Odanacatib, a Cathepsin K Inhibitor Accelerates Wound Healing in a Diabetic Porcine Model

Anne Chenchar[1], Brenda M Alexander[2], Zhaojie J Zhang[3], Amanda E. Louiselle[4], Stephen M. Niemiec[4], James Bardill[4], Tanner Lehmann[4], Carlos Zgheib[4], Kenneth. W. Liechty[4], Sreejayan Nair[1].

[1]School of Pharmacy, College of Health Sciences, [2]Department of Animal Science, [3]Zoology & Physiology, University of Wyoming, WY, USA; [4]University of Colorado Denver-Anschutz Medical Campus and Children's Hospital Colorado, Laboratory for Fetal and Regenerative Biology, Department of Surgery, Aurora, CO, USA.

Abstract

Introduction: We have previously shown that the genetic knockout of the cysteine protease cathepsin K facilitates the healing of wounds in diabetic mice. The objective of this study was to assess the efficacy of odanacatib, a small molecule inhibitor of cathepsin K to accelerate diabetic wound healing in a clinically relevant porcine model.
Methods: Eight female Yorkshire pigs (3-months-old) were rendered diabetic by streptozocin (150 mg/Kg/iv). Four weeks following the induction of diabetes, ten full-thickness excision wounds were created on the backs of the pigs. Individual wounds were treated intradermally with odanacatib (300 or 10 ng/wound), PBS, or becaplermin (Regranex®, 100 ug/g; 0.6 inch/wound) on days 0, 7, and 14 post-wounding. Wounds were photographed for wound area measurement. Histological sections were stained with H&E, Mason's trichome, CD45, and CD31. Statistical analysis was performed using an ANOVA followed by Tukey's post hoc test, and a p-value ≤ 0.05 was considered statistically significant.
Results: Our data showed the average day to healing for the wounds for the control (non-diabetic vehicle-treated animals) was 26.75 ± 1.5 days, while the wounds of the diabetic animals healed at 30.2 ± 2.4 days (mean ± SEM, n=4-5 wounds). In contrast, full closure of the becaplermin-treated diabetic wounds averaged 30.4 ± 2.5 days (n=5). Wounds treated with the higher dose of odanacatib exhibited accelerated healing, at rates like that of the vehicle-treated wounds, exhibiting a full closure at 25.4 ± 1.5 days (n=5). The lower dose of odanacatib did not alter the rate of wound healing in diabetic pigs.
Discussion: Our results demonstrate that odanacatib accelerates wound healing in diabetic pigs, suggesting its potential clinical utility.

Introduction

- Patients with diabetes mellitus have a 15-20% lifetime risk of developing a diabetic foot ulcer (DFU), with over 70% of the patients who develop DFUs requiring lower-limb amputation.[1,2]
- CatK is a lysosomal cystine protease known for its activity in degrading collagen and elastin within osteoclasts[3].
- Our lab has previously shown an increase in cathepsin K (CatK) levels in diabetic human skin tissue, and the knockout of the cathepsin K gene (*CTSK*$^{-/-}$) accelerates healing in streptozocin-treated mice.
- Odanacatib is a selective, reversible inhibitor of Catk that binds to the active site of the enzyme, preventing collagen binding[4].
- We **hypothesize** that the inhibition of CatK using odanacatib will accelerate wound healing within a diabetic porcine model.

Methodology

Eight female Yorkshire pigs (3 months old) were rendered diabetic by a single intravenous infusion of streptozocin (150 mg/Kg) or received citrate buffer as control. Four weeks following the induction of diabetes, ten full-thickness excision wounds measuring 2.5 x 2.5 cm were created on the backs of the pigs. Individual wounds were treated with either odanacatib at either 300 ng or 10ng/wound via intradermal injections, vehicle (PBS), or becaplermin (Regranex®) gel (0.65g per inch of the wound). Treatments were performed on days 0, 7, and 14 post-wounding. Wounds were photographed, and ImageJ was used to measure the wound area. The skin tissues were subjected to histological analyses on both day 14 and at full closure for collagen (Mason's trichome staining). Statistical analysis was performed using an ANOVA followed by Tukey's post hoc test (GraphPad Prism 9), a p-value ≤ 0.05 was considered statistically significant.

Results

Odanacatib Facilitates Wound Healing in Diabetic Pigs

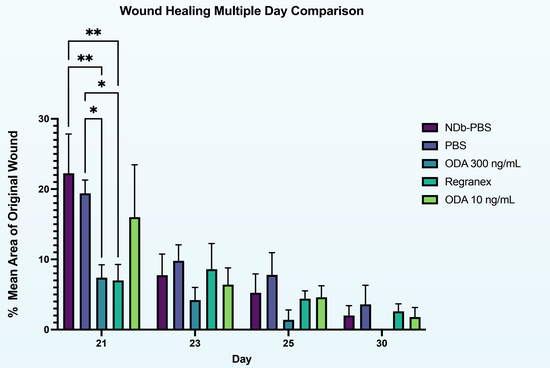

Fig 1. Accelerated wound healing in diabetic pigs by odanacatib. Diabetic pigs were subjected to full-thickness wounds and treated with PBS, odanacatib, or Regrenex®, and wound closure was determined from day 21 to day 30. On day 21, odanacatib 300 ng/mL exhibited improved wound healing compared to Regranex® and vehicle. Data are expressed as mean± SEM, n= 5). In contrast to Regranex and the PBS treatment, odanacatib exhibited complete healing on day 30. ND-non-diabetic, PBS– phosphate-buffered saline; ODA- odanacatib.

Results

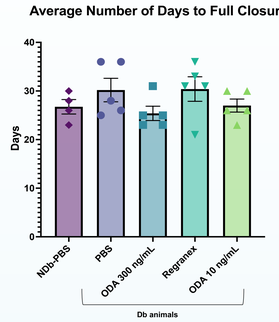

Fig 2. Time to complete wound closure. Diabetic pigs were subjected to full-thickness wounds and treated with PBS, odanacatib, or Regrenex®, and the time to full wound closure was determined. Data are expressed as mean± SEM, n= 5). No statistically significant difference was seen between the treatments for full wound closure although ODA exhibited a trend towards a shorter time to closure.

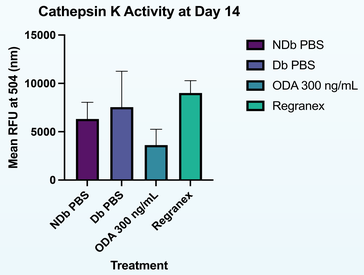

Fig 3. Cathepsin K enzyme activity in diabetic wounds Diabetic pigs were subjected to full-thickness wounds and treated with PBS, odanacatib, or Regrenex®. Odanacatib-treated wound samples show reduced levels of cathepsin K activity compared to both non-diabetic and diabetic PBS and diabetic Regranex®, treated animals. Data are expressed as (mean ± SEM, n=4-5). No Statistical significance between treatments was seen.

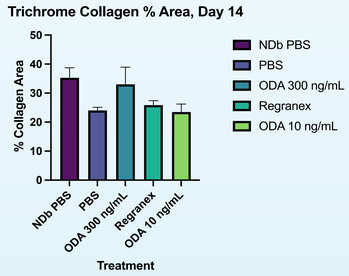

Fig 4. Immunohistochemistry of collagen in diabetic wounds. Diabetic pigs were subjected to full-thickness wounds and treated with PBS, odanacatib, or Regrenex®. Collagen percent area was determined by Masson's trichome staining and ImageJ anaylsis of 1x4 mm sections in the center of the wounded area. (n=5, mean ±SEM). No statistical significance present, although Non-diabetic PBS treated, and diabetic ODA 300 ng/mL animals exhibited greater levels of collagen deposition.

Conclusion

- Odanacatib (300 ng/wound) treatment accelerated healing of wounds in a porcine model of diabetes
- On day 21 post-wounding, odanacatib-treated wounds had a significantly lower wound area compared to Regranex®-treated wounds, the healing with odanacatib was comparable to that of the vehicle-treated group.
- A trend toward reduction in cathepsin K levels and increased collagen levels were observed in the odanacatib-treated wounds.
- Inhibition of cathepsin K is a potential strategy to treat diabetic wounds.

References

1. Gao, D., Zhang, Y., Bowers, D. T., Liu, W. & Ma, M. Functional hydrogels for diabetic wound management. *APL Bioeng.* **5**, 031503 (2021).
2. Glover, K., Stratakos, A. C., Varadi, A. & Lamprou, D. A. 3D scaffolds in the treatment of diabetic foot ulcers: New trends vs conventional approaches. *Int. J. Pharm.* **599**, 120423 (2021).
3. Dai, R. *et al.* Cathepsin K: The Action in and Beyond Bone. *Front. Cell Dev. Biol.* **8**, (2020).
4. Stone, J. A., McCrea, J. B., Witter, R., Zajic, S. & Stoch, S. A. Clinical and translational pharmacology of the cathepsin K inhibitor odanacatib studied for osteoporosis. *Br. J. Clin. Pharmacol.* **85**, 1072–1083 (2019).

Acknowledgements

This work was supported by a grant from NIH NIDDK (1R41DK125213-01A1)