Acute Toxicity Evaluation on a Novel Transdermal Metformin Lotion for Tendinopathy Treatment

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INTRODUCTION

Tendinopathy is a common tendon disorder affecting millions of Americans and costing billions annually in healthcare expenditures. We previously demonstrated that intraperitoneal (IP) injection of metformin (Met), a first-line diabetes medication, can prevent tendinopathy development in a mouse model [1]. However, daily IP injections are impractical for clinical use and can cause skin trauma, while oral dosing is associated with gastrointestinal side effects [2]. Transdermal drug delivery offers an attractive alternative, enabling local administration, reduced dosing, and avoidance of gastrointestinal complications. In this study, we developed a novel transdermal Met lotion (ML) and evaluated its safety and acute dermal toxicity in rats.

METHODS

ML was formulated in-house using a proprietary composition (**Fig. 1A**) and tested in 66 female Sprague Dawley rats (8 weeks old). Female rats were selected in accordance with acute toxicology guidelines, which recommend the more sensitive sex to maximize detection of adverse effects while minimizing animal numbers. Animals were assigned to three groups receiving 2 g of either control lotion (0% ML), 3% ML, or 6% ML (~300–600 mg/kg) applied to the shaved dorsal skin (**Fig. 1C**). Four untreated rats served as additional controls (**Fig. 1B**). Clinical signs—including mortality, edema, erythema, and dermal changes—were recorded up to 96 h post-application. At each time point, subsets of animals were euthanized for gross organ inspection, histology (H&E, Safranin O/Fast Green), and immunostaining for collagen I and phosphorylated AMP-activated protein kinase (p-AMPK). All procedures were approved by the University of Pittsburgh IACUC.

RESULTS

No mortality, edema, erythema, or other visible skin abnormalities were observed in any group (**Fig. 1D–O**). Gross examination revealed no pathological changes in the skin or major organs, including liver, lung, kidney, spleen, and heart. Heart size and heart-to-body weight ratios were comparable across all groups (**Fig. 2A-J**). Histological analysis showed preserved skin architecture in all treated and control animals (**Fig. 3A–F**), with similar collagen I expression levels (**Fig. 3G–I**). Notably, p-AMPK expression was increased in ML-treated skin compared with untreated controls (**Fig. 3J–L**), confirming local activation of Met's primary molecular target.

DISCUSSION

Our data demonstrate that topical ML, even at the highest tested dose (6%), does not induce acute dermal or systemic toxicity in rats. The absence of edema, erythema, and histological alterations indicates good dermal tolerance, while preserved organ morphology confirms systemic safety. The observed p-AMPK activation suggests that ML engages its intended mechanism of action in skin tissue, which is known to suppress inflammatory responses and reduce fibrosis. This dual safety–efficacy profile supports further evaluation of ML as a localized, noninvasive therapy for tendinopathy and potentially wound healing.

SIGNIFICANCE

Topical ML is a safe, effective, and convenient delivery strategy for metformin, with potential to transform tendinopathy management and improve patient compliance. These findings warrant continued preclinical development and eventual translation into clinical studies.

REFERENCES

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ACKNOWLEDGEMENTS

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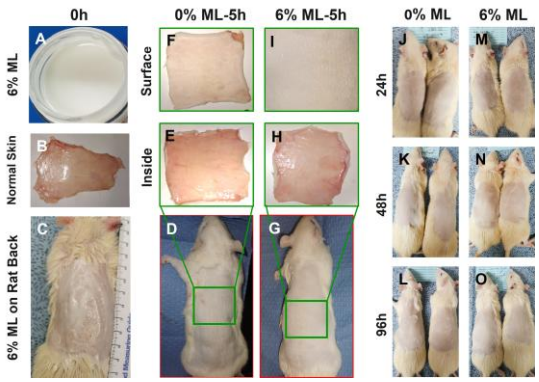

Fig. 1 ML does not cause acute dermal toxicity in rats. (**A**) 6% ML appears as a white, homogeneous cream. (**B**) Normal rat skin. (**C**) Application of 6% ML to the dorsal skin at 0 h. No signs of acute dermal toxicity are observed on the skin surface or underlying tissue after short-term (5 h) or long-term (4 days) exposure in any group (**D–O**).

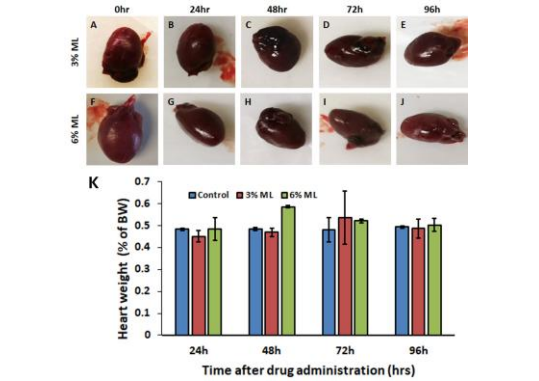

Fig. 2 ML does not alter heart size or weight in rats compared to normal controls. (**A–J**) No visible changes in heart size after ML treatment. (**K**) The heart weight-to-body weight (BW) ratio is comparable among the three groups.

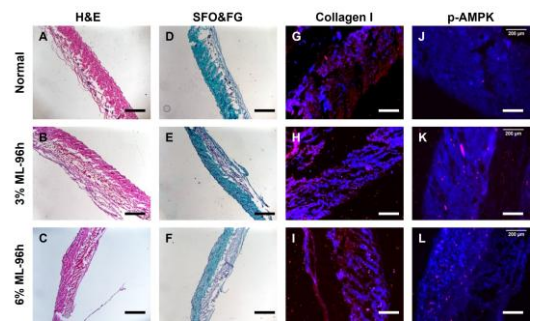

Fig. 3 ML-treated rat skin exhibits a similar structure to that of normal rats, with increased p-AMPK expression in skin tissues. (**A–C**) H&E staining. (**D–F**) SFO & FG staining. (**G–I**) Collagen I staining. (**J–L**) p-AMPK staining. Black scale bars: 500 µm; white scale bars: 200 µm.