negative effects on regenerative processes. Studies of this nature are extremely relevant because it appears as if inflammation is a necessary, yet complex, phase postinjury, and manipulating inflammation to provide benefit involves more than a single intervention during the acute inflammatory response.

It is imperative that we continue to unravel the complex nature of pro- and anti-inflammatory signaling as a functional network. This approach emphasizes the importance of collaborative expertise from benchtop biologists and computational scientists. These efforts will lay the foundation for the discovery and successful implementation of combinatoric therapeutics that target inflammatory and regenerative processes in skeletal muscle. Implicit in this approach is the idea that combinatoric therapeutics will incorporate specificity to the various aspects of injury to include time postinjury, nature of the injury, and magnitude of the inflammatory response. To this end, treatments that may be appropriate after exercise-induced muscle injury may not be appropriate for acute muscle trauma or disease and vice versa.

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AUTHOR CONTRIBUTIONS

Author contributions: M.L.U. conception and design of research; M.L.U. prepared figures; M.L.U. drafted manuscript; M.L.U. edited and revised manuscript; M.L.U. approved final version of manuscript.

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