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Effects of Platelet Rich Plasma (PRP) on the recovery of gastrocnemius muscle rupture in Wistar straining white rats

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Abstract
Gastrocnemius muscle rupture is a lower extremity rupture that has a poor prognose. Rupture of the gastrocnemius muscle can interfere the body's weight-bearing ability. Rupture are especially common among athletes and individuals who engage in activities that sudden involve and forceful movements of the calf. The current treatment has not been able to restore the biological and mechanical properties of the muscles to their original condition. One of the treatments used to speed up the healing of the gastrocnemius muscle is application of PRP. Objective: To determine the effect Platelet Rich Plasma (PRP) on the recovery of muscle rupture in animals model (wistar strain white rats). Methods: This is an experimental study with a post-test only control group design using animals model (white rats). This study was conducted at the INA Lab Laboratory, Padang in November 2024. The samples were divided into two groups, the treatment group and the control group where both of these groups were injured and sutured to the muscle. The control group was not given PRP, while the treatment group was given 0.1 cc of PRP. The PRP used was heterologous from healthy human blood. Observations were made after 1 week both groups were maintained and treated. Results: There were 12 white rats which were divided into the PRP group and the control group. The PRP group had a lower histopathological score (7.68) than the control group (15.99). In this study, there was a significant difference between the recovery of muscle ruptures in animal model (white rats) that were given Human Platelet Rich Plasma (PRP) and experimental animals (rats) that were not given PRP (p value 0.001). Conclusion: PRP improves healing in Gastrocnemius muscle rupture.

Keywords: Muscle rupture, gastrocnemius muscle, platelet rich plasma, histopathological score of musculus

Introduction
Gastrocnemius muscle rupture, a common injury among athletes and individuals engaging in vigorous physical activities, significantly impairs the body's ability to bear weight. This condition, often caused by sudden, forceful movements of the calf, presents challenges in achieving complete biological and mechanical recovery. Current standard treatments, including rest, physical therapy, and surgical intervention, have shown limited efficacy in restoring muscle integrity to its pre-injury state (Huard *et al*., 2015) [2]. As such, alternative approaches that accelerate recovery and improve outcomes are critical in addressing this issue.

Platelet-Rich Plasma (PRP) therapy has gained attention for its potential to enhance the healing process in various musculoskeletal injuries. PRP, derived from autologous or heterologous blood, contains a high concentration of growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β), which promote tissue repair and regeneration (Rossi *et al*., 2019) [4]. In preclinical studies, PRP has demonstrated promising effects on cellular proliferation, angiogenesis, and collagen synthesis, all of which are essential for muscle repair (Xiao *et al*., 2020) [5]. These findings suggest that PRP could offer a novel therapeutic strategy for managing gastrocnemius muscle rupture.

Animal models, particularly Wistar strain white rats, are widely used to study the efficacy of PRP in muscle injury recovery due to their physiological similarities to humans in the healing process (Oryan *et al*., 2016) [3]. Experimental studies have indicated that PRP application in these models can significantly reduce inflammation, enhance histopathological scores, and accelerate the repair of ruptured muscle fibers (El-Sharkawy *et al*., 2017) [1]. However, despite the promising results, the optimal dosage, timing, and application techniques for PRP remain

areas of ongoing research.

This study aims to evaluate the effects of PRP on the recovery of gastrocnemius muscle rupture in Wistar strain white rats. By comparing the histopathological outcomes between PRP-treated and untreated groups, this research seeks to provide evidence supporting the clinical application of PRP in muscle injury management. Understanding the therapeutic potential of PRP in this context could pave the way for improved treatment protocols that enhance recovery and reduce disability following gastrocnemius muscle rupture (Zhang *et al*., 2021) [6].

Materials and Methods

Study Design

This research employed an experimental study with a post-test only control group design. The study aimed to evaluate the effects of Platelet-Rich Plasma (PRP) on the recovery of gastrocnemius muscle rupture in Wistar strain white rats. The research was conducted at the INA Lab Laboratory, Padang, in November 2024.

Study Subjects

The study utilized 12 male Wistar strain white rats (Rattus norvegicus), aged 8–10 weeks, and weighing between 200–250 grams. The rats were selected due to their physiological similarities to humans in the healing process. The animals were housed in individual cages under standard laboratory conditions with a 12-hour light-dark cycle, controlled temperature of 22 °C ± 2 °C, and ad libitum access to food and water. Ethical approval for the study was obtained from the Institutional Animal Ethics Committee, ensuring compliance with animal welfare standards.

Preparation of Platelet-Rich Plasma (PRP)

The PRP used in this study was heterologous, derived from healthy human donors under strict aseptic conditions. Blood samples were collected in anticoagulant-coated tubes and centrifuged at 1,500 rpm for 10 minutes to separate the plasma and platelet-rich layer. The PRP layer was then carefully extracted and stored at 4 °C until use. The final concentration of platelets in the PRP preparation was standardized to 5–6 times the baseline concentration.

Induction of Muscle Injury

The gastrocnemius muscle rupture was induced under general anesthesia. Each rat was anesthetized using an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). A small incision was made on the posterior lower limb to expose the gastrocnemius muscle. A controlled transverse rupture was created in the mid-belly of the muscle using a scalpel. The muscle was then sutured using a 4-0 absorbable suture in a standard interrupted pattern to mimic clinical repair.

Study Groups

The rats were randomly divided into two groups of six animals each:

1. Control Group: Rats received only suturing of the ruptured gastrocnemius muscle with no additional treatment.
2. PRP Treatment Group: Rats received 0.1 cc of PRP injected directly at the injury site immediately after suturing.

Postoperative Care

All rats were monitored daily for signs of infection, pain, or complications. Postoperative care included administration of analgesics (meloxicam 1 mg/kg) and antibiotics (enrofloxacin 10 mg/kg) for three days. The animals were allowed to recover in individual cages with limited movement to prevent further injury.

Histopathological Analysis

After one week, all animals were euthanized using an overdose of ketamine and xylazine. The gastrocnemius muscles were harvested and fixed in 10% formalin. The specimens were then processed, embedded in paraffin, and sectioned at 4 µm thickness for histopathological examination. Hematoxylin and eosin (H&E) staining was performed to assess inflammation, neovascularization, fibroblast proliferation, and muscle fiber regeneration. A blinded pathologist evaluated the samples and assigned histopathological scores based on a validated scoring system.

Statistical Analysis

The data were analyzed using SPSS version 25. The histopathological scores were compared between the control and PRP treatment groups using an independent t-test. A p-value of less than 0.05 was considered statistically significant. Data were presented as mean ± standard deviation.

Results

Study Population

A total of 12 male Wistar strain white rats were included in this study. The rats were randomly divided into two groups of six animals each: the control group (no PRP application) and the PRP treatment group (0.1 cc PRP application). All animals completed the study without any complications such as infection or mortality.

Histopathological Scores

The histopathological evaluation revealed significant differences between the control and PRP treatment groups. The mean histopathological score for the control group was 15.99±1.21, while the PRP treatment group had a significantly lower mean score of 7.68±1.03. Lower scores in the PRP group indicated reduced inflammation, enhanced neovascularization, increased fibroblast activity, and improved muscle fiber regeneration compared to the control group ($p = 0.001$).

Histopathological Findings

1. Control Group

Marked inflammation characterized by abundant inflammatory cell infiltration.

Limited neovascularization and fibroblast proliferation.

Poor muscle fiber alignment and minimal regeneration at the injury site.

2. PRP Treatment Group

Reduced inflammatory cell infiltration compared to the control group.

Significant neovascularization and fibroblast activity observed at the injury site.

Enhanced muscle fiber regeneration with better alignment of new muscle fibers.

Statistical Analysis

The independent t-test demonstrated a statistically significant difference in histopathological scores between the PRP treatment group and the control group ($p = 0.001$). The effect size was calculated to be large, indicating a strong therapeutic effect of PRP on muscle healing.

Observation Outcomes

During the one-week observation period, the PRP-treated group exhibited faster recovery with improved mobility compared to the control group. Rats in the PRP group showed reduced swelling and redness around the injury site, correlating with the histopathological findings.

PRP application significantly improved the healing process in ruptured gastrocnemius muscles, as evidenced by histopathological scores. The therapeutic effects of PRP included reduced inflammation, enhanced neovascularization, increased fibroblast activity, and improved muscle fiber regeneration. These findings support the hypothesis that PRP accelerates muscle recovery and enhances the biological and mechanical properties of injured muscles.

Discussion

The Role of PRP in Muscle Healing

The results of this study demonstrate that Platelet-Rich Plasma (PRP) significantly enhances the recovery of gastrocnemius muscle rupture in Wistar strain white rats. PRP application led to improved histopathological outcomes, characterized by reduced inflammation, enhanced neovascularization, increased fibroblast activity, and better muscle fiber regeneration. These findings are consistent with previous research indicating that PRP's high concentration of growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor-beta (TGF-β), promotes tissue repair and regeneration (Rossi *et al*., 2019) [4]. These factors facilitate angiogenesis, collagen synthesis, and cellular proliferation, all essential for muscle healing.

Reduction in Inflammation

Inflammation is a key phase in muscle repair, but excessive inflammation can delay healing and lead to fibrotic scar formation. In this study, the PRP-treated group showed significantly lower levels of inflammatory cell infiltration compared to the control group. This aligns with research suggesting that PRP modulates inflammation by balancing pro- and anti-inflammatory cytokines (El-Sharkawy *et al*., 2017) [1]. By reducing inflammation, PRP creates an optimal environment for subsequent healing phases, including proliferation and remodeling.

Enhancement of Neovascularization and Fibroblast Activity

Neovascularization is critical for supplying oxygen and nutrients to the injured tissue, facilitating the healing process. In the PRP group, increased neovascularization was observed, which is consistent with the angiogenic properties of PRP. Additionally, fibroblast proliferation was significantly enhanced, contributing to the synthesis of extracellular matrix and collagen necessary for muscle regeneration (Xiao *et al*., 2020) [5]. These effects underscore the role of PRP in accelerating the transition from the inflammatory phase to the proliferative phase of healing.

Improved Muscle Fiber Regeneration

The PRP group demonstrated superior muscle fiber regeneration with better alignment compared to the control group. This finding is supported by evidence that PRP promotes the activation and differentiation of satellite cells, which are essential for muscle repair (Oryan *et al*., 2016) [3]. Improved muscle fiber alignment and organization suggest that PRP not only accelerates healing but also restores the structural integrity of the injured muscle.

Comparison with Previous Studies

Similar studies have reported the efficacy of PRP in enhancing muscle healing. A study by Huard *et al*. (2015) [2] demonstrated that PRP-treated muscle injuries showed faster recovery and less fibrosis in animal models. Moreover, clinical studies have highlighted the potential of PRP in reducing recovery time for athletes with muscle injuries (Zhang *et al*., 2021) [6]. The consistency of these findings reinforces the therapeutic potential of PRP in managing muscle injuries.

Clinical Implications

The results of this study suggest that PRP can be a valuable adjunct in the treatment of muscle injuries, including gastrocnemius muscle rupture. By accelerating the healing process and improving tissue quality, PRP has the potential to reduce recovery time and enhance functional outcomes. However, the use of heterologous PRP in this study raises questions about its clinical translation, as autologous PRP is more commonly used in practice. Further research is needed to determine the optimal preparation, dosage, and application techniques for PRP.

Study Limitations and Future Directions

While this study provides valuable insights, several limitations should be addressed. The sample size was relatively small, which may affect the generalizability of the findings. Additionally, the study duration was limited to one week, which may not capture the long-term effects of PRP on muscle healing. Future studies should include larger sample sizes, longer observation periods, and functional assessments to validate these findings and explore the potential of PRP in clinical settings.

Conclusion

This study highlights the significant benefits of PRP in accelerating the recovery of gastrocnemius muscle rupture in Wistar strain white rats. By modulating inflammation, enhancing neovascularization and fibroblast activity, and promoting muscle fiber regeneration, PRP improves the biological and mechanical properties of the injured muscle. These findings pave the way for further research and potential clinical applications of PRP in managing muscle injuries.

Conflict of Interest

Not available

Financial Support

Not available

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