



Pharmacologic approaches to prevent skeletal muscle atrophy after spinal cord injury

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Abstract

Skeletal muscle atrophy is a hallmark of severe spinal cord injury (SCI) that is precipitated by the neural insult and paralysis. Additionally, other factors may influence muscle loss, including systemic inflammation, low testosterone, low insulin-like growth factor (IGF)-1, and high-dose glucocorticoid treatment. The signaling cascades that drive SCI-induced muscle loss are common among most forms of disuse atrophy and include ubiquitin-proteasome signaling and others. However, differing magnitudes and patterns of atrophic signals exist after SCI versus other disuse conditions and are accompanied by endogenous inhibition of IGF-1/PI3K/Akt signaling, which combine to produce exceedingly rapid atrophy. Several well-established anabolic agents, including androgens and myostatin inhibitors, display diminished ability to prevent SCI-induced atrophy, while ursolic acid and β 2-agonists more effectively attenuate muscle loss. Strategies combining physical rehabilitation regimens to reload the paralyzed limbs with drugs targeting the underlying molecular pathways hold the greatest potential to improve muscle recovery after severe SCI.

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Introduction

In the United States, ~80,000–120,000 individuals are living with a severe motor-complete spinal cord injury (SCI) [1], which induces immediate and permanent paralysis in muscles innervated below the spinal lesion. Rapid skeletal muscle atrophy is a hallmark of severe SCI that is precipitated by the neural insult and the resulting neuromuscular impairment, with 25–60% lower muscle cross-sectional area (CSA) and muscle fiber (f)CSA in paralyzed muscles 3–6 months post-injury [2]. Changes in the molecular signaling cascades that regulate muscle size are distinct after severe SCI, with muscle loss being more rapid than in other disuse conditions, such as hindlimb immobilization [3] or sciatic transection [4]. Therefore, pharmacologic strategies intending to limit SCI-induced muscle loss must target the initiating atrophy pathways in the paralyzed limbs, while also addressing systemic physiologic consequences of SCI that have the potential to exacerbate muscle loss and/or inhibit muscle recovery. This mini-review provides overviews of the SCI muscle phenotype, the molecular signaling pathways, and secondary factors that influence muscle atrophy, and recent pharmacologic approaches to lessen muscle loss in the paralyzed limbs after severe SCI.

2. Pathophysiology of skeletal muscle loss following spinal cord injury

Severe SCI results in impaired neural drive, motoneuron atrophy, and pathologic changes to the neuromuscular junction that combine to produce low muscle force generating capacity and/or paralysis [2]. Collectively, these deficits impact the rapid muscle atrophy and the development of the SCI muscle phenotype, which is

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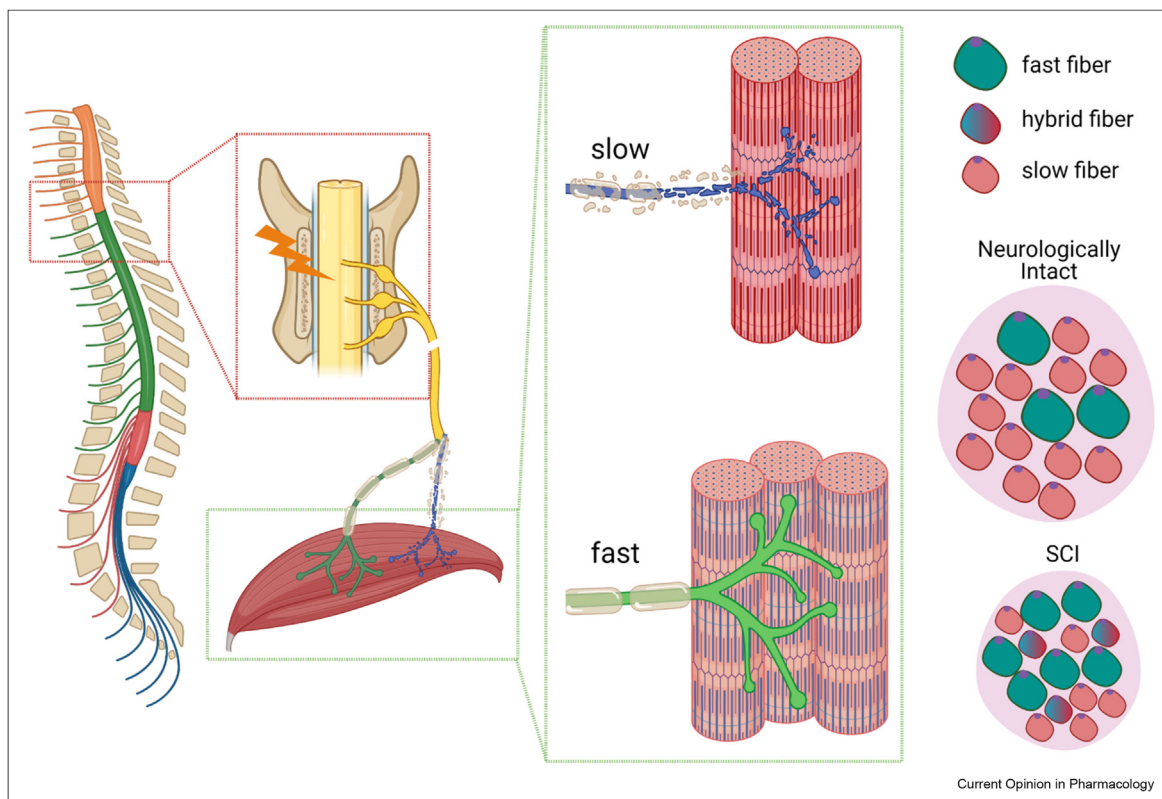
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characterized by mitochondrial dysfunction, a slow-oxidative to fast-glycolytic muscle fiber-type transition, and the development of muscle fibrosis (Figure 1) [2,5]. The atrophic signals that initiate muscle loss after SCI are thought to be common among most forms of disuse atrophy and include the ubiquitin-proteasome and transforming growth factor (TGF) β /Smad-3 signaling pathways, among others [6]. However, after severe SCI the rate of muscle loss is more rapid than in other disuse conditions [3,4], likely because the magnitude and pattern of atrophic signals differ in response to SCI. For example, in rodents, mRNA expression of the muscle-specific E₃ ubiquitin ligases muscle atrophy F-box (MAFbx or atrogin-1) and muscle ring finger-1 (MuRF1) are twofold to threefold higher after spinal transection vs. sciatic nerve transection, resulting in a two-fold increase in the muscle atrophy rate over the initial 7-d post-injury [4]. Thereafter, atrophy signals revert to the levels of sham-operated controls and muscle atrophy slows. Similarly, in persons with chronic complete SCI, muscle expression of MAFbx and MuRF1 was equal to or less than that of similarly aged able-bodied persons [7] and nuclear localization of forkhead box O (FOXO)1 and

FOXO3a (atrophic transcription factors) and MAFbx were lower [8], suggesting that proteasomal degradation is not central to the sustained atrophy after SCI.

Although atrophy signaling initiates muscle loss after severe SCI, reduced anabolic signaling likely contributes to the sustained muscle deficits. Key proteins involved in anabolic signaling are those downstream of the insulin-like growth factor (IGF)-1/PI3K/Akt pathway [9] and of other anabolic stimuli, with the nexus of protein synthesis in all cells being the intracellular kinase mechanistic target of rapamycin (mTOR). Once phosphorylated (p), mTOR targets and phosphorylates downstream proteins involved in translation initiation and efficiency that increases protein synthesis and, if sustained, produces muscle growth [10]. However, total and (p)mTOR decline within the muscle in persons over the initial 3–12 months post-SCI [11], likely contributing to lower anabolic signaling that persists for decades in this population [8]. Similarly, (p)PI3K, (p)Akt, and (p)mTOR levels are ~50–75% lower in the soleus muscle of rodent SCI models versus controls within only a few days to weeks post-injury [12,13].

Figure 1



Pathophysiology of skeletal muscle loss after severe spinal cord injury (SCI). SCI results in impaired neural drive, motor neuron atrophy, and pathological changes to the neuromuscular junction that combine to produce low muscle force generating capacity and/or paralysis. Collectively, these deficits impact the rapid rate of muscle atrophy and the repeated denervation–reinnervation cycles that influence the slow-oxidative to fast-glycolytic muscle fiber-type transition in paralyzed muscles. Figure was generated in BioRender.

The reasons why differing atrophic and anabolic signaling patterns exist after SCI versus other disuse conditions are unknown, although it is likely that secondary factors that occur in response to SCI are involved. For example, it is possible that the systemic inflammation that develops in response to the direct SCI trauma (e.g., car crash or fall) or to the subsequent surgical interventions [14] exacerbates muscle atrophy. In this regard, high-dose methylprednisolone (systemic glucocorticoid) is routinely administered to persons with severe SCI over the acute–subacute recovery phase to suppress inflammatory processes that influence the secondary injury cascade within the spinal cord, despite the questionable safety and efficacy of this regimen [15]. However, preclinical research indicates that high-dose glucocorticoid treatment directly stimulates muscle atrophy by initiating atrophic pathways and/or suppressing anabolic signaling [16] and that methylprednisolone exacerbates the increase in FOXO1, MAFbx, MuRF1, and REDD1 (an inhibitor of mTOR signaling) expression in response to SCI, along with the subsequent muscle loss [17]. Several hormonal irregularities that are associated with muscle atrophy (e.g., low testosterone and low IGF-1) also occur secondary to SCI [2,18]. These are important factors to consider because circulating testosterone [19] and IGF-1 [20] have both been positively correlated with thigh muscle CSA in persons with chronic complete SCI.

3. Pharmacologic approaches to ameliorate muscle atrophy after SCI

No drugs are currently approved to counter muscle atrophy after severe SCI and no pharmacologic strategy has been definitively shown to attenuate muscle loss in the paralyzed limbs of persons with SCI; however, several promising preclinical strategies have been identified. The following sections discuss the most investigated anabolic agents in relation to SCI (i.e., androgens and β 2-adrenergic agonists), along with several other promising pharmaceuticals. Because of the brevity of this mini-review, we focus on drugs that have been tested in persons with paralyzed limbs or in animal models with complete/severe SCI because muscle atrophy after incomplete SCI is less drastic, due to the presence of spared spinal tracts that permit voluntary musculoskeletal loading in the impaired limbs [2].

4. Androgens

Testosterone is the most abundant bioactive androgen within the circulation. Pharmacologic testosterone increases muscle mass in able-bodied hypogonadal men when administered in sufficient doses [21], either via direct androgen receptor engagement and/or indirectly via androgen-induced alterations in anabolic (e.g., IGF-1/PI3K/Akt) or catabolic signaling (e.g., myostatin/Smad-3) pathways [2]. In the 1950s, Cooper et al. [22] reported elevated urinary nitrogen excretion and a

negative nitrogen balance that persisted for several months in persons with SCI and that high-dose testosterone (50–100 mg/day) normalized nitrogen balance by mitigating nitrogen excretion, suggesting that high-dose testosterone may limit muscle wasting after SCI. However, this possibility has yet to be verified, likely because high-dose testosterone produces several health risks, including prostate enlargement [23]. Alternatively, moderate-dose testosterone (5–10 mg/day for 12 months) was shown to increase whole body and lower extremity lean mass in a small cohort of hypogonadal men with chronic complete SCI [24], with improvements persisting for 6 months [25]. In contrast, low-dose testosterone (2–6 mg/day for 16 weeks) did not increase whole body lean mass, lower extremity muscle CSA [26], or muscle fCSA [27] in eugonadal and hypogonadal men with chronic complete SCI. These small trials did not observe prostate enlargement nor reported any serious adverse events. In comparison, some preclinical SCI studies reported that high-dose testosterone increased the mass of the prostate and of the levator ani-bulbocavernosus (LABC) muscle (involved in sexual function) and various hindlimb muscles [2], while others have reported that testosterone did not increase muscle mass or fCSA in the paralyzed hindlimbs [28–31]. The reasons for these inconsistencies are unknown, although androgen receptor expression is greater than threefold higher in the prostate and LABC (androgen-responsive tissues) versus soleus [30] and other non-androgen-responsive hindlimb muscles [2]. Regardless, testosterone has been shown to suppress muscle FOXO1, MAFbx, MuRF1, and REDD1 expression and lessen the excess atrophy associated with methylprednisolone treatment in a rodent spinal transection model [17]. Moreover, testosterone attenuated gastrocnemius muscle loss after spinal transection, when given in combination with nandrolone (non-5 α -reducible androgen), with muscle preservation being associated with reduced *ACVR2B* (myostatin receptor) expression and reduced nuclear content of Smad2/3 (downstream effectors of myostatin signaling) [31]. In a rodent severe SCI model, high-dose testosterone with finasteride (US Food and Drug Administration (FDA)-approved 5 α -reductase inhibitor) also lessened prostate enlargement versus testosterone alone and did not impede androgen-induced LABC growth [32], indicating that the 5 α reduction of testosterone mediates prostate growth but not muscle growth. However, these preclinical findings remain to be verified in clinical trials.

5. β 2-Adrenergic agonists

β 2-agonists are traditionally used to treat bronchospasm resulting from asthma or chronic obstructive pulmonary disease (COPD) through smooth muscle relaxation and are categorized as short- or long-acting agonists, with treatment effects lasting 3–6 h or 12–24 h, respectively.

Select β 2-agonists also increase protein synthesis and suppress protein degradation in skeletal muscle by activating the PI3K/Akt/mTOR pathway and suppressing FOXO transcriptional activation of the ubiquitin-proteasome and autophagy-lysosome pathways [33]. In this regard, short-acting β 2-agonist metaproterenol (80 mg/day for 4 weeks) improved muscle size and strength in a small cohort of men with muscle atrophy following SCI [34] and short-acting clenbuterol (2 μ g/kg/day for 3 months) attenuated the reduction in type I and II fCSA by \sim 40% in persons with acute denervation due to traumatic cervical brachial plexus injury [35]. Additionally, in a mouse spinal transection model, short-acting clenbuterol (1 mg/kg/day) and high-dose testosterone produced additive improvement in hindlimb muscle fCSA when delivered for 1–8 weeks [36], although the signaling changes mediating this effect remain to be determined. Interestingly, in a mouse contusion SCI model, the long-acting β 2-agonist formoterol (0.3 mg/kg/day) did not prevent gastrocnemius muscle loss 3 days post-SCI, despite completely preventing myostatin mRNA induction and producing 100% higher muscle *Igf1* expression, likely because formoterol did not prevent the rapid increase in MuRF1 protein nor the dramatic (p)Akt suppression after SCI [13]. In comparison, formoterol-treated mice displayed similar MuRF1 and (p)Akt protein levels to controls at 21 days and higher muscle mass versus untreated SCI mice [13]. It is important to note that considerable locomotor recovery occurred in formoterol-treated mice after SCI, which introduced hindlimb reloading. However, delaying formoterol treatment for 24-h post-SCI induced preservation of gastrocnemius mass in the absence of locomotor recovery [37]. Although promising, it remains unknown whether formoterol lessened fCSA atrophy in these studies. Regardless, the formoterol-induced locomotor improvements, muscle signaling changes, and muscle mass preservation appeared dependent on the β 2-adrenergic receptor (*ADRB2*), as no neuromuscular improvements were observed in global *Adbr2*^{-/-} knockout mice treated with formoterol after SCI [13]. The above-mentioned pre-clinical findings have not yet been verified in clinical trials, although formoterol is FDA approved to control COPD symptoms.

6. Myostatin inhibitors

Myostatin (also known as growth and differentiation factor 8 (GDF-8)) is a member of the TGF- β superfamily and a muscle-derived negative regulator of muscle growth that acts via the activin IIB receptors [38]. Elevated myostatin gene expression has been observed in persons with chronic SCI [39] and in rodent SCI models several days post-injury [13]. Interestingly, in a rodent spinal transection model, administration of a soluble activin IIB receptor that inhibits myostatin (RAP-031, 10 mg/kg, 2 \times /week) increased whole body

lean mass \sim 15% and increased mass of the fully loaded forelimb muscles \sim 20–40%, without attenuating muscle loss in the paralyzed hindlimbs [40]. Similarly, others have reported that pharmacologic myostatin inhibition did not attenuate muscle loss after sciatic nerve transection but prevented disuse (immobilization) muscle atrophy [41]. Collectively, these results suggest that intact innervation may be required for muscle growth in response to myostatin inhibition.

7. SS-31/Elamipretide

Mitochondrial reactive oxygen species (ROS) generation triggers muscle atrophy signaling in response to prolonged immobilization [42] and has been proposed a contributing factor to SCI-induced mitochondrial dysfunction and muscle atrophy [5]. SS-31, a mitochondrial-targeting tetrapeptide, prevents atrophy in response to immobilization and reduces markers of mitochondrial ROS and oxidative stress [42]. SS-31 has been shown to attenuate ROS levels, to reverse mitochondrial dysfunction, and to lessen lung edema and damage in a rodent model of SCI-induced lung injury [43]. However, SS-31 (5-mg/kg/day) did not lessen hindlimb muscle atrophy in mice after moderate contusion SCI [44]. This suggests that ROS generation may not contribute extensively to SCI-induced muscle atrophy and/or that locomotor recovery due to the moderate SCI may have confounded any positive benefits of SS-31.

8. Natural products

To identify novel small molecule muscle atrophy inhibitors Adams et al. developed an SCI-centric drug discovery strategy that (1) surveyed genome-wide mRNA expression patterns that were conserved across normal human and mouse muscle and that were altered in atrophic muscle collected after SCI or fasting and (2) searched for small molecules with established safety profiles that induced inverse mRNA patterns in human skeletal muscle cell lines [45]. This strategy identified ursolic acid (UA), a natural plant metabolite with previously unrecognized anabolic properties, which has since been shown to stimulate muscle growth in mice in an IGF-1-dependent manner and to lessen disuse atrophy, with effects dependent on repression of MAFbx and MuRF1 [46]. Interestingly, UA (200 mg/kg/day) lessened FOXO1 protein and MAFbx expression in the mouse soleus 1 week after moderate–severe SCI and prevented the SCI-induced suppression of (p)P13K, (p)Akt, (p)mTOR, and (p)70s6K for several weeks thereafter. This resulted in higher soleus masses in SCI+UA versus untreated SCI mice [12]. However, UA induced some locomotor recovery after SCI, which may have influenced these muscle responses. Although promising, it remains unknown whether UA preserved fCSA in this study or whether UA can improve muscle mass in the paralyzed limbs after severe SCI.

Epicatechin, a flavanol that is present in tea and other edible plants, has also been shown to improve muscle performance in several atrophy models [47]. Recently, epicatechin (1 mg/kg/day) was shown to lower ubiquitin and MuRF1 protein by 33–50% in mice within 7 days of spinal transection and to return ubiquitin, FOXO1, MAFbx, and MuRF1 to control levels within 30 days, which lessened muscle CSA and fCSA atrophy ~50% [48]. Similarly, acteoside (verbascoside), a phenylethanoid glycoside found in tea and other plants [49], was shown to stimulate skeletal muscle cell proliferation in culture by increasing secretion of pyruvate kinase isoform M2 [50]. In a mouse moderate–severe SCI, acteoside (0.1 mg, 3×/week) increased hindlimb muscle mass versus untreated SCI animals, when initiated 30 days post-SCI [50]. However, acteoside also improved hindlimb locomotor function, which likely influenced the observed findings. Although these natural compounds have shown promise in preclinical studies, their clinical efficacy remains to be established.

9. Future directions

Activity-based physical therapies (ABPTs) have been used to combat muscle atrophy and the deleterious muscle phenotype that develops following SCI [2]. For example, both bodyweight-supported treadmill training (BWSTT) and neuromuscular electrical stimulation (NMES) are known to increase muscle CSA in persons with chronic complete SCI and to facilitate a fast-glycolytic to slow-oxidative fiber-type conversion [2,5], although ABPT effectiveness wanes as injury severity increases and continual training is needed to maintain muscular gains. Given these limitations it seems relevant to assess pharmacologic adjuvants combined with established ABPTs. For example, in a rodent severe SCI model high-dose testosterone combined with quadrupedal (q)BWSTT (40 min/day, 5×/week) attenuated soleus fCSA atrophy, prevented the soleus slow-to-fast fiber-type transition, and maintained isolated muscle force production better than testosterone alone [28]. Similarly, in a rodent spinal transection model, a multimodal therapy involving high-dose testosterone with electrical stimulation (1.5 V, 40 Hz, 2 s:18 s on:off) suppressed MAFbx and MuRF1 expression better than testosterone alone and produced slightly better muscle recovery [29]. Moreover, in men with chronic complete SCI low-dose testosterone in combination with a 16-week NMES-based progressive resistance training protocol produced greater knee extensor CSA and fCSA than testosterone alone [26,27]. Collectively, these studies provide evidence that multimodal therapies combining ABPTs with pharmacologic adjuvants provide improved muscle recovery after severe SCI.

10. Conclusion

Numerous pharmacologic agents stimulate hypertrophy in fully innervated and loaded muscles. However, most

anabolic agents display a diminished ability to lessen atrophy in the paralyzed limbs after severe SCI for yet to be identified reasons, although several possibilities exist. First, most anabolic drugs target specific signaling pathways but not the plethora of molecular changes in atrophic muscle after SCI, highlighting the need to elucidate the complexity of signaling pathways that drive SCI-induced muscle loss and to identify pharmaceuticals that target these pathways. Second, increased atrophy signaling coincides with reduced anabolic signaling after SCI, as detailed above, implying that effective drugs may need to suppress atrophy and simultaneously stimulate anabolic pathways, which has proven difficult in the absence of innervation and loading in the paralyzed limbs. Third, muscle atrophy occurs more rapidly after severe SCI than in other disuse conditions, suggesting that the ideal window to prevent muscle loss is limited. Given these possibilities, compounds that target the molecular signatures present in atrophic muscle after SCI appear to hold the greatest potential to lessen muscle loss and/or promote muscle recovery, especially when combined with established ABPTs that reload the paralyzed limbs.

Conflict of interest statement

Nothing declared.

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