

Title: Small Molecule Antagonists of RAGE/DIAPH1 Interaction: Novel Therapeutic Opportunities in Impaired Wound Healing in Type 2 Diabetic Mice

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Introduction: Previous work in our laboratory has shown that administration of the soluble extracellular domains of the receptor for Advanced Glycation Endproducts, RAGE, thereby blocking the access of ligands to cell surface RAGE, restored effective wound healing in diabetic mice⁽¹⁾. Our recent work has also shown that the cytoplasmic tail (ct) of RAGE is essential for RAGE ligand-mediated signal transduction and consequent modulation of gene expression and cellular properties and RAGE signaling requires interaction of ctRAGE with the intracellular effector, mammalian diaphanous 1, or DIAPH1⁽²⁾. Prompted by our earlier discovery of 13 small molecules that block the interaction of ctRAGE with DIAPH1⁽³⁾, we synthesized a novel compound (RAGE229) and performed structure-activity relationship experiments to identify its ability to delay or prevent the progression of RAGE-related diabetic wound healing, as impaired wound healing in diabetes is a major cause of morbidity and mortality and the therapeutic landscape is currently limited.

Methods: Wound surgery: 8 week old male and female Lep^(ob) (BTBR background) mice underwent surgery to introduce a 1cm x 1cm full-thickness wound located in the upper back. **rt-PCR levels of DIAPH1 in wound healing tissue:** DIAPH1 levels were measured in either whole skin or newly formed wound tissue.

Wound treatment with RAGE229. Beginning on day 3 and through to day 10 post-op, animals were either treated with novel compound RAGE229 or vehicle by topical administration. Photographs were taken weekly and wound closure was measured using Image J. All animals were sacrificed at Day 21.

Results:

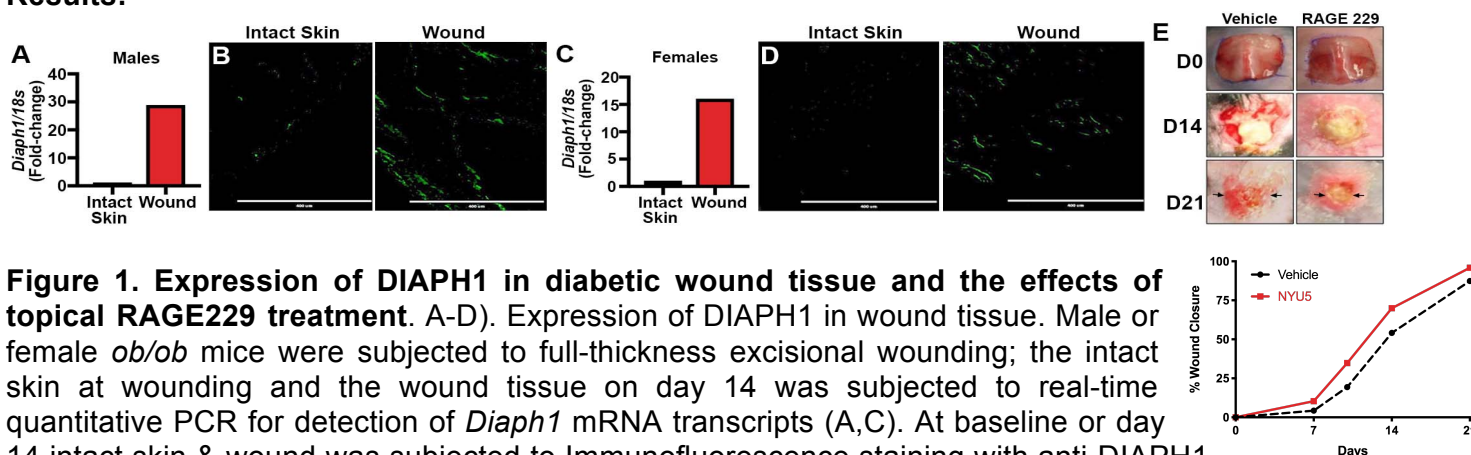


Figure 1. Expression of DIAPH1 in diabetic wound tissue and the effects of topical RAGE229 treatment. A-D). Expression of DIAPH1 in wound tissue. Male or female *ob/ob* mice were subjected to full-thickness excisional wounding; the intact skin at wounding and the wound tissue on day 14 was subjected to real-time quantitative PCR for detection of *Diaph1* mRNA transcripts (A,C). At baseline or day 14 intact skin & wound was subjected to Immunofluorescence staining with anti-DIAPH1 IgG (B,D). Scale bar: 400 μ m. In E, male *ob/ob* mice were subjected to full-thickness excisional wound and on Day 3, mice began treatment with either topical RAGE229 (5 mg/kg/2x per day) vs. vehicle, placed under the tegaderm through Day 10. RAGE229-treated wound exhibited better organized wound closure (arrowheads) vs. vehicle-treated mice, where the wound tissue appeared thin and disorganized through Day 21.

Conclusions: We conclude that these preliminary data suggest that this novel compound inhibits the interaction of ctRAGE with DIAPH1, thereby accelerating wound healing. Furthermore, the development of the RAGE-DIAPH1 antagonist, RAGE229, presents an attractive molecular scaffold for the development of therapeutics against RAGE-mediated diabetic complications.

References:

- 1) Goova *et al.* Am. J. of Path. 2001; 159(2)
- 2) Hudson *et al.* JBC 2008; 283:49
- 3) Manigrasso *et al.* Sci Reports 2016 6:22450