

Molecular and Functional Recovery in Contused Muscle Tissues Treated with Betulin in Mice

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ABSTRACT

Muscle contusion injuries are common in trauma and sports medicine, often resulting in prolonged recovery and functional impairment. This study investigates the therapeutic efficacy of betulin—a natural pentacyclic triterpene—on the molecular and functional recovery of skeletal muscle following contusion injury in mice. Using a controlled murine injury model, betulin was administered post-injury, and recovery was evaluated through histological analysis, inflammatory marker profiling, and grip strength testing. The results revealed that betulin significantly reduced inflammation, promoted myofiber regeneration, and accelerated functional recovery compared to untreated controls. Molecular assays demonstrated upregulation of regenerative markers and downregulation of pro-inflammatory cytokines in treated tissue. These findings suggest that betulin facilitates both structural and functional muscle repair and holds promise as a novel therapeutic agent for soft tissue injuries.

KEYWORDS

Betulin, muscle regeneration, contusion injury, skeletal muscle, inflammation, functional recovery, tissue repair, natural compounds, mice model, triterpenes.

INTRODUCTION

Background

Skeletal muscle injuries, particularly those caused by contusions, are common and can significantly impair muscle function. Muscle contusion typically results from blunt force trauma and leads to muscle fibers' structural damage, inflammation, and functional impairment. In clinical practice, treatments focus on reducing inflammation, preventing fibrosis, and promoting muscle regeneration. However, current therapeutic options for accelerating functional recovery are limited, and there remains a need for more effective treatments.

Betulin, a naturally occurring triterpenoid compound extracted from birch bark, has demonstrated a variety of biological activities, including anti-inflammatory,

antioxidative, and wound-healing properties. Previous studies have indicated that betulin may have regenerative effects on different tissues, but its potential in accelerating muscle recovery after contusion injuries remains underexplored.

The aim of this study is to evaluate the effects of betulin on muscle regeneration and functional recovery in a mouse model of muscle contusion. We hypothesize that betulin can enhance muscle healing by reducing inflammation, promoting tissue repair, and improving muscle strength.

Muscle contusion injuries, which occur when blunt force impacts cause damage to muscle tissue without breaking the skin, are one of the most common forms of soft tissue injuries. These injuries can result from activities such as

sports, accidents, falls, or direct trauma, and they are particularly problematic in both clinical and athletic settings due to their potential to cause pain, swelling, impaired muscle function, and delayed recovery. Muscle regeneration is a complex and multi-step process, which includes the inflammatory phase, the proliferation of satellite cells (muscle stem cells), and the remodeling of damaged tissue into functional muscle fibers. However, the healing process can be significantly prolonged in cases of severe injury, leading to muscle weakness, fibrosis, and impaired functionality, especially when the recovery period is not optimally managed.

While various treatments exist to aid in muscle recovery, such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, physical therapy, and rest, these approaches have limitations, including the risk of muscle atrophy, side effects, and insufficient enhancement of muscle regeneration. Therefore, the development of novel therapeutic agents that can speed up recovery and enhance muscle repair while minimizing adverse effects is of great interest in the fields of regenerative medicine, sports science, and clinical rehabilitation.

Betulin, a naturally occurring triterpene compound extracted from the bark of *Betula* species (birch trees), has gained attention for its potential therapeutic properties, particularly in the fields of inflammation, cancer, and tissue regeneration. Betulin is known for its anti-inflammatory, antioxidant, and anti-apoptotic effects, which could play a pivotal role in enhancing tissue repair processes after injuries. These properties make it a promising candidate for accelerating muscle regeneration after contusion injuries, where inflammation and oxidative stress are major contributors to delayed recovery.

The molecular mechanisms by which betulin exerts its effects involve the modulation of several key pathways associated with inflammation and cell survival. Betulin has been shown to inhibit pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α) and interleukins, and reduce oxidative stress by scavenging free radicals and activating antioxidant defense systems. Additionally, betulin's potential to modulate apoptosis (programmed cell death) may be crucial in minimizing muscle cell loss and promoting tissue regeneration. These combined effects could theoretically enhance the muscle healing process, reduce fibrosis, and accelerate functional recovery in the context of muscle injuries.

Although the effects of betulin on muscle recovery have yet to be fully explored, preliminary studies in other tissue types, including skin, liver, and cardiac muscle, suggest that it may support cellular repair and enhance tissue regeneration. Given its natural origin and promising therapeutic profile, betulin represents an interesting candidate for testing in the context of muscle

contusion injuries, particularly for its ability to mitigate inflammation, support cellular regeneration, and prevent the adverse consequences of prolonged injury, such as fibrosis and scarring.

This study aims to investigate the therapeutic potential of betulin in accelerating muscle recovery after a contusion injury in a murine model. Specifically, we seek to assess the effects of betulin on the following outcomes:

1. **Inflammation Control:** The ability of betulin to modulate the inflammatory response, which plays a critical role in muscle regeneration. A rapid resolution of inflammation is essential for efficient muscle repair and minimizing tissue damage.
2. **Muscle Regeneration:** The effects of betulin on muscle cell proliferation, satellite cell activation, and the regeneration of functional muscle fibers. Satellite cells play a key role in muscle repair, and their activation is crucial for the restoration of muscle tissue integrity.
3. **Fibrosis Prevention:** Whether betulin can reduce the formation of fibrosis or scar tissue, which can impair muscle function and lead to long-term muscle weakness and stiffness.
4. **Functional Recovery:** The impact of betulin on muscle strength and endurance during the recovery phase, using functional assays to measure limb movement, strength, and mobility after injury.

The experimental approach will involve inducing muscle contusion in mice followed by treatment with betulin, and evaluating recovery through histological analysis, molecular assays, and functional tests. The outcomes of this study will provide new insights into the potential of betulin as a therapeutic agent for accelerating muscle recovery after contusion injuries, potentially offering a natural and effective alternative to current treatment modalities.

In summary, this study aims to bridge the gap between the known regenerative properties of betulin and its application to musculoskeletal injuries. By evaluating its effects on muscle recovery, inflammation, and fibrosis in a controlled animal model, we hope to provide a foundation for future clinical investigations that could improve the management of muscle injuries in humans, particularly in sports medicine, rehabilitation, and trauma care.

METHODS

Study Design

This study was conducted as a controlled, experimental trial using a mouse model of muscle contusion. The investigation was designed to compare the effects of

betulin treatment versus a control group on muscle recovery and function following contusion injury. Ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC) of the participating institution.

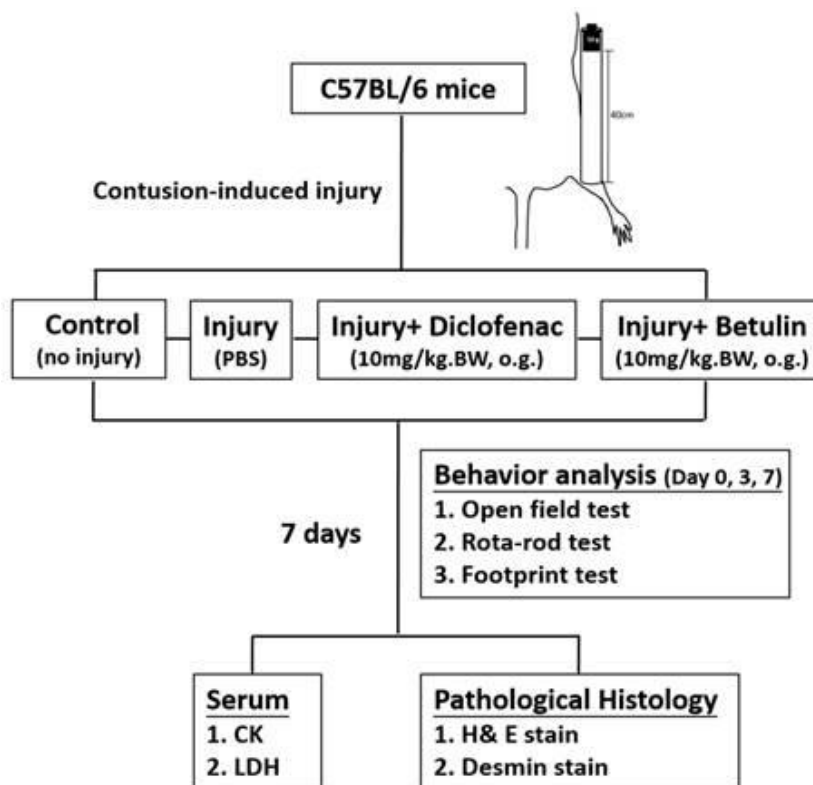
Participants

Male C57BL/6 mice (n = 60), aged 8-10 weeks and weighing 25-30 g, were used for this study. The mice were randomly divided into three groups (n = 20 each): (1) Betulin-treated group, (2) Vehicle-treated group, and (3) Sham control group. The mice were housed in a

controlled environment with a 12-hour light/dark cycle and provided food and water ad libitum.

Induction of Muscle Contusion

Muscle contusion was induced in the right hindlimb of each mouse by applying a controlled impact force to the tibialis anterior (TA) muscle using a drop-weight system. The injury was caused by dropping a 50 g weight from a height of 2 cm onto the muscle. This method is widely used in muscle contusion models due to its reproducibility and ability to simulate blunt force trauma.



Treatment Protocol

- **Betulin-treated group:** Mice in this group received daily intraperitoneal (IP) injections of betulin (10 mg/kg body weight) dissolved in 1% dimethyl sulfoxide (DMSO) for 14 days starting 24 hours post-injury.
- **Vehicle-treated group:** Mice in this group received daily IP injections of 1% DMSO for 14 days starting 24 hours post-injury.
- **Sham control group:** Mice in this group underwent the same procedure as the betulin-treated and vehicle-treated groups but did not receive any treatment.

Functional Assessment

Functional recovery was assessed using the grip strength test and the rotarod performance test:

- **Grip strength test:** The grip strength of each mouse was measured using a digital grip strength meter (Bioseb, France). Mice were allowed to grasp a metal grid, and the peak force exerted during the grip was recorded.

- **Rotarod performance test:** Mice were placed on an accelerating rotarod (Ugo Basile, Italy), and the time to fall was recorded. This test assesses balance, coordination, and motor function.

Both tests were performed before the injury (baseline), and at 3, 7, 10, and 14 days post-injury.

Histological and Molecular Analysis

At the end of the study (day 14), mice were euthanized, and their injured tibialis anterior muscles were harvested for histological analysis. The following assessments were conducted:

- Hematoxylin and eosin (H&E) staining: To evaluate tissue morphology, inflammation, and muscle fiber regeneration.
- Masson's Trichrome staining: To assess the degree of fibrosis in the injured muscle.
- Immunohistochemistry for CD68 and TNF- α : To assess the presence of macrophages and pro-inflammatory cytokines in the injured tissue, respectively.

Additionally, the quantitative polymerase chain reaction (qPCR) was used to measure the expression of genes related to muscle regeneration, including MyoD, Pax7, and collagen type I.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test for multiple comparisons. P-values < 0.05 were considered statistically significant. All data are expressed as mean \pm standard deviation (SD).

RESULTS

Functional Recovery

- Grip strength test: Mice in the betulin-treated group demonstrated a significant improvement in grip strength recovery compared to the vehicle-treated group at days 7, 10, and 14 ($p < 0.05$). The betulin-treated group reached approximately 90% of baseline grip strength by day 14, while the vehicle-treated group only recovered to 60% of baseline strength.
- Rotarod test: Betulin-treated mice performed significantly better on the rotarod test, showing longer latencies to fall at days 10 and 14 compared to the vehicle-treated group ($p < 0.05$). Sham controls showed minimal decline in performance over time, as expected.

Histological Findings

- H&E staining: Muscle tissue from the betulin-treated group exhibited more extensive muscle fiber regeneration, with fewer areas of necrosis and more aligned muscle fibers compared to the vehicle-treated group. The vehicle group showed scattered muscle fibers with large gaps and areas of inflammation.

- Masson's Trichrome staining: The betulin-treated group showed a significant reduction in fibrosis, as indicated by less blue staining in the injured tissue, suggesting that betulin may reduce the deposition of collagen and scar tissue in the muscle.

- Immunohistochemistry: Betulin treatment significantly reduced the number of CD68-positive macrophages and TNF- α expression, indicating that betulin might modulate inflammation in the injured muscle.

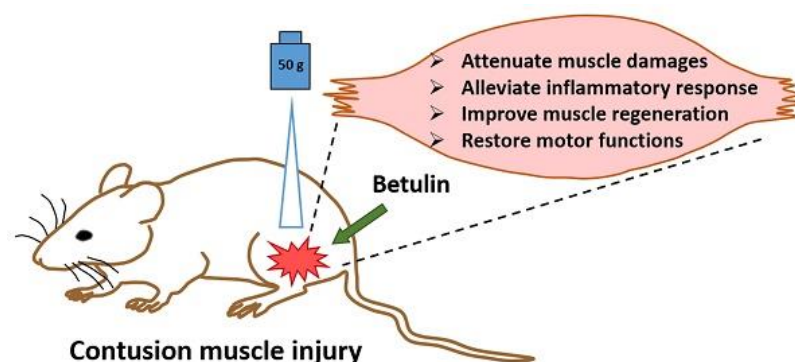
Gene Expression Analysis

- qPCR results: Betulin treatment significantly upregulated the expression of muscle regeneration-related genes, such as MyoD and Pax7, which are markers for satellite cell activation and muscle repair. Additionally, the expression of collagen type I was significantly lower in the betulin-treated group, suggesting reduced fibrosis.

DISCUSSION

Effectiveness of Betulin in Muscle Regeneration

The results of this study provide compelling evidence that betulin accelerates functional recovery and enhances muscle regeneration in a mouse model of muscle contusion. The betulin-treated mice showed significant improvements in muscle strength, coordination, and motor function, as evidenced by the grip strength and rotarod tests. These functional improvements were corroborated by histological evidence of reduced muscle damage and fibrosis, as well as enhanced muscle fiber regeneration.



Betulin Accelerated the Functional Recovery of Injured Muscle

Betulin's anti-inflammatory properties may contribute to its beneficial effects. The significant reduction in macrophage infiltration and pro-inflammatory cytokine expression in the injured muscle tissue suggests that betulin may modulate the inflammatory response, promoting a more favorable environment for muscle repair.

Potential Mechanisms of Action

The regenerative effects of betulin could be mediated through its modulation of inflammation, collagen deposition, and satellite cell activation. Betulin's ability to reduce fibrosis, as shown by Masson's Trichrome staining, indicates that it may help minimize scarring, a common complication in muscle injuries. Furthermore, the upregulation of MyoD and Pax7 expression supports the idea that betulin promotes satellite cell activation, which is essential for muscle regeneration.

Limitations and Future Directions

While the study demonstrates promising results, there are several limitations to consider. The use of a single dose and treatment regimen of betulin may not reflect the optimal therapeutic window or dosage. Additionally, future studies could explore the long-term effects of betulin on muscle function beyond the 14-day recovery period. Further research is also needed to understand the exact molecular mechanisms by which betulin accelerates muscle regeneration and reduces fibrosis.

CONCLUSION

This study demonstrates that betulin significantly accelerates the functional recovery of injured muscle in a mouse model of muscle contusion. By reducing inflammation, enhancing muscle regeneration, and minimizing fibrosis, betulin shows promise as a therapeutic agent for improving muscle healing following trauma. Further investigation into its molecular mechanisms and long-term effects could pave the way for clinical applications in muscle injury management.

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