Topical Metformin Lotion Enhances Muscle Regeneration Following Volumetric Muscle Loss in Mice

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INTRODUCTION

Skeletal muscle injuries involving volumetric muscle loss (VML) pose unique challenges for surgical repair. Although skeletal muscle has substantial regenerative potential, severe tissue loss can overwhelm the satellite cell pool, leading to persistent muscle mass loss, functional impairment, and intramuscular fibrosis characterized by collagen deposition. Our laboratory previously demonstrated that metformin lotion (ML) enhances skin wound healing in rats. Here, we investigated whether topical ML accelerates early healing of muscle injury and improves muscle regeneration.

METHODS

Metformin lotion (ML): Prepared in-house using a proprietary formulation. **Animal model:** VML was created in the gastrocnemius of 10 female mice using a 2-mm sterile biopsy punch (**Fig. 1A-C**). Because this was a pilot study, male mice were not included. Mice were randomly assigned to two groups (n=5/group): (1) control (0% ML, vehicle only; 20 mg/mouse daily) or (2) treatment (6% ML; 20 mg/mouse daily). Beginning on postoperative day 1, lotion was applied topically to the skin surrounding the injured gastrocnemius (**Fig. 1D**). Mice were euthanized at 4 weeks for histological and immunohistochemical analyses. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

RESULTS

H&E staining showed that in the muscular injury area treated with 0% ML, large areas of defect remained visible (**Fig. 2A–C**). In contrast, the injury area treated with 6% ML showed a significantly reduced defect area (**Fig. 2G–I**). Collagen deposition was clearly observed by Masson's trichrome (MT) staining. Compared with the 0% ML control group (**Fig. 2D–F**), the 6% ML treatment group exhibited lower levels of collagen staining (**Fig. 2J–L**). Moreover, the 6% ML-treated group displayed much less robust α-SMA expression (**Fig. 3G–I**) compared with the 0% ML group (**Fig. 3A–C**), suggesting reduced myofibroblast activation and improved muscle regeneration. Finally, collagen III expression in the 6% ML group (**Fig. 3J–L**) was lower than in the 0% ML group (**Fig. 3D–F**), where a large defect area persisted (**Fig. 3M**).

DISCUSSION

Early application of ML around muscle injury promoted repair and regeneration by reducing defect size, lowering collagen III levels, and decreasing α-SMA–positive myofibroblast activity, thereby limiting fibrosis and supporting tissue integrity. These beneficial effects are likely mediated by ML's local anti-inflammatory and antifibrotic actions. Previously, we showed that ML decreased levels of HMGB1 and IL-1β and enhanced AMPK activity in skin wound healing. Similar mechanisms may underlie the improvements observed in skeletal muscle, contributing to preservation of muscle mass and function. Overall, these findings highlight the therapeutic potential of ML in muscle repair and regeneration.

SIGNIFICANCE

Topical ML administration is a simple, effective, and convenient approach for treating muscular injuries.

ACKNOWLEDGEMENTS

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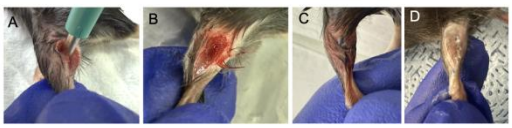

Fig. 1 Intraoperative imaging of the VML surgical procedure and postoperative ML application. A VML defect was created in the gastrocnemius muscle using a 2-mm sterile biopsy punch (**A–C**). ML was then applied topically to the skin surrounding the gastrocnemius muscle injury (**D**). VML, volumetric muscle loss.

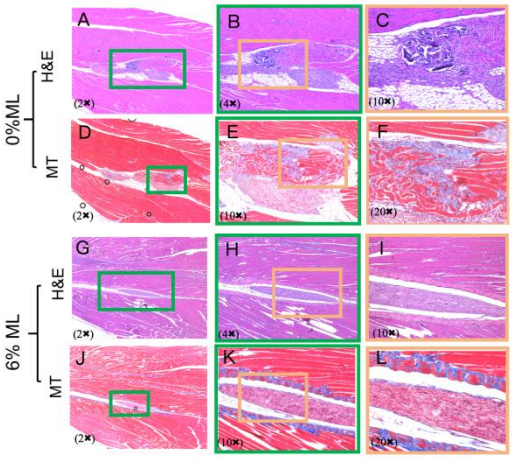

Fig. 2 ML reduces muscle defect area and collagen deposition in injured mouse gastrocnemius muscle, as assessed by H&E and Masson's trichrome (MT) staining.

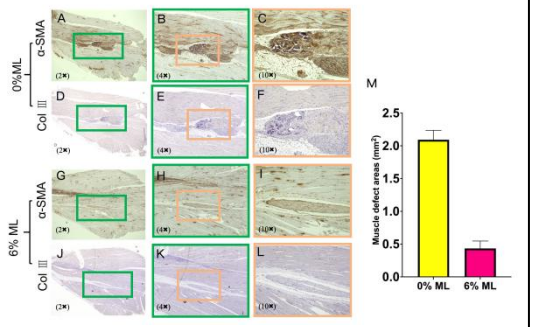

Fig. 3 ML reduces α-SMA and collagen III expression as shown by immunohistochemical staining (**A–L**). Quantification of muscle defect areas in ML-treated versus untreated controls is shown in the chart (**M**).