Skeletal Muscle Regeneration in Cardiotoxin-Induced Muscle Injury

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Skeletal muscle injuries occur frequently in daily life and exercise. Understanding the mechanisms of regeneration is critical for accelerating the repair and regeneration of muscle. The process of regeneration is similar in different mouse strains and is inhibited by aging, obesity, and diabetes. Exercise, microcurrent electrical neuromuscular stimulation, and mechanical loading improve regeneration. The mechanisms of regeneration are complex and strain-dependent, and changes in functional proteins involved in the processes of necrotic fiber debris clearance, M1 to M2 macrophage conversion, SC activation, myoblast proliferation, differentiation and fusion, and fibrosis and calcification influence the final outcome of the regenerative activity.

cardiotoxin injury skeletal muscle regeneration mechanism

1. Introduction

Skeletal muscle, the main organ of systemic metabolism in the body, is composed of differentiated fibers and displays a strong ability to regenerate after injury. Skeletal muscle injuries occur frequently in daily life and exercise, and the capacity of regeneration is critical for the repair and functional maintenance of skeletal muscle. The regeneration of adult muscle is based on the activation of satellite cells (SCs), which are mononuclear progenitors of skeletal muscle and are located between the sarcolemma and basal lamina [1]. After injury, the regeneration of muscle occurs in three overlapping stages: in the first stage, inflammatory cells infiltrate into damaged sites, and necrotic fiber fragments are removed; in the second stage, SCs are activated and proliferate into myoblasts, thereafter differentiating and fusing to form new muscle cells and replace damaged fibers; the last stage involves the maturation of newly formed fibers and the remodeling of damaged muscle [2][3]. The processes of regeneration are highly coordinated, and the expression of genes involved in regeneration are spatially and temporally regulated [4][5]. Numerous studies have been conducted to investigate the molecular mechanisms underlying muscle regeneration. A comprehensive understanding of the events involved in muscle regeneration will facilitate the treatment of skeletal muscle diseases.

In order to achieve a better understanding of muscle regeneration following physiological injury, the innervation, tendons, vascularization, and SCs should not be injured in mouse models because they contribute to myogenesis following injury. Cardiotoxin (CTX), derived from *Naja pallida*, induces a transient and reproducible acute injury without affecting the vasculature or nerves, and then produces a consistent injury in the whole muscle followed by synchronized regeneration [6][7][8]. Its application also has the advantages of allowing molecular and biochemical analyses to be performed on the whole muscle in contrast to physiological injury models induced by exercise [9][10].

Additionally, CTX injury models have relatively low harmfulness for animals compared with other non-physiological models such as crushing models [11]. Due to these characteristics, the CTX-induced skeletal muscle injury model is a suitable model for exploring the mechanisms of skeletal muscle regeneration.

2. The Characteristics and Positions of Injury in CTX-Induced Skeletal Muscle Injury Models

CTX, a natural amphiphilic peptide derived from *Naja pallida*, can affect membrane calcium binding sites, and lower the threshold of calcium-modulated calcium ion release from the sarcoplasmic reticulum, thereafter inducing the destruction of skeletal muscle [12][13]. Muscle injury occurs at days 1 to 2 after CTX injection, where inflammatory cells infiltrate and SCs are activated to proliferate; at days 3–5, the myoblasts are induced to differentiate; at days 5–7, the new fibers with a central nucleus begin to form; and at days 10–14, the major muscle structures are restored; at day 28, the damaged muscles have almost completely recovered [14][15][16]. Due to its characteristics of transience and reproducibility, the CTX-induced injury model has been widely used to explore the mechanisms of skeletal muscle regeneration.

In CTX-induced injury models, the damaged sites are hindlimb muscles, where injuries also often occur in humans. In the related literature, the tibialis anterior is the most widely studied site in CTX-induced injury models. This is because of its obvious location and the characteristics of having a mixture of fiber types. Additionally, as a highly heterogenous muscle, tibialis anterior has only one belly, which results in uniform injury. Gastrocnemius consists mainly of fast-twitch fibers and is a bicep muscle, which may result in nonuniform injury despite the obvious location. Furthermore, other hindlimb muscles were also used in the studies such as the extensor digitorum longus, soleus, and quadriceps. The characteristics of only one type of muscle fiber and the muscle group may lead to a preference for position.

Notably, there are still some limitations in CTX-induced skeletal muscle injury models. First, the skeletal muscles include antigravity (e.g., gastrocnemius, quadriceps) and non-antigravity muscles (e.g., tibialis anterior, biceps brachii) [17]. The mechanisms identified in CTX-induced non-antigravity muscle injury models may not apply directly to the CTX-induced antigravity muscles. Second, in CTX-induced injury models, it always does not affect the vasculature or nerves in muscles [8]. In contrast, the vasculature or nerve damage often occurs during the pathogenesis of human muscle injuries [18]. This discrepancy limits the exploration of the contribution of vasculature or nerves in muscle regeneration using CTX-induced muscle injury models. Third, CTX may induce a complete necrosis of the small muscles such as EDL when examined in cross-section 48 h after injection [19]. This may make it impossible to explore the mechanisms involved in the early stages of these muscles.

3. Skeletal Muscle Regeneration in Different Mouse Models after CTX-Induced Skeletal Muscle Injury

CTX has been used to induce skeletal muscle injury in many mouse models including that of diabetes, obesity, aging, exercise training, mechanical loading, and nutrition intervention, among others. Studies have shown that streptozocin and gene mutation-induced diabetes [20][21][22][23], high fat diet-induced obesity and ob/ob mice [22][24][25], cancer cachexia [26], aging [27][28], irradiation [29], elevated carbon dioxide (CO_2) level [30], and hindlimb suspension [31][32] lead to impaired regeneration, whereas exercise training [33][34], microcurrent electrical neuromuscular stimulation [35], microelement zinc [36], and overloading [37][38] improve the regeneration of CTX-induced damaged muscle. The accumulation of mitochondrial DNA alterations activates muscle regeneration in myofibers during aging, but leads to reduced muscle mass [39].

Gender and sex hormone levels also influence the regeneration processes in CTX-induced muscle injuries. Males exhibit larger newly formed fibers than females at the same age after injury, whereas females show higher fat deposition than males during regeneration [40][41] and also remove necrotic tissue more rapidly [41]. Castration of males increases the cross-sectional areas (CSAs) of the newly formed fibers and fat accumulation, whereas ovariectomized mice exhibit inhibited regeneration and decreased adipocyte accumulation, and estrogen supplementation rescues regeneration in ovariectomized mice [41][42]. Lack of estrogen-related receptor α also impairs the recovery of mitochondrial energetic capacity and decreases the activity of adenosine 5′-moophpsphate (AMP)–activated protein kinase (AMPK), which then also leads to delayed regeneration [43].

Additionally, the studies also revealed that different mouse strains have similar regeneration processes with no significant morphological and functional differences. However, the mechanisms of skeletal muscle regeneration may be strain-dependent. For instance, toll-like receptor 4 (TLR4) plays distinct roles in the injured muscle of C57BL/6 and C3H/HeJ [44][45].

It was also reported that the regeneration of skeletal muscle is position-specific: after CTX injury in tibialis anterior and the masseter, head muscles recover slowly and eventually return to the base level, whereas limb muscles show quicker recovery and eventually excessive growth [46].

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