Histol Histopathol (2003) 18: 1243-1256

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Muscle injuries and repair: The role of prostaglandins and inflammation

V. Prisk[1] and J. Huard[1,2]

[1]Growth and Development Laboratory, Department of Orthopaedic Surgery Children's Hospital of Pittsburgh and University of Pittsburgh, Pittsburgh, USA and [2]Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, PA, USA

Summary. Skeletal muscle injuries are a common problem in trauma and orthopaedic surgery. Muscle injuries undergo the healing phases of degeneration, inflammation, regeneration, and fibrosis. Current and experimental therapies to improve muscle regeneration and limit muscle fibrosis include conservative and surgical principles with the adjuvant use of non-steroidal anti-inflammatory drugs (NSAIDs) and growth factor manipulation. NSAIDs appear to have a paradoxical effect on the healing of muscle injuries with early signs of improvement and subsequent late impairment in functional capacity and histology. In vitro and in vivo studies have explored the role of the cyclooxygenases and prostaglandins in the biological processes of healing muscle, including precursor cell activation, myoblast proliferation, myoblast fusion, and muscle protein synthesis. Through use of more specific cyclooxygenase inhibitors, we may be able to better understand the role of inflammation in muscle healing.

Key words: Muscle injury, Prostaglandins, NSAIDs, Cyclooxygenase

Introduction

Skeletal muscle injuries constitute the majority of sports-related injuries in many epidemiological studies (Garrett, 1996; Croisier et al., 2002). Moderate to severe muscle injuries may result in the inability to train or compete for several weeks and have a high tendency to recur (Verrall et al., 2001; Orchard and Best, 2002). Muscle injuries result from a variety of mechanisms, including direct and indirect causes. Direct causes include traumatic processes such as lacerations, contusions, and strains (Hughes et al., 1995; Kasemkijwattana et al., 1998, 2000; Fukushima et al., 2001). Indirect causes are those that result from another medical condition such as ischemia or neurologic dysfunction (Day et al., 2002; Paoni et al., 2002).

Additionally, repeated eccentric muscle contractions can result in delayed-onset muscle soreness (DOMS) with symptoms similar to muscle injuries, including decreased function, stiffness, and pain (Warren et al., 2002). DOMS is attributable to a distinct process in muscle that includes an inflammatory response and changes in the structural integrity of muscle resulting in the loss of functional capacity (Barash et al., 2002; Lieber et al., 2002). Moreover, mechanical damage and leukocyte infiltration after intense eccentric exercise are known to coincide with torque reductions (MacIntyre et al., 1996).

Years of research have clarified the time-dependent and interrelated processes that occur after skeletal muscle is injured (Hurme et al., 1991; Kaariainen et al., 2000; Huard et al., 2002). Muscle injuries undergo a distinct set of healing phases, including degeneration, inflammation, regeneration, and fibrosis. In this paper, we describe these biological events, which occur during the first few weeks following muscle injury. We then outline the latest discoveries made with regard to improving the regenerative process and reducing fibrosis formation both experimentally and therapeutically to improve muscle functional recovery. Finally, we focus on the role of the inflammatory phase of muscle healing, giving the most attention to the cyclooxygenases, the prostaglandins, and their inhibitors.

The phases of muscle healing after injury

Injured skeletal muscle undergoes the healing phases of degeneration, inflammation, regeneration, and fibrosis. Although the biological processes of these phases have a great deal of overlap, the different phases of muscle healing, as depicted by histological analysis, can be very distinct at sequential time points (Fig. 1). Below, we discuss these phases in more detail.

Offprint requests to: Johnny Huard, Ph.D., Director, Growth and Development Laboratory, 4151 Rangos Research Center, Children's Hospital of Pittsburgh, 3705 Fifth Avenue Pittsburgh, PA 15213-2583. Fax: 412-692-7095. e-mail: jhuard+@pitt.edu

Degeneration

Trauma to muscle tissue disrupts the integrity of the sarcomere, sarcolemma, and basal lamina, leading to the ingress of extracellular calcium as well as the activation of the complement cascade (Carpenter and Karpati, 1989; Orimo et al., 1991). Intrinsic proteases autodigest disrupted and subsequently necrotic myofibers (Ebisui et al., 1995; Mbebi et al., 1999). The tendon-myofiber-tendon units are disrupted and the ruptured myofibers retract forming a gap (Kaariainen et al., 2000). Skeletal muscle is richly vascularized, and capillary injury results in a hematoma that fills this gap. The upregulation of adhesion molecules and cytokines influences local vascular permeability and blood flow, thus accelerating the ensuing inflammatory response and resultant edema. Toxic free radical species develop and can impair excitation-contraction coupling, induction of proteolysis, and subsequent necrosis of myofibers both in the traumatized tissue and in healthy tissue located nearby (Clanton et al., 1999). In addition to mechanical damage, the myofibers become denervated through destruction of intramuscular nerve branches and separation of the fiber segment containing the neuromuscular junction from the remainder of the myofiber (Rantanen et al., 1995b).

Little attention has been paid to finding ways to limit the degeneration that occurs with muscle injury. In order to affect this phase, one must employ preventative medicine techniques. Some have suggested the use of antioxidant substances prior to sporting activities. However, studies on antioxidant or free radical inactivating substances like vitamin E, vitamin C, and N-acetyl cysteine have failed to show promising results (Childs et al., 2001; Beaton et al., 2002).

Inflammation

Inflammation is an early response to muscle tissue injury and involves coordination between the immune system and the injured tissue. This phase is, perhaps, the least distinct phase as its processes overlap with all of the other phases of muscle injury and repair (Fig. 1). It is known that within the first day after muscle injury, small blood vessels, neutrophils, activated macrophages, and

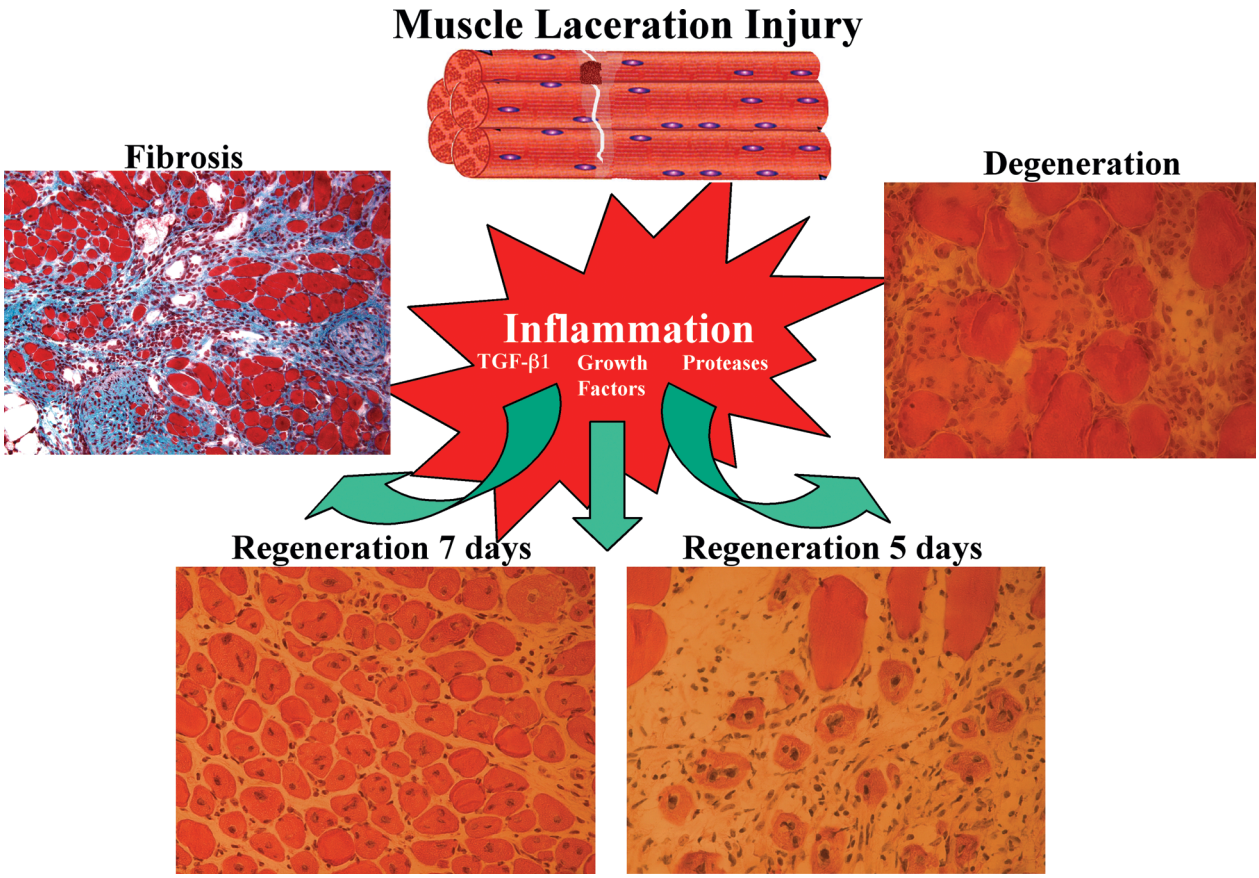

Fig. 1. Injured muscle undergoes the healing phases of degeneration, inflammation, regeneration, and fibrosis. Muscle inflammation after injury results in the release of growth factors, cytokines, free radicals, and other mediators, which set up a microenvironment that overlaps with all of the healing phases. Muscle degeneration involves marked myofiber necrosis and early inflammatory cell infiltration. Muscle regeneration is most clearly defined by the presence of centronucleated myofibers. Muscle fibrosis results from collagen deposition between regenerated myofibers.

T-lymphocytes infiltrate the hematoma between the ruptured myofibers (Fielding et al., 1993; Tidball, 1995; Frenette et al., 2000; MacIntyre et al., 2000). The invading neutrophils are followed by different populations of macrophages (Lapointe et al., 2002). Some macrophages are believed to be involved mainly in phagocytosis and removal of cellular debris, though they also may participate with neutrophils in evoking nonspecific tissue damage due to the spillage of degradative enzymes (Lapointe et al., 2002). Like neutrophils, these activated macrophages release pro-inflammatory cytokines, prostanoids, collagenases, and many potentially cytotoxic compounds such as peroxynitrite, which can lead to further muscle degeneration and excitation-contraction uncoupling outside of the zone of injury (Beckman and Koppenol, 1996; Witko-Sarsat et al., 2000). Other populations of macrophages do not phagocytose degenerating skeletal muscle fibers; rather, they produce early cytokine and growth factor signals during the regenerative process (McLennan, 1993; St Pierre and Tidball, 1994).

In addition to the inflammatory cell response, the myogenic cells are potentially capable of secreting growth factors, cytokines, and prostanoids, which affect the regenerative microenvironment and contribute to the symptomatology of muscle injuries. Various growth factors released during the inflammatory phase have well described roles in both the muscle regeneration phase and the formation of muscle fibrosis after severe injury (Huard et al., 2002; Li and Huard, 2002). Altering the inflammatory process may have both beneficial and detrimental effects. Limiting inflammation may theoretically reduce excessive muscle degeneration and signals for scar formation, but reducing the availability of growth factors, cytokines, and prostaglandins may inhibit strong signals that promote the regenerative process as well. This paradox is explored in more detail later in this review.

Regeneration

Whereas active muscle degeneration and inflammation are occurring at the injury site in the first few days, muscle regeneration begins to take precedence during the first week post-injury (Kaariainen et al., 2000; Huard et al., 2002). Satellite cells are activated as early as 24 hours post-injury, but the beginning of the regenerative phase is marked by the subsequent myoblast proliferation, which culminates in myoblast differentiation and fusion into multinucleated myofibers and eventually mature myofibers (Rantanen et al., 1995a; Zammit and Beauchamp, 2001; Huard et al., 2002). In fact, some researchers theorize that the disruption of the sarcolemma and basal lamina after muscle injury releases and activates previously quiescent satellite cells and muscle stem cells residing between these structures (Hurme and Kalimo, 1992; Bischoff, 1994; Qu-Petersen et al., 2002). Growth factors released at the injury site, including Insulin-like Growth Factor-1 (IGF-1), basic Fibroblast Growth Factor (bFGF), Epidermal Growth Factor (EGF), Hepatocyte Growth Factor (HGF), and Transforming Growth Factor Beta-1 (TGF-ß1), have been shown to influence the proliferation and differentiation of myoblasts and muscle stem cells in vitro (Sheehan and Allen, 1999; Deasy et al., 2002; Huard et al., 2002). In vivo studies of muscle laceration, contusion, and strain have shown that IGF-1, bFGF, and, to a lesser extent, Nerve Growth Factor (NGF) injected at early time points post-injury (2, 5, and 7 days) are all capable of enhancing muscle regeneration (Kasemkijwattna et al., 1998, 2000; Menetrey et al., 2000). Of particular interest, IGF-1 plays an influential role in the muscle regenerative process by stimulating myoblast proliferation, differentiation, and, eventually, myofiber protein synthesis and hypertrophy (Engert et al., 1996; Damon et al., 1998). As the local environment during muscle regeneration overlaps with the inflammatory process, prostaglandins released in the injured muscle may also contribute or be essential to the action of growth factors in myofiber regeneration. We discuss the role of inflammatory mediators in satellite cell activation, growth factor regulation, and myoblast differentiation in further detail below.

Fibrosis

The growth factors released from resident macrophages, myogenic precursor cells, and other cellular mediators of the injury process do not always act to augment the regenerative process. In fact, EGF, myostatin, and TGF-ß1 have been shown to inhibit skeletal muscle regenerative processes both in vitro and in vivo (Doumit et al., 1993; Mendler et al., 2000; Yamanouchi et al., 2000; Fukushima et al., 2001; Taylor et al., 2001; Rios et al., 2002; Langley et al., 2002; Li and Huard, 2002). It has been documented that the hematoma in the necrotic muscle gap begins to be replaced by a connective tissue scar made of type III and then type I collagen starting as early as the third day post-injury (Kaariainen et al., 2000). This fibrotic tissue provides early support for ruptured myofibers, but as it becomes increasingly dense over the course of seven to fourteen days post-injury, it restricts the regenerative growth of myofibers (Kaariainen et al., 2000; Li and Huard, 2002). The dense fibrotic tissue that develops after severe muscle injuries not only prevents the myofiber stumps from rejoining but also may prevent new axons from reaching muscle fibers to create neuromuscular junctions (Kaariainen et al., 2000). Thus, those fibers may undergo atrophy following denervation.

TGF-ß1 has been implicated in the pathogenesis of fibrosis in many tissues, including those of the lung, kidney, central nervous system, heart, and liver (Czaja et al., 1989; Khalil et al., 1993; Logan et al., 1994; Lijnen et al., 2000; Ina et al., 2002; Venkatesan et al., 2002). Similarly, TGF-ß1 has been found to be associated with the muscle necrosis and fibrosis that occur in Duchenne muscular dystrophy and dermatomyositis as documented

via human muscle biopsy specimens (Yamazaki et al., 1994; Confalonieri et al., 1997; Bernasconi et al., 1999; Amemiya et al., 2000). TGF-ß1 acts during inflammation and fibrosis to stimulate the production of extracellular matrix proteins and, concurrently, to inhibit their degradation (Broekelmann et al., 1991; Seeland et al., 2002). Additionally, TGF-ß1 appears to lead to the differentiation of myoblasts and muscle-derived stem cells into a myofibroblast lineage that eventually contributes to the development of fibrosis (Li and Huard, 2002). The high risk of injury recurrence and loss of muscle strength after muscle injuries may be attributable to the lack of congruity and structural integrity that results from TGF-ß1–induced fibrosis within the muscle.

Current and experimental therapeutics of muscle injuries

Recurrence of injury, persistent weakness, and inflexibility all prolong the disability that occurs with muscle injury. For several decades, clinicians and scientists have been looking for ways to limit this disability in order to help patients return to their previous level of function. Clinically, mild to moderate acute muscle injuries are treated in accordance with the R.I.C.E. principle (Rest, Ice, Compression, Elevation), other physical modalities (e.g., therapeutic ultrasound), and non-steroidal anti-inflammatory drugs (NSAIDs) (Kellett, 1986). However, some physical modalities have shown only limited benefit experimentally—e.g., the ability of therapeutic ultrasound to stimulate satellite cell proliferation (Rantanen et al., 1999). In terms of more novel therapies, research has documented that stimulating muscle regeneration with growth factors or, alternatively, inhibiting the formation of muscle fibrosis leads to improvement in muscle functional recovery. Blocking TGF-ß1 and thus reducing scar tissue through use of the agents suramin, decorin, or γ-interferon also facilitate improved muscle functional recovery (Fukushima et al., 2001; Chan et al., 2002; Foster et al., 2003). For more severe injuries, particularly laceration injuries, suture repair conveys some benefit over immobilization, in that it also reduces scar formation and helps in the recovery of more muscle strength (Menetrey et al., 1999).

Although it is possible to enhance muscle regeneration via the addition of growth factors like IGF-I and limit muscle fibrosis via the utilization of anti-TGF-ß1 agents, it is unclear as to how the inflammatory process should be manipulated. Recent data suggest that treatment of skin wounds with newer NSAIDs, the cyclooxygenase-2 selective inhibitors, can reduce scar tissue formation, but whether this occurs in skeletal muscle is unknown (Wilgus et al., 2003). Previous experimental results have suggested a paradox with early improvement but subsequent decline in muscle function following both corticosteroid and NSAID drug treatments (Mishra et al.,1995; Beiner et al., 1999). Although the regenerative and fibrotic phases of muscle healing have been extensively studied in the past, the summative effects of the inflammatory process and its overlap with these phases of healing remain unclear. For the remainder of this review, we focus on the NSAIDs and their effects on the muscle healing process.

NSAIDs and cyclooxygenases

Prostaglandins have been identified and implicated as major factors in tissue inflammation for many years after the discovery that aspirin and other NSAIDs, which inhibit prostaglandin synthesis, also attenuate acute inflammation (Vane, 1971). Furthermore, injection of prostaglandins into various tissues can potentiate the signs of inflammation induced by bradykinnin and histamine (Lewis et al., 1974, 1975). Many prostaglandins are synthesized by the cyclooxygenase enzyme. Cyclooxygenase comes in multiple isoforms and catalyzes multiple steps in the conversion of arachidonic acid to various prostaglandins. Pro-inflammatory mediators induce the synthesis of prostaglandins through phospholipase A2 (PLA2)-mediated release of membrane-associated arachidonic acid and induction of cyclooxygenase enzyme activity (Murakami et al., 2000a). Three isoforms of cyclooxygenase have been described to date. Cyclooxygenase-1 (COX-1) is produced constitutively, synthesizes prostaglandins important for homeostasis, and appears to play a small role in early inflammation (Murakami et al., 2000b; Tilley et al., 2001). Cyclooxygenase-2 (COX-2) is an inducible isoform that plays a major role in the mediation of pain and inflammation after injury. Increased COX-2 products, such as PGE_2, appear to sensitize local nociceptor terminals, thereby increasing peripheral hypersensitivity to pain (Mense, 1981; Zhang et al., 1997; Smith et al., 1998; Hedenberg-Magnusson et al., 2002). There is also evidence that interleukins and other inflammatory mediators lead to prostaglandin-mediated sensitization to pain in the spinal cord and other areas of the central nervous system (Smith et al., 1998; Samad et al., 2001). Likewise, multiple studies have proven the effectiveness of selective COX-2 inhibitors in the reduction of postoperative and arthritic pain (Sinatra, 2002). Cyclooxygenase-3 (COX-3) is a recently described isoform of cyclooxygenase that appears to be involved in processes such as fever and is inhibited by acetaminophen (Botting, 2000; Chandrasekharan et al., 2002). Unlike COX-1 and COX-2, COX-3 does not appear to have significant involvement in tissue inflammation.

The older cyclooxygenase inhibitors display variable selectivity for the COX-1 and COX-2 isoforms, usually with greater selectivity for COX-1 than COX-2. However, newer drugs (e.g., celecoxib, rofecoxib, valdecoxib) demonstrate far greater COX-2 selective inhibition, and with their superior gastrointestinal safety and lack of clinical effect on platelet function, are now prescribed for arthritis, postoperative pain, and,

occasionally, acute soft tissue injury. These agents are clearly capable of reducing the pain associated with various insults, but it remains unclear whether they have beneficial or detrimental effects on the complex process of muscle repair and recovery of strength. Although many studies have looked at the role of non-selective NSAIDs in muscle healing, there is a paucity of information regarding the effects of the COX-2 selective inhibitors in this process.

NSAIDs and muscle functional recovery

In addition to the R.I.C.E. principle, clinicians often recommend that NSAIDs be taken in the first few days after injury to limit inflammation and pain. However, some studies indicate early functional and histological improvement with the use of NSAIDs but concomitant loss of functional capacity at later time points (Fig. 2). In animal models and humans, NSAIDs are capable of decreasing prostaglandin concentrations, limiting edema, and delaying the inflammatory process in traumatized skeletal muscle (Almekinders, 1999; Trappe et al., 2001). Salminen and Kihlstrom (1987) noted that NSAIDs provide a cytoprotective effect on exercised muscle with a reduction in myofibrillar inflammatory cells and myonecrosis at 48 hours post-exercise injury in mice. However, Almekinders and Gilbert (1986) reported a delay in muscle regeneration and no significant effect on tensile strength recovery (force to rupture) in a rat tibialis anterior strain injury when treated with the NSAID piroxicam.

In a later study, Obremsky et al. (1994) investigated contractile strength recovery and tensile strength, and conducted histological analysis of strain-injured muscle in rabbits. In their study, contractile forces at day 1 post-injury showed approximately 20% improvement with piroxicam treatment as compared to untreated controls. No further significant improvements were observed at 2, 4, or 7 days follow-up. As with Almekinders and Gilbert (1986), no change in tensile strength was noted at any time point. The rabbit model utilized by Obremsky et al. also demonstrated similar histologic responses to

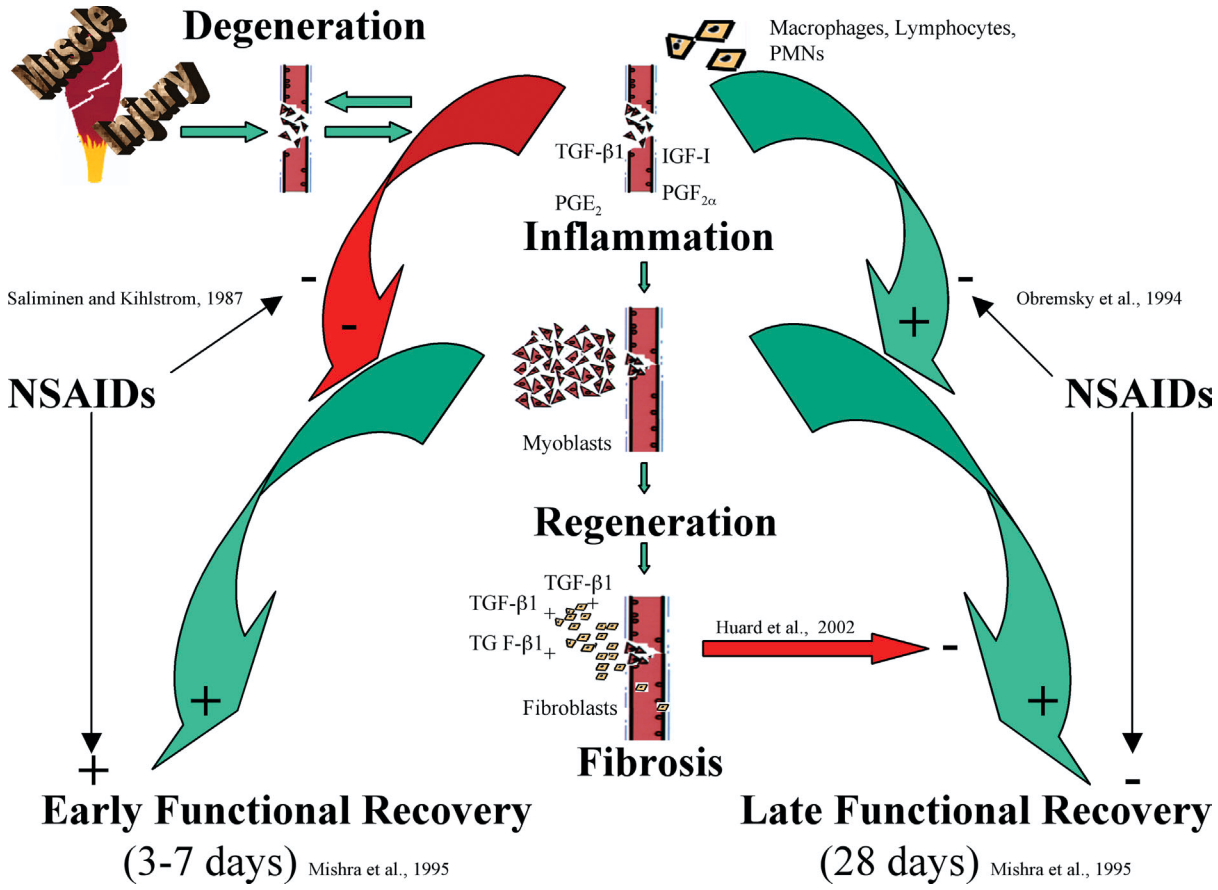

Fig. 2. The NSAIDs have been shown to improve early functional capacity of injured muscle but lead to deficits in late functional capacity. NSAIDs may improve early outcome by limiting the destructive effects of inflammation, while NSAID inhibition of regenerative signals from the inflammatory process may result in late functional deficits.

piroxicam treatment, including the inhibition of inflammatory cell infiltration, myonecrosis, and collagen deposition but limited myofiber regeneration. Thus, though the NSAID piroxicam improved contractile forces and limited inflammation very early, it is uncertain as to whether the associated inhibition of myofiber regeneration could lead to deficits in functional capacity at later time points post-injury.

To investigate this matter further, Mishra et al. (1995) studied the effects of the NSAID flurbiprofen on eccentric contraction–induced muscle injury in the extensor digitorum longus of the rabbit up to 28 days post-injury. They found that flurbiprofen conveyed a short-term protective effect in the first week with more complete recovery of muscle strength. Yet, again, their results also suggested a delay in the regenerative process between 3 and 7 days. Even though functional recovery was improved at early time points upon flurbiprofen treatment, at 28 days the researchers recorded an unexplained reduction in torque production by the treated muscles. The reason for this occurrence is unknown as no follow-up studies were performed at time points between 7 and 28 days.

When NSAID therapies have been examined in human muscle injuries, more conflicting results have been observed. Reynolds et al. (1995) studied the effects of the NSAIDs meclofenamate and diclofenac in a double-blind, placebo-controlled study of healing after acute hamstring muscle strains. Unexpectedly, the groups with severe muscle strains experienced significantly more persistent pain when treated with the NSAIDs as compared to the control group. Similarly, Bourgeois et al. (1999) found no significant difference in perceived, post-exercise muscle soreness upon treatment with the NSAID naproxen, though significant improvement occurred at 48 hours, including the return of voluntary knee extension torque to baseline. Conversely, a more recent study by Sayers et al. (2001) showed that ketoprofen treatment 36 hours after intense eccentric muscle contraction exercise reduced soreness and improved maximal isometric force production. NSAIDs are typically given to patients after muscle injury to limit pain. Whether or not this practice results in a premature return to activity and subsequent early re-

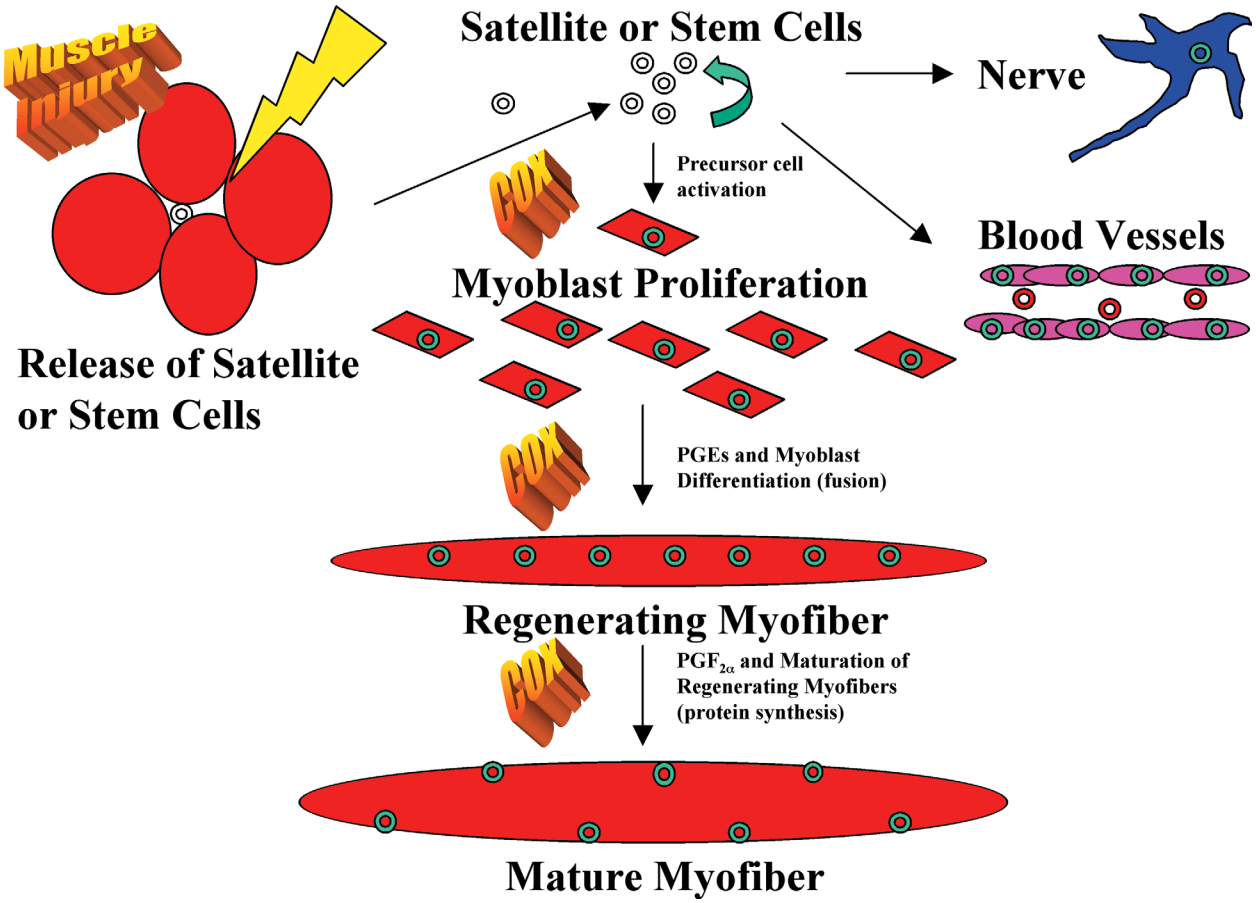

Fig. 3. Muscle injuries result in the release and activation of satellite cells and muscle stem cells that proliferate, fuse, and differentiate into mature myofibers. The muscle stem cells also can differentiate into other tissues, including nerve and blood vessel. Cyclooxygenase activity (COX) by isoform 1 or 2 produces prostaglandins that play a role in each of these processes.

injury of the previously traumatized muscle is unclear.

Making comparisons amongst the many studies using NSAIDs to treat muscle injuries is complex due to a great deal of variability in the experimental methods. Inconsistencies among the mentioned studies are confounded by the use of different NSAIDs with different specificities for various cyclooxygenase isoforms, timing of outcome measurements, dosage and administration routes of drugs, magnitude or type of damaging force (i.e., laceration, contusion, strain), and/or muscle type injured. Intervention with NSAIDS during the early inflammatory process may significantly affect the long-term outcomes of skeletal muscle injuries, and thus necessitates further investigation to understand the effects of inactivating the cyclooxygenase enzymes during skeletal muscle repair.

Prostaglandins and muscle repair biology

In the remainder of this paper, we focus on the biological processes in which prostaglandins are known to play a role during muscle repair. The prostaglandins, which are largely produced during the inflammatory phase after muscle injury, also have a role in the regenerative and fibrosis phases of muscle repair. Mechanistically, the regenerative phase of muscle healing can be broken down into the components of precursor cell activation, proliferation, fusion, and differentiation into mature myofibers (Fig. 3). Through further examination of past research it may be possible to formulate an understanding of the paradoxical early functional improvement and later functional impairment occasionally seen with NSAID therapies.

Prostaglandins in muscle inflammation

Skeletal muscle can produce PGE_1, PGE_2, $PGF_{2\alpha}$, and other prostaglandins both *in situ* and *in vitro* (Young and Sparks, 1979; Berlin et al., 1979; Rodemann and Goldberg, 1982; Palmer et al., 1983; Wennmalm and Fitzgerald, 1988; McLennan, 1991). In particular, PGE_2 concentrations have been shown to be increased in injured or painful muscle (McArdle et al., 1994; Hedenberg-Magnusson et al., 2002). Additionally, both dystrophin-deficient mdx mouse muscle and muscle in patients with Duchenne muscular dystrophy release an increased amount of PGE_2 in response to contractile activity (Jackson et al., 1991; McArdle et al., 1991, 1992). McArdle et al. (1994) further noted that regenerating myofibers display enhanced phospholipase activity and release more PGE_2.

PGE_2 appears to have multiple functions in the muscle inflammatory process, including chemotaxis of inflammatory cells, stimulation of pro-inflammatory cytokines, induction of nitric oxide synthase, and vasodilatation with increased vascular permeability (Murata et al., 1997; Lapointe et al., 2002). Many inflammatory mediators (IL1ß, TNFα, and others) are capable of enhancing PGE_2 production by a variety of cell types (Jourdan et al., 1999; Walch and Morris, 2002; Bradbury et al., 2002). Further enhancement of the inflammatory phase by the presence of PGE_2 may lead to excess degradation of myofibers within the injured area. The added inflammation, pain, disruption of excitation-contraction coupling, and calcium homeostasis attributable to PGE_2 likely reduces early recovery of muscle functional capacity in the injured athlete. Thus, inhibition of this process through the use of NSAIDs could explain the early functional improvement that may occur with these drugs.

Prostaglandins in precursor cell activation and myoblast proliferation

Prostaglandins released during the inflammatory phase contribute toward the microenvironment that overlaps with precursor cell activation and proliferation during the regenerative phase of muscle healing. A complex interaction appears to exist between growth factors and prostaglandins in control of cell proliferation. Past experiments commonly employed a peptide growth factor with a prostaglandin, like $PGF_{2\alpha}$, to achieve the passage of a larger number of cells through the cell cycle (de Asua et al., 1977; O'Farrell et al., 1979). The connection between $PGF_{2\alpha}$ and cell proliferation was described many years ago but is still not completely understood (Taylor and Polgar, 1977). Although it is clear that prostaglandins have a function in cellular proliferation *in vitro*, it is unclear what role prostaglandins play in muscle precursor cell activation and/or subsequent myoblast proliferation and differentiation after injury *in situ*.

Immediately after muscle injury, satellite cells and stem cells within the basal lamina of myofibers are released and activated from the quiescent state to the activated state to divide and eventually participate in the regeneration of myofibers (Rantanen et al., 1995a; Huard et al., 2002). Mitogenic stimulation of multiple quiescent cell types by the addition of serum to medium typically results in increased expression of COX-2 *in vitro* (O'Banion et al., 1992; Pilbeam et al., 1993). This finding correlates with the complex role of COX-2 in the cell cycle dynamics of neoplasia, and one could postulate that COX-2 plays a related role in initiating proliferation of satellite cells or muscle stem cells (Rossi et al., 1989; Steiner et al., 1995; Cao and Prescott, 2002). Moreover, Steiner et al. (1995) noted that the rise in COX-2 protein levels after changing from serum depletion to serum stimulation of myoblasts is only transient, suggesting that COX-2 expression might only be of significance in stimulating re-entry of satellite cells into the cell cycle rather than being essential for continued exponential proliferation of those cells. Studies in human fibroblasts have shown that COX-2 induction by IL1-b is more pronounced in quiescent cells within the G_0 phase than in cells in cycle (Gilroy et al., 2001). Theoretically, NSAID inhibition of COX-2 may lead to a decreased number of active muscle stem cells,

satellite cells, or myofibroblasts contributing to myogenic, vascular, neurologic, or fibrotic tissue synthesis. How this might contribute to early versus late recovery of muscle functional capacity is too complex to decipher at this time.

Prostaglandins in myoblast fusion and differentiation

Cyclooxygenase products not only affect cell proliferation and growth, but also display complex mechanisms in muscle differentiation and maturation. After muscle injury, activated, previously quiescent satellite cells proliferate and fuse to form multinucleated myofibers that later differentiate into mature myofibers (Huard et al., 2002). Early work by Zalin (1977) revealed that NSAIDs prevent cell fusion in cultured chick myoblasts. Zalin (1987) later suggested that a change from $PGF_{2\alpha}$ production to E series prostaglandin production contributed to the transition from the proliferating state to the differentiated state. Overall, the control of myoblast fusion involves a complex interaction between ion channels, prostaglandins, and acetylcholine receptors (Entwistle et al., 1988a,b). Entwistle et al. (1988a) suggested that fusion is controlled by depolarization initiated through activation of the acetylcholine receptor or prostaglandin actions on chloride channels.

Hausman et al. have published numerous studies on the role of prostaglandins in early cell surface events leading to the fusion of myoblasts (Hausman and Velleman, 1981; Hausman et al., 1986, 1990; Hausman and Berggrun, 1987; Santini et al., 1987, 1988; Elgendy and Hausman, 1990). These studies have led to the conclusion that a G-protein–mediated event results from the binding of prostaglandins to their receptors and subsequent membrane organization events, which allow for cell-cell adhesion and fusion into myotubes (Santini et al., 1987, 1988; Hausman et al., 1990; Elgendy and Hausman, 1990). Myoblasts grown in the NSAID indomethacin fail to differentiate into myotubes, and this block of cell-cell adhesion can be reversed via addition of exogenous prostaglandin to the cell culture medium (Santini et al., 1988). Furthermore, administration of indomethacin or aspirin to chick embryos has been found to decrease the number of myonuclei incorporated into the embryo muscles (McLennan, 1987b). Overall, the prostaglandins appear to be important mediators of myoblast fusion and formation of multinucleated myofibers.

Schutzle et al. (1984) have suggested that E series prostaglandins may contribute to muscle differentiation by stimulating the production of muscle-specific proteins. They observed that indomethacin treatment reduced creatine kinase accumulation in cultured chick myotubes and that this outcome could be reversed by adding PGE_1 and PGE_2 to the medium (Schutzle et al., 1984). Similarly, chick embryos treated with aspirin or indomethacin develop disrupted myofibrils and lack creatine kinase, resulting in a muscular dystrophy-like myopathy (McLennan, 1985, 1987a). McLennan (1987a) also observed the loss of both thick and thin filaments from the myofibrillar apparatus with abnormal contacts between the developing myofibers. The simultaneous administration of PGE_1 reversed the effects of the NSAIDs on creatine kinase levels and muscle structure (Schutzle et al., 1984; McLennan, 1985, 1987a). Even though prostaglandins may be essential for embryonic myogenesis, their importance to adult muscle cell differentiation from myoblast to myofiber after injury is unclear. For instance, Thorsson et al. (1998) used a rat gastrocnemius contusion injury model to demonstrate that intramuscular injection of the NSAID naproxen did not appear to affect the formation of regenerating myofibers from satellite cells. In looking at the NSAID paradox of early versus late effects on the recovery of functional capacity in injured muscle, it is conceivable that inhibition or delay of myoblast differentiation due to inhibition of prostaglandin synthesis could lead to demonstrated losses at later time points.

Prostaglandins and muscle protein synthesis

$PGF_{2\alpha}$ and PGE_2 appear to perform conflicting actions when it comes to protein turnover rates in skeletal muscle. Early on it was discovered that PGE_2 could lead to net protein degradation, possibly via activation of the lysosomal apparatus, and that $PGF_{2\alpha}$ played a role in stimulating muscle protein synthesis and growth, especially under insulin stimulation (Palmer et al., 1989; Thompson et al., 1993; Hussey and Tisdale, 2000). Additionally, prostaglandins appear to act as messengers in the regulation of tension-induced muscle protein turnover rates (Smith et al., 1983; Palmer et al., 1983; Vandenburgh et al., 1990, 1995; Trappe et al., 2002). McMillan et al. (1987) showed that a stimulus for muscle hypertrophy increased muscle protein synthesis and that the NSAID fenbufen inhibited this increase. Furthermore, Trappe et al. (2002) reported that over-the-counter dosing of both ibuprofen and acetaminophen could suppress the normal rise in protein synthesis that occurs after eccentric contraction exercise in humans. Their follow-up studies revealed that $PGF_{2\alpha}$ levels were elevated significantly in human vastus lateralis biopsies following a high intensity eccentric contraction exercise protocol and that these levels could be reduced by both ibuprofen and acetaminophen (Trappe et al., 2001).

Vandenburgh et al. (1993) revealed that more than 90% of the prostaglandin production during muscle stretch arises from the myofibers rather than from fibroblasts. Vandenburgh et al. (1995) further demonstrated that cyclooxygenase activity and $PGF_{2\alpha}$ production are elevated greatly within 24 hours by mechanical stretch of mature avian myoblast cultures. Moreover, the stretched skeletal myofibers displayed higher COX-2 expression as compared to nearly undetectable constitutive COX-1 expression. Although conflicting studies exist, the aforementioned data suggest that the inducible COX-2 enzyme and its

products play an important role in muscle protein synthesis and that inhibition of this enzyme may lead to a reduction in the accumulation of contractile or bioenergetic proteins required for later recovery of full functional capacity (Turinsky and Loegering, 1985; McKinley and Turinsky, 1986; Barnett and Ellis, 1987; McElligott et al., 1988).

Prostaglandins and muscle fibrosis

Work in our laboratory has demonstrated that TGF-ß1 is a significant contributor to the formation of scar tissue in injured muscle (Li and Huard, 2002). The relationship between inflammation and scar tissue formation has been extensively studied in models of pulmonary fibrosis and wound healing. However, the role of inflammatory prostaglandins in the formation of muscle fibrosis after injury is undefined. By looking at other tissue types, however, it is possible to theorize how prostaglandins and myofibroblasts might interact after muscle injury.

Multiple studies have demonstrated a lack of inflammation in early trimester fetal wound healing that correlates with scarless repair (Liechty et al., 2000a,b). Likewise, several studies have shown that the addition of pro-inflammatory cytokines, PGE_2, and TGF-ß1 transformed the process of scarless wound healing into one of fibrotic scar formation (Haynes et al., 1994; Lanning et al., 2000). As previously mentioned, Wilgus et al. (2003) demonstrated that the COX-2 specific inhibitor celecoxib reduced scar tissue formation in skin wounds with a concomitant reduction in inflammatory cell infiltration and TGF-ß1 production. However, it remains to be determined whether inhibition of COX-2 and inflammation limits the formation of scar tissue after injury in muscle as it appears to do in skin wounds.

As previously mentioned in regards to satellite cells, COX-2 appears to play a role in mitogen-stimulated fibroblast cell proliferation (Kujubu et al., 1991; Scheuren et al., 2002; Frungieri et al., 2002). The COX-2 product, PGE_2, is the most prevalent prostaglandin produced by fibroblasts and is a potent regulator of TGF-ß1–stimulated fibroblast proliferation and collagen synthesis (Saltzman et al., 1982; Goldstein and Polgar, 1982; Elias, 1988; McAnulty et al., 1997). Although PGE2 has been shown to increase fibroblast proliferation and collagen production, higher concentrations of PGE2 play a negative feedback role on stimulators of collagen synthesis in fibroblasts (Goldstein and Polgar, 1982; Elias, 1988; McAnulty et al., 1997). In fact, TGF-ß1 upregulates COX-2 expression and increases PGE_2 production in fibroblasts (Diaz et al., 1989; Keerthisingam et al., 2001). Lower concentrations of PGE_2 appear to contribute to fibroblast proliferation and collagen production, while higher concentrations inhibit this process. Because regenerating skeletal muscle produces high levels of PGE2, it is difficult to determine whether the fibrotic phase of muscle repair is augmented or inhibited by COX-2 specific inhibition. All things considered, the role of cyclooxygenases and prostaglandins in TGF-ß1 negative feedback, combined with their roles in fibroblast proliferation and extracellular matrix synthesis, makes it difficult to predict the effect of NSAIDs on muscle fibrosis.

Conclusion

In 1971, Vane proposed that aspirin-like drugs inhibit cyclooxygenase and thus block the formation of the prostaglandins, essential mediators of inflammation (Vane, 1971). These aspirin-like drugs, now known as NSAIDs, since have been shown to perform actions independent of their inhibition of cyclooxygenase (Tegeder et al., 2001). The role of NSAIDs in the inhibition of NF-κB and many other transcription factors strengthens the argument that there is much more behind the anti-inflammatory action of NSAIDs than simply changes in prostaglandin synthesis (Tegeder et al., 2001). Some NSAIDs act directly on the cell membrane to alter fluidity (Abramson and Weissmann, 1989). Thus, NSAIDs may independently influence immune cell proliferation, differentiation, lysosomal enzyme release, and chemotaxis without affecting cyclooxygenase. In trying to understand the role of prostaglandins in muscle cell biology, it is best to take into consideration which NSAIDs are being used to manipulate the synthetic pathways. Most of the studies mentioned in this review used non-selective COX inhibitors that limit the synthesis of COX-1 and COX-2 products. We are unaware of any studies examining post-traumatic muscle repair with new, more selective COX-2 inhibitors.

There are a few aspects of this review that should be highlighted. First, the possibility that COX-2 may drive the quiescent cell into an activated state is of great interest. Our laboratory has characterized a population of muscle-derived stem cells that display an improved transplantation capacity (relative to satellite cells) partially attributable to their high self-renewal ability and multipotent behavior (Qu-Petersen et al., 2002). An improved understanding of the physiologic processes driving the proliferation and differentiation of these cells during the regeneration of musculoskeletal tissues should prove quite valuable to the basic and clinical sciences. Second, it appears as though COX-2 and its products are important mediators of cellular responses to growth factors. Thus, the prostaglandins produced during the inflammatory phase after muscle injury may play an integral role in the subsequent regenerative and fibrotic phases of muscle healing. Research has clearly shown that NSAID inhibition of prostaglandin production after muscle injury results in changes in the regenerative process and long-term deficits in muscle functional capacity. The exact mechanism by which this occurs remains unknown, but disruption of growth factor–driven processes is a likely culprit. Third, it appears as though COX-2 and the prostaglandins have an important function with regards to the formation of fibrosis within diseased tissues. Whether or not they play

a similar role in injured skeletal muscle is unclear.

Recent studies have already shown that the acute administration of COX-2 inhibitors can limit the healing of fractured bone (Simon et al., 2002). Is it possible that the same will occur with muscle healing? Will the COX-2 inhibitors limit muscle fibrosis after injury as they appear to do in skin wound healing? By using specific COX-1 or COX-2 inhibitors, as well as COX-1 and -2 knockout mice, we are currently attempting to clarify some of the mechanisms behind the paradox of early functional improvement and late functional impairment observed with use of NSAIDs in muscle injuries and repair.

Acknowledgements. The authors wish to thank Ryan Sauder for his editorial assistance with the manuscript.

References

Abramson S.B. and Weissmann G. (1989). The mechanisms of action of nonsteroidal antiinflammatory drugs. Arthritis Rheum. 32, 1-9.

Almekinders L.C. (1999). Anti-inflammatory treatment of muscular injuries in sport. An update of recent studies. Sports Med. 28, 383-388.

Almekinders L.C. and Gilbert J.A. (1986). Healing of experimental muscle strains and the effects of nonsteroidal antiinflammatory medication. Am. J. Sports Med. 14, 303-308.

Amemiya K., Semino-Mora C., Granger R.P. and Dalakas M.C. (2000). Downregulation of TGF-beta1 mRNA and protein in the muscles of patients with inflammatory myopathies after treatment with high-dose intravenous immunoglobulin. Clin. Immunol. 94, 99-104.

Barash I.A., Peters D., Friden J., Lutz G.J. and Lieber R.L. (2002). Desmin cytoskeletal modifications after a bout of eccentric exercise in the rat. Am. J. Physiol. Regul. Integr. Comp. Physiol. 283, R958-R963.

Barnett J.G. and Ellis S. (1987). Prostaglandin E2 and the regulation of protein degradation in skeletal muscle. Muscle Nerve 10, 556-559.

Beaton L.J., Allan D.A., Tarnopolsky M.A., Tiidus P.M. and Phillips S.M. (2002). Contraction-induced muscle damage is unaffected by vitamin E supplementation. Med. Sci. Sports Exerc. 34, 798-805.

Beckman J.S. and Koppenol W.H. (1996). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am. J. Physiol. 271, C1424-C1437.

Beiner J.M., Jokl P., Cholewicki J. and Panjabi M.M. (1999). The effect of anabolic steroids and corticosteroids on healing of muscle contusion injury. Am. J. Sports Med. 27, 2-9.

Berlin T., Cronestrand R., Nowak J., Sonnenfeld T. and Wennmalm A. (1979). Conversion of arachidonic acid to prostaglandins in homogenates of human skeletal muscle and kidney. Acta Physiol. Scand. 106, 441-445.

Bernasconi P., Di Blasi C., Mora M., Morandi L., Galbiati S., Confalonieri P., Cornelio F. and Mantegazza R. (1999). Transforming growth factor-beta1 and fibrosis in congenital muscular dystrophies. Neuromuscul. Disord. 9, 28-33.

Bischoff R. (1994). The satellite cell and muscle regeneration. In: Myology. Basic and Clinical. 2nd ed. Engel A.G. and Franzini-Armstrong C. (eds). McGraw-Hill. New York. pp 97-118.

Botting R.M. (2000). Mechanism of action of acetaminophen: is there a cyclooxygenase 3? Clin. Infect. Dis. 31, S202-S210.

Bourgeois J., MacDougall D., MacDonald J. and Tarnopolsky M. (1999). Naproxen does not alter indices of muscle damage in resistance-exercise trained men. Med. Sci. Sports Exerc. 31, 4-9.

Bradbury D.A., Newton R., Zhu Y.M., Stocks J., Corbett L., Holland E.D., Pang L.H. and Knox A.J. (2002). Effect of bradykinin, TGF-beta1, IL-1beta, and hypoxia on COX-2 expression in pulmonary artery smooth muscle cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 283, L717-L725.

Broekelmann T.J., Limper A.H., Colby T.V. and McDonald J.A.(1991). Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. Proc. Natl. Acad. Sci. USA 88, 6642-6646.

Cao Y. and Prescott S.M. (2002). Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. J. Cell. Physiol. 190, 279-286.

Carpenter S. and Karpati G. (1989). Segmental necrosis and its demarcation in experimental micropuncture injury of skeletal muscle fibers. J. Neuropathol. Exp. Neurol. 48, 154-170.

Chan Y., Li Y., Horaguchi T. and Foster W. (2002) The antifibrotic effects of suramin in injured skeletal muscle [abstr]. 48th Annual Meeting of the Orthopaedic Research Society. Dallas, TX.

Chandrasekharan N.V., Dai H., Roos K.L., Evanson N.K., Tomsik J., Elton T.S. and Simmons D.L. (2002). COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc. Natl. Acad. Sci. USA 99, 13926-13931.

Childs A., Jacobs C., Kaminski T., Halliwell B. and Leeuwenburgh C. (2001). Supplementation with vitamin C and N-acetyl-cysteine increases oxidative stress in humans after an acute muscle injury induced by eccentric exercise. Free Radic. Biol. Med. 31, 745-753.

Clanton T.L., Zuo L. and Klawitter P. (1999). Oxidants and skeletal muscle function: physiologic and pathophysiologic implications. Proc. Soc. Exp. Biol. Med. 222, 253-262.

Confalonieri P., Bernasconi P., Cornelio F. and Mantegazza R. (1997). Transforming growth factor-beta 1 in polymyositis and dermatomyositis correlates with fibrosis but not with mononuclear cell infiltrate. J. Neuropathol. Exp. Neurol. 56, 479-484.

Croisier J.L., Forthomme B., Namurois M.H., Vanderthommen M. and Crielaard J.M. (2002). Hamstring muscle strain recurrence and strength performance disorders. Am. J. Sports Med. 30, 199-203.

Czaja M.J., Weiner F.R., Flanders K.C., Giambrone M.A., Wind R., Biempica L. and Zern M.A. (1989). In vitro and in vivo association of transforming growth factor-beta 1 with hepatic fibrosis. J. Cell Biol. 108, 2477-2482.

Damon S.E., Haugk K.L., Birnbaum R.S. and Quinn L.S. (1998). Retrovirally mediated over-expression of insulin-like growth factor binding protein 4: evidence that insulin-like growth factor is required for skeletal muscle differentiation. J. Cell. Physiol. 175, 109-120

Day C.S., Buranapanitkit B., Riano F.A., Tomaino M.M., Somogyi G., Sotereanos D.G., Kuroda R. and Huard J. (2002). Insulin growth factor-1 decreases muscle atrophy following denervation. Microsurgery 22, 144-151.

de Asua L.J., O'Farrell M.K., Clingan D. and Rudland P.S. (1977). Temporal sequence of hormonal interactions during the prereplicative phase of quiescent cultured 3T3 fibroblasts. Proc. Natl. Acad. Sci. USA 74, 3845-3849.

Deasy B.M., Qu-Peterson Z., Greenberger J.S. and Huard J. (2002). Mechanisms of muscle stem cell expansion with cytokines. Stem Cells 20, 50-60.

Diaz A., Varga J. and Jimenez S.A. (1989). Transforming growth factor-beta stimulation of lung fibroblast prostaglandin E2 production. J. Biol. Chem. 264, 11554-11557.

Doumit M.E., Cook D.R. and Merkel R.A. (1993). Fibroblast growth factor, epidermal growth factor, insulin-like growth factors, and platelet-derived growth factor-BB stimulate proliferation of clonally derived porcine myogenic satellite cells. J. Cell. Physiol. 157, 326-332.

Ebisui C., Tsujinaka T., Morimoto T., Kan K., Iijima S., Yano M., Kominami E., Tanaka K. and Monden M. (1995). Interleukin-6 induces proteolysis by activating intracellular proteases (cathepsins B and L, proteasome) in C2C12 myotubes. Clin. Sci. (Lond). 89, 431-439.

Elgendy H. and Hausman R.E. (1990). Prostaglandin-dependent phosphatidylinositol signaling during embryonic chick myogenesis. Cell Differ. Dev. 32, 109-115.

Elias J.A. (1988). Tumor necrosis factor interacts with interleukin-1 and interferons to inhibit fibroblast proliferation via fibroblast prostaglandin-dependent and -independent mechanisms. Am. Rev. Respir. Dis. 138, 652-658.

Engert J.C., Berglund E.B. and Rosenthal N. (1996). Proliferation precedes differentiation in IGF-1 stimulated myogenesis. J. Cell Biol. 135, 431-440.

Entwistle A., Zalin R.J., Bevan S. and Warner A.E. (1988a). The control of chick myoblast fusion by ion channels operated by prostaglandins and acetylcholine. J. Cell Biol. 106, 1693-1702.

Entwistle A., Zalin R.J., Warner A.E. and Bevan S. (1988b). A role for acetylcholine receptors in the fusion of chick myoblasts. J. Cell Biol. 106, 1703-1712.

Fielding R.A., Manfredi T.J., Ding W., Fiatarone M.A., Evans W.J. and Cannon J.G. (1993). Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 265, R166-R172.

Foster W., Li Y., Usas A., Somogyi G. and Huard J. (2003). Gamma interferon as an antifibrosis agent in skeletal muscle. J. Orthop. Res. (In press).

Frenette J., Cai B. and Tidball J. (2000). Complement activation promotes muscle inflammation during modified muscle use. Am. J. Pathol. 156, 2103-2110.

Frungieri M.B., Weidinger S., Meineke V., Kohn F.M. and Mayerhofer A. (2002). Proliferative action of mast-cell tryptase is mediated by PAR2, COX2, prostaglandins, and PPARgamma: possible relevance to human fibrotic disorders. Proc. Natl. Acad. Sci. USA 99, 15072-15077.

Fukushima K., Badlani N., Usas A., Riano F., Fu F. and Huard J. (2001). The use of an antifibrosis agent to improve muscle recovery after laceration. Am. J. Sports Med. 29, 394-402.

Garrett W.E. (1996). Muscle strain injuries. Am. J. Sports Med. 24, S2-S8.

Gilroy D.W., Saunders M.A. and Wu K.K. (2001). COX-2 expression and cell cycle progression in human fibroblasts. Am. J. Physiol. Cell Physiol. 281, C188-C194.

Goldstein R.H. and Polgar P. (1982). The effect and interaction of bradykinin and prostaglandins on protein and collagen production by lung fibroblasts. J. Biol. Chem. 257, 8630-8633.

Hausman R.E. and Berggrun D.A. (1987). Prostaglandin binding does not require direct cell-cell contact during chick myogenesis in vitro. Exp. Cell Res. 168, 457-462.

Hausman R.E., Dobi E.T., Woodford E.J., Petrides S., Ernst M. and Nichols E.B. (1986). Prostaglandin binding activity and myoblast fusion in aggregates of avian myoblasts. Dev. Biol. 113, 40-48.

Hausman R.E., Elgendy H. and Craft F. (1990). Requirement for G protein activity at a specific time during embryonic chick myogenesis. Cell Differ. Dev. 29, 13-20.

Hausman R.E. and Velleman S.G. (1981). Prostaglandin E1 receptors on chick embryo myoblasts. Biochem. Biophys. Res. Commun. 103, 213-218.

Haynes J.H., Johnson D.E., Mast B.A., Diegelmann R.F., Salzberg D.A., Cohen I.K. and Krummel T.M. (1994). Platelet-derived growth factor induces fetal wound fibrosis. J. Pediatr. Surg. 29, 1405-1408.

Hedenberg-Magnusson B., Ernberg M., Alstergren P. and Kopp S. (2002). Effect on prostaglandin E2 and leukotriene B4 levels by local administration of glucocorticoid in human masseter muscle myalgia. Acta Odontol. Scand. 60, 29-36.

Huard J., Li Y. and Fu F.H. (2002). Muscle injuries and repair: current trends in research. J. Bone Joint Surg. Am. 84-A, 822-832.

Hughes C. 4th, Hasselman C.T., Best T.M., Martinez S. and Garrett W.E. (1995). Incomplete, intrasubstance strain injuries of the rectus femoris muscle. Am. J. Sports Med. 23, 500-506.

Hurme T., Kalimo H., Lehto M. and Jarvinen M. (1991). Healing of skeletal muscle injury: an ultrastructural and immunohistochemical study. Med. Sci. Sports Exerc. 23, 801-810.

Hurme T. and Kalimo H. (1992). Activation of myogenic precursor cells after muscle injury. Med. Sci. Sports Exerc. 24, 197-205.

Hussey H.J. and Tisdale M.J. (2000). Effect of the specific cyclooxygenase-2 inhibitor meloxicam on tumour growth and cachexia in a murine model. Int. J. Cancer 87, 95-100.

Ina K., Kitamura H., Tatsukawa S., Takayama T., Fujikura Y. and Shimada T. (2002). Transformation of interstitial fibroblasts and tubulointerstitial fibrosis in diabetic nephropathy. Med. Electron Microsc. 35, 87-95.

Jackson M.J., Brooke M.H., Kaiser K. and Edwards R.H. (1991). Creatine kinase and prostaglandin E2 release from isolated Duchenne muscle. Neurology 41, 101-104.

Jourdan K.B., Evans T.W., Goldstraw P. and Mitchell J.A.(1999). Isoprostanes and PGE2 production in human isolated pulmonary artery smooth muscle cells: concomitant and differential release. FASEB J. 13, 1025-1030.

Kaariainen M., Jarvinen T., Jarvinen M., Rantanen J. and Kalimo H. (2000). Relation between myofibers and connective tissue during muscle injury repair. Scand. J. Med. Sci. Sports 10, 332-337.

Kasemkijwattna C., Menetrey J., Bosch P., Moreland M.S., Fu F., Buranapanitkit B., Watkins S.C. and Huard J. (2000). The use of growth factors to improve muscle healing after strain injury. Clin. Orthop. 370, 272-285.

Kasemkijwattana C., Menetrey J., Somogyi G., Moreland M.S., Fu F., Buranapanitkit B., Watkins S.C. and Huard J. (1998). Development of approaches to improve the healing following muscle contusion. Cell Transplant. 7, 585-598.

Keerthingam C.B., Jenkins R.G. Harrison N.K., Hernandez-Rodriguez N.A., Booth H., Laurent G.J., Hart S.L., Foster M.L. and McAnulty R.J. (2001). Cyclooxygenase-2 deficiency results in a loss of the anti-prloiferative response to transforming growth factor-beta in human fibrotic lung fibroblasts and promotes bleomycin-induced pulmonary fibrosis in mice. Am. J. Pathol. 158, 1411-1422.

Kellett J. (1986). Acute soft tissue injuries—a review of the literature. Med. Sci. Sports Exerc. 18, 489-500.

Khalil N., Whitman C., Zuo L., Danielpour D. and Greenberg A. (1993).

Regulation of alveolar macrophage transforming growth factor-beta secretion by corticosteroids in bleomycin-induced pulmonary inflammation in the rat. J. Clin. Invest. 92, 1812-1818.

Kujubu D.A., Fletcher B.S., Varnum B.C., Lim R.W. and Herschman H.R. (1991). TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J. Biol. Chem. 266, 12866-12872.

Langley B., Thomas M., Bishop A., Sharma M., Gilmour S. and Kambadur R. (2002). Myostatin inhibits myoblast differentiation by down regulating MyoD expression. J. Biol. Chem. 277, 49831-49840.

Lanning D.A., Diegelmann R.F., Yager D.R., Wallace M.L., Bagwell C.E. and Haynes J.H. (2000). Myofibroblast induction with transforming growth factor-beta1 and -beta3 in cutaneous fetal excisional wounds. J. Pediatr. Surg. 35, 183-187.

Lapointe B.M., Frenette J. and Cote C.H. (2002). Lengthening contraction-induced inflammation is linked to secondary damage but devoid of neutrophil invasion. J. Appl. Physiol. 92, 1995-2004.

Lewis A.J., Nelson D.J. and Sugrue M.F. (1974). Proceedings: Potentiation by prostaglandin E1 and arachidonic acid of oedema in the rat paw induced by various phlogogenic agents. Br. J. Pharmacol. 50, 468P-469P.

Lewis A.J., Nelson D.J. and Sugrue M.F.(1975). On the ability of prostaglandin E1, and arachidonic acid to modulate experimentally induced oedema in the rat paw. Br. J. Pharmacol. 55, 51-56.

Li Y. and Huard J. (2002). Differentiation of muscle-derived cells into myofibroblasts in injured skeletal muscle. Am. J. Pathol. 161, 895-907.

Lieber R.L., Shah S. and Friden J. (2002). Cytoskeletal disruption after eccentric contraction-induced muscle injury. Clin. Orthop. 403, S90-S99.

Liechty K.W., Adzick N.S. and Crombleholme T.M. (2000a). Diminished interleukin 6 (IL-6) production during scarless human fetal wound repair. Cytokine 12, 671-676.

Liechty K.W., Kim H.B., Adzick N.S. and Crombleholme T.M. (2000b). Fetal wound repair results in scar formation in interleukin-10-deficient mice in a syngeneic murine model of scarless fetal wound repair. J. Pediatr. Surg. 35, 866-872.

Lijnen P.J., Petrov V.V. and Fagard R.H. (2000). Induction of cardiac fibrosis by transforming growth factor-beta(1). Mol. Genet. Metab. 71, 418-435.

Logan A., Berry M., Gonzalez A.M., Frautschy S.A., Sporn M.B. and Baird A. (1994). Effects of transforming growth factor beta 1 on scar production in the injured central nervous system of the rat. Eur. J. Neurosci. 6, 355-363.

MacIntyre D.L., Reid W.D., Lyster D.M. and McKenzie D.C. (2000). Different effects of strenuous eccentric exercise on the accumulation of neutrophils in muscle in women and men. Eur. J. Appl. Physiol. 81, 47-53.

MacIntyre D.L., Reid W.D., Lyster D.M., Szasz I.J. and McKenzie D.C. (1996). Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise. J. Appl. Physiol. 80, 1006-1013.

Mbebi C., Hantai D., Jandrot-Perrus M., Doyennette M.A. and Verdiere-Sahuque M. (1999). Protease nexin I expression is up-regulated in human skeletal muscle by injury-related factors. J. Cell. Physiol. 179, 305-314.

McAnulty R.J., Hernandez-Rodriguez N.A., Mutsaers S.E., Coker R.K. and Laurent G.J. (1997). Indomethacin suppresses the anti-proliferative effects of transforming growth factor-beta isoforms on fibroblast cell cultures. Biochem. J. 321, 639-643.

McArdle A., Edwards R.H. and Jackson M.J. (1991). Effects of contractile activity on muscle damage in the dystrophin-deficient mdx mouse. Clin. Sci. (Lond). 80, 367-371.

McArdle A., Edwards R.H. and Jackson M.J. (1992). Accumulation of calcium by normal and dystrophin-deficient mouse muscle during contractile activity in vitro. Clin. Sci. (Lond). 82, 455-459.

McArdle A., Edwards R.H. and Jackson MJ. (1994). Release of creatine kinase and prostaglandin E2 from regenerating skeletal muscle fibers. J. Appl. Physiol. 76, 1274-1278.

McElligott M.A., Chaung L.Y., Baracos V. and Gulve E.A. (1988). Prostaglandin production in myotube cultures. Influence on protein turnover. Biochem. J. 253, 745-749.

McKinley C.J. and Turinsky J. (1986). Prostaglandin E2 and muscle proteolysis: effect of burn injury and cycloheximide. Am. J. Physiol. 250, R207-R210.

McLennan I.S. (1985). Inhibition of prostaglandin synthesis produces a muscular dystrophy-like myopathy. Exp. Neurol. 89, 616-621.

McLennan I.S. (1987a). Characterization of a prostaglandin dysfunction myopathy. Muscle Nerve 10, 801-809.

McLennan I.S. (1987b). Hormonal regulation of myoblast proliferation and myotube production in vivo: influence of prostaglandins. J. Exp. Zool. 241, 237-245.

McLennan I.S. (1991). E and F alpha series prostaglandins in developing muscles. Prostaglandins Leukot. Essent. Fatty Acids 43, 77-82.

McLennan I.S. (1993). Resident macrophages (ED2- and ED3-positive) do not phagocytose degenerating rat skeletal muscle fibres. Cell Tissue Res. 272, 193-196.

McMillan D.N., Reeds P.J., Lobley G.E. and Palmer R.M. (1987). Changes in protein turnover in hypertrophying plantaris muscles of rats: effect of fenbufen—an inhibitor of prostaglandin synthesis. Prostaglandins 34, 841-852.

Mendler L., Zador E., Ver Heyen M., Dux L. and Wuytack F. (2000). Myostatin levels in regenerating rat muscles and in myogenic cell cultures. J. Muscle Res. Cell Motil. 21, 551-563.

Menetrey J., Kasemkijwattana C., Day C.S., Bosch P., Vogt M., Fu F.H., Moreland M.S. and Huard J. (2000). Growth factors improve muscle healing in vivo. J. Bone Joint Surg. Br. 82, 131-137.

Menetrey J., Kasemkijwattana C., Fu F.H., Moreland M.S. and Huard J. (1999). Suturing versus immobilization of a muscle laceration. A morphological and functional study in a mouse model. Am. J. Sports Med. 27, 222-229.

Mense S. (1981). Sensitization of group IV muscle receptors to bradykinin by 5-hydroxytryptamine and prostaglandin E2. Brain Res. 225, 95-105.

Mishra D.K., Friden J., Schmitz M.C. and Lieber R.L. (1995). Anti-inflammatory medication after muscle injury. A treatment resulting in short-term improvement but subsequent loss of muscle function. J. Bone Joint Surg. Am. 77, 1510-1519.

Murakami M., Nakatani Y., Kuwata H. and Kudo I. (2000a). Cellular components that functionally interact with signaling phospholipase A(2)s. Biochim. Biophys. Acta 1488, 159-166.

Murakami M., Naraba H., Tanioka T., Semmyo N., Nakatani Y., Kojima F., Ikeda T., Fueki M., Ueno A., Oh S. and Kudo I. (2000b). Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in

concert with cyclooxygenase-2. J. Biol. Chem. 275, 32783-32792.

Murata T., Ushikubi F., Matsuoka T., Hirata M., Yamasaki A., Sugimoto Y., Ichikawa A., Aze Y., Tanaka T., Yoshida N., Ueno A., Oh-ishi S. and Narumiya S. (1997). Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. Nature. 388, 678-682.

O'Banion M.K., Winn V.D. and Young D.A. (1992). cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc. Natl. Acad. Sci. USA 89, 4888-4892.

Obremsky W.T., Seaber A.V., Ribbeck B.M. and Garrett W.E. Jr. (1994). Biomechanical and histologic assessment of a controlled muscle strain injury treated with piroxicam. Am. J. Sports Med. 22, 558-561.

O'Farrell M.K., Clingan D., Rudland P.S. and Jimenez de Asua L. (1979). Stimulation of the initiation of DNA synthesis and cell division in several cultured mouse cell types. Effect of growth-promoting hormones and nutrients. Exp. Cell. Res. 118, 311-321.

Orchard J. and Best T. (2002). The management of muscle strain injuries: an early return versus the risk of recurrence. Clin. J. Sport Med. 12, 3-5.

Orimo S., Hiyamuta E., Arahata K. and Sugita H. (1991). Analysis of inflammatory cells and complement C3 in bupivacaine-induced myonecrosis. Muscle Nerve. 14, 515-520.

Palmer R.M., Campbell G.P., Whitelaw P.F., Brown D.S., Bain P.A. and Hesketh J.E. (1989). The cyclo-oxygenase inhibitors indomethacin and ibuprofen inhibit the insulin-induced stimulation of ribosomal RNA synthesis in L6 myoblasts. Biochem. J. 264, 101-106.

Palmer R.M., Reeds P.J., Atkinson T. and Smith R.H. (1983). The influence of changes in tension on protein synthesis and prostaglandin release in isolated rabbit muscles. Biochem. J. 214, 1011-1014.

Paoni N.F., Peale F., Wang F., Errett-Baroncini C., Steinmetz H., Toy K., Bai W., Williams M., Bunting S., Gerritsen M.E. and Powell-Braxton L. (2002). Time course of skeletal muscle repair and gene expression following acute hind limb ischemia in the mouse. Physiol. Genomics. 11, 263-272.

Pilbeam C.C., Kawaguchi H., Hakeda Y., Voznesensky O., Alander C.B. and Raisz L.G. (1993). Differential regulation of inducible and constitutive prostaglandin endoperoxide synthase in osteoblastic MC3T3-E1 cells. J. Biol. Chem. 268, 25643-25649.

Qu-Petersen Z., Deasy B., Jankowski R., Ikezawa M., Cummins J., Pruchnic R., Mytinger J., Cao B., Gates C., Wernig A. and Huard J. (2002). Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. J. Cell. Biol. 157, 851-864.

Rantanen J., Ranne J., Hurme T. and Kalimo H. (1995a). Satellite cell proliferation and expression of myogenin and desmin in regenerating skeletal muscle: evidence for two different populations of satellite cells. Lab. Invest. 72, 341-347.

Rantanen J., Ranne J., Hurme T. and Kalimo H. (1995b). Denervated segments of injured skeletal muscle fibers are reinnervated by newly formed neuromuscular junctions. J. Neuropathol. Exp. Neurol. 54, 188-194.

Rantanen J., Thorsson O., Wollmer P., Hurme T. and Kalimo H. (1999). Effects of therapeutic ultrasound on the regeneration of skeletal myofibers after experimental muscle injury. Am. J. Sports Med. 27, 54-59.

Reynolds J.F., Noakes T.D., Schwellnus M.P., Windt A. and Bowerbank P. (1995). Non-steroidal anti-inflammatory drugs fail to enhance healing of acute hamstring injuries treated with physiotherapy. S. Afr. Med. J. 85, 517-522.

Rios R., Carneiro I., Arce V.M. and Devesa J. (2002). Myostatin is an inhibitor of myogenic differentiation. Am. J. Physiol. Cell Physiol. 282, C993-C999.

Rodemann H.P. and Goldberg A.L. (1982). Arachidonic acid, prostaglandin E2 and F2 alpha influence rates of protein turnover in skeletal and cardiac muscle. J. Biol. Chem. 257, 1632-1638.

Rossi M.J., Clark M.A. and Steiner S.M. (1989). Possible role of prostaglandins in the regulation of mouse myoblasts. J. Cell. Physiol. 141, 142-147.

Salminen A. and Kihlstrom M. (1987). Protective effect of indomethacin against exercise-induced injuries in mouse skeletal muscle fibers. Int. J. Sports Med. 8, 46-49.

Saltzman L.E., Moss J., Berg R.A., Hom B. and Crystal R.G. (1982). Modulation of collagen production by fibroblasts. Effects of chronic exposure to agonists that increase intracellular cyclic AMP. Biochem. J. 204, 25-30.

Samad T.A., Moore K.A., Sapirstein A., Billet S., Allchorne A., Poole S., Bonventre J.V. and Woolf C.J. (2001). Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature. 410, 471-475.

Santini M.T., Indovina P.L. and Hausman R.E. (1987). Changes in myoblast membrane order during differentiation as measured by EPR. Biochim. Biophys. Acta 896, 19-25.

Santini M.T., Indovina P.L. and Hausman R.E. (1988). Prostaglandin dependence of membrane order changes during myogenesis in vitro. Biochim. Biophys. Acta 938, 489-492.

Sayers S.P., Knight C.A., Clarkson P.M., Van Wegen E.H. and Kamen G. (2001). Effect of ketoprofen on muscle function and sEMG activity after eccentric exercise. Med. Sci. Sports Exerc. 33, 702-710.

Scheuren N., Jacobs M., Ertl G. and Schorb W. (2002). Cyclooxygenase-2 in myocardium stimulation by angiotensin-II in cultured cardiac fibroblasts and role at acute myocardial infarction. J. Mol. Cell. Cardiol. 34, 29-37.

Schutzle U.B., Wakelam M.J. and Pette D. (1984). Prostaglandins and cyclic AMP stimulate creatine kinase synthesis but not fusion in cultured embryonic chick muscle cells. Biochim. Biophys. Acta. 805, 204-210.

Seeland U., Haeuseler C., Hinrichs R., Rosenkranz S., Pfitzner T., Scharffetter-Kochanek K. and Bohm M. (2002). Myocardial fibrosis in transforming growth factor-beta(1) (TGF-beta(1)) transgenic mice is associated with inhibition of interstitial collagenase. Eur. J. Clin. Invest. 32, 295-303.

Sheehan S.M. and Allen R.E. (1999). Skeletal muscle satellite cell proliferation in response to members of the fibroblast growth factor family and hepatocyte growth factor. J. Cell. Physiol. 181, 499-506.

Simon A.M., Manigrasso M.B. and O'Connor J.P. (2002). Cyclo-oxygenase 2 function is essential for bone fracture healing. J. Bone Miner. Res. 17, 963-976.

Sinatra R. (2002). Role of COX-2 inhibitors in the evolution of acute pain management. J. Pain Symptom Manage. 24, S18-S27.

Smith C.J., Zhang Y., Koboldt C.M., Muhammad J., Zweifel B.S., Shaffer A., Talley J.J., Masferrer J.L., Seibert K. and Isakson P.C. (1998). Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc. Natl. Acad. Sci. USA. 95, 13313-13318.

Smith R.H., Palmer R.M. and Reeds PJ. (1983). Protein synthesis in isolated rabbit forelimb muscles. The possible role of metabolites of arachidonic acid in the response to intermittent stretching. Biochem. J. 214, 153-161.

St Pierre B.A. and Tidball J.G. (1994). Differential response of macrophage subpopulations to soleus muscle reloading after rat hindlimb suspension. J. Appl. Physiol. 77, 290-297.

Steiner S.M., Hu Y. and Steiner MR. (1995). Regulation of prostaglandin H synthase 1 and 2 in MyoD transfected cells. Exp. Cell. Res. 218, 389-393.

Taylor L. and Polgar P. (1977). Self regulation of growth by human diploid fibroblasts via prostaglandin production. FEBS Lett. 79, 69-72.

Taylor W.E., Bhasin S., Artaza J., Byhower F., Azam M., Willard D.H. Jr, Kull F.C. Jr and Gonzalez-Cadavid N. (2001). Myostatin inhibits cell proliferation and protein synthesis in C2C12 muscle cells. Am. J. Physiol. Endocrinol. Metab. 280, E221-E228.

Tegeder I., Pfeilschifter J. and Geisslinger G. (2001). Cyclooxygenase-independent actions of cyclooxygenase inhibitors. FASEB J. 15, 2057-2072.

Thompson M.G., Acamovic F., Mackie S.C., Morrison K.S. and Palmer R.M. (1993). Arachidonate activation of protein kinase C may be involved in the stimulation of protein synthesis by insulin in L6 myoblasts. Biosci. Rep. 13, 359-366.

Thorsson O., Rantanen J., Hurme T. and Kalimo H. (1998). Effects of nonsteroidal antiinflammatory medication on satellite cell proliferation during muscle regeneration. Am. J. Sports Med. 26, 172-176.

Tidball J.G. (1995). Inflammatory cell response to acute muscle injury. Med. Sci. Sports Exerc. 27, 1022-1032.

Tilley S.L., Coffman T.M. and Koller B.H. (2001). Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. J. Clin. Invest. 108, 15-23.

Trappe T.A., Fluckey J.D., White F., Lambert C.P. and Evans W.J. (2001). Skeletal muscle PGF(2)(alpha) and PGE(2) in response to eccentric resistance exercise: influence of ibuprofen acetaminophen. J. Clin. Endocrinol. Metab. 86, 5067-5070.

Trappe T.A., White F., Lambert C.P., Cesar D., Hellerstein M. and Evans W.J. (2002). Effect of ibuprofen and acetaminophen on postexercise muscle protein synthesis. Am. J. Physiol. Endocrinol. Metab. 282, E551-E556.

Turinsky J. and Loegering D.J. (1985). Prostaglandin E2 and muscle protein turnover in Pseudomonas aeruginosa sepsis. Biochim. Biophys. Acta. 840, 137-140.

Vandenburgh H.H., Hatfaludy S., Sohar I. and Shansky J. (1990). Stretch-induced prostaglandins and protein turnover in cultured skeletal muscle. Am. J. Physiol. 259, C232-C240.

Vandenburgh H.H., Shansky J., Karlisch P. and Solerssi R.L. (1993). Mechanical stimulation of skeletal muscle generates lipid-related second messengers by phospholipase activation. J. Cell. Physiol. 155, 63-71.

Vandenburgh H.H., Shansky J., Solerssi R. and Chromiak J. (1995). Mechanical stimulation of skeletal muscle increases prostaglandin F2 alpha production, cyclooxygenase activity, and cell growth by a pertussis toxin sensitive mechanism. J. Cell. Physiol. 163, 285-294.

Vane J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat. New Biol. 231, 232-235.

Venkatesan N., Roughley P.J. and Ludwig M.S. (2002). Proteoglycan expression in bleomycin lung fibroblasts: role of transforming growth factor-beta(1) and interferon-gamma. Am. J. Physiol. Lung Cell. Mol. Physiol. 283, L806-L814.

Verrall G.M., Slavotinek J.P., Barnes P.G., Fon G.T. and Spriggins A.J. (2001). Clinical risk factors for hamstring muscle strain injury: a prospective study with correlation of injury by magnetic resonance imaging. Br. J. Sports Med. 35, 435-439; discussion 440.

Walch L. and Morris P.L. (2002). Cyclooxygenase 2 pathway mediates IL-1beta regulation of IL-1alpha, -1beta, and IL-6 mRNA levels in Leydig cell progenitors. Endocrinology 143, 3276-3283.

Warren G.L., Ingalls C.P., Lowe D.A. and Armstrong R.B. (2002). What mechanisms contribute to the strength loss that occurs during and in the recovery from skeletal muscle injury? J. Orthop. Sports Phys. Ther. 32, 58-64.

Wennmalm A. and Fitzgerald G.A. (1988). Excretion of prostacyclin and thromboxane A2 metabolites during leg exercise in humans. Am. J. Physiol. 255, H15-H18.

Wilgus T.A., Vodovotz Y., Vittadini E., Clubbs E.A. and Oberyszyn T.M. (2003). Reduction of scar formation in full-thickness wounds with topical celecoxib treatment. Wound Repair Regen. 11, 25-34.

Witko-Sarsat V., Rieu P., Descamps-Latscha B., Lesavre P. and Halbwachs-Mecarelli L. (2000). Neutrophils: molecules, functions and pathophysiological aspects. Lab. Invest. 80, 617-653.

Yamanouchi K., Soeta C., Naito K. and Tojo H. (2000). Expression of myostatin gene in regenerating skeletal muscle of the rat and its localization. Biochem. Biophys. Res. Commun. 270, 510-516.

Yamazaki M., Minota S., Sakurai H., Miyazono K., Yamada A., Kanazawa I. and Kawai M. (1994). Expression of transforming growth factor-beta 1 and its relation to endomysial fibrosis in progressive muscular dystrophy. Am. J. Pathol. 144, 221-226.

Young E.W. and Sparks H.V. (1979). Prostaglandin E release from dog skeletal muscle during restricted flow exercise. Am. J. Physiol. 236, H596-H599.

Zalin R.J. (1977). Prostaglandins and myoblast fusion. Dev. Biol. 59, 241-248.

Zammit P. and Beauchamp J. (2001). The skeletal muscle satellite cell: stem cell or son of stem cell? Differentiation 68, 193-204.

Zhang Y., Shaffer A., Portanova J., Seibert K. and Isakson P.C. (1997). Inhibition of cyclooxygenase-2 rapidly reverses inflammatory hyperalgesia and prostaglandin E2 production. J. Pharmacol. Exp. Ther. 283, 1069-1075.

Accepted May 7, 2003