

Metformin Lotion Reduces Scar Tissue Formation in Rats by Activating AMPK and Inhibiting TGF- β 1

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INTRODUCTION: Skin wounds and compromised wound healing are major concerns for the public health sector. In the United States alone, 6.5 million patients need treatment for chronic wounds and an estimated US \$25 billion is spent annually [1]. Following injury, the skin undergoes a wound healing process that forms a mature scar. Scars can be painful, disfiguring and disabling. Most commercial wound healing products fail to initiate skin regeneration. High levels of α -smooth muscle actin (α -SMA) and collagen III can result in loose collagen fibers that cause scar formation in skin. Adenosine monophosphate-activated protein kinase (AMPK) is known to regulate tissue inflammatory signaling, and activation of AMPK can inhibit scar tissue formation [2]. Metformin (Met), an oral hypoglycemic drug commonly used for the treatment of type-2 diabetes, can activate AMPK. Met has been shown to prevent lung fibrosis previously [3]. However, oral Met has side effects on stomach, liver and kidney. In this study, we formulated Met lotion as a novel topical drug to deliver Met directly into the injured skin area to enhance the skin healing quality. The effect of Met lotion on wounded skin healing was also studied by using a rat skin injury model.

METHODS: Met lotion was formulated in our lab. Ten Sprague Dawley (SD) rats were used for this study. An incised wound (2 cm) was made on the skin of each Achilles tendon area by a scalpel. Then the wounds were sutured. The wounded rats were divided into two groups and the skin surface of the wound area was treated for 10 days as follows: **Group 1:** smeared the control lotion daily (**0% Met-lotion**); **Group 2:** smeared 6% Met-lotion daily (**6% Met-lotion**). The animals were sacrificed at 10 days post-surgery. The Met effect on wounded skin healing was investigated by histological analysis on rat skin tissue sections. **Statistical analysis** – Data were analyzed by One-way ANOVA followed by Fisher's LSD test for multiple comparisons. A p-value less than 0.05 was considered to be a significant difference between the two groups. This study was approved by IACUC.

RESULTS: H&E staining results showed the dense and thick epidermis formed at the wound area treated with 0% Met-lotion (yellow arrows in **Fig. 1A, B**). However, normal skin-like tissues were formed at the wound area treated with 6% Met lotion (red arrows in **Fig. 1C, D**). Trichrome Masson staining showed the large empty gap at the wound area treated with 0% Met-lotion (black arrows in **Fig. 1E, F**). However, no gap was found in the wound areas treated with 6% Met-lotion (white arrows in **Fig. 1G, H**). Semi-quantification indicated that the epidermis of the healed skin tissue at the wound area treated with 6% Met-lotion was 6 times thinner than the epidermis of the skin at the wound area treated with 0% Met-lotion (**Fig. 1I**). Picro Sirius Red staining indicated that under bright light microscope, large gap was found in the 0% Met-lotion treated wound areas (black arrow in **Fig. 2A**), while there was no gap found in the wound area treated with 6% Met-lotion (**Fig. 2C**). Under polarized light microscope, high levels of collagen III were found at the wound area treated with 0% Met-lotion (green fluorescence in **Fig. 2B**), however, the tissues at the wound area treated with 6% Met-lotion were positively stained with collagen I (red fluorescence in **Fig. 2D**). Immunostaining results showed that Met-lotion enhanced AMPK activity (**Fig. 3A**), inhibited α -SMA (**Fig. 3B**) and TGF- β 1 (**Fig. 3C**), decreased collagen III (**Fig. 3D**) levels and increased collagen I (**Fig. 3E**) expression in wounded skin areas. In contrast, the wound treated with control lotion (0% Met-lotion) expressed lower levels of AMPK (**Fig. 3F**), higher levels of α -SMA (**Fig. 3G**), TGF- β 1 (**Fig. 3H**) and collagen III (**Fig. 3I**), and lower levels of collagen I when compared to 6% Met-lotion treated wounds.

DISCUSSION: This study used a novel Met lotion as a topical drug for the prevention of scar tissue formation in skin wound healing. Our results demonstrated that the skin wound treated with control lotion without Met healed slowly and formed scar-like tissues. In contrast, the wound treated with Met lotion healed much faster than control lotion and formed normal-skin-like tissues. Moreover, Met lotion application enhanced AMPK activity, decreased TGF- β 1 expression, inhibited α -SMA⁺ cell numbers, and decreased collagen III production. These findings indicate that inhibition of scar tissue formation by Met lotion is likely through activation of AMPK, reduction of TGF- β 1 expression, inhibition of α -SMA-expressing myofibroblasts and reduction of collagen III production.

SIGNIFICANCE/CLINICAL RELEVANCE: Metformin lotion may be used in clinical settings to prevent scar formation in tissues such as skin and connective tissues, such that a high quality of tissue regeneration in wounded skin can be achieved.

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