4-aminopyridine Accelerates Wound Healing Through Re-epithelialization, Hair Follicle Neogenesis, Myofibroblast Differentiation, Angiogenesis, and Reinnervation Following Full-Thickness Dorsal Skin Wounds

John C. Elfar, Jagadeeshaprasad M. G., and Prem Kumar Govindappa

Department of Orthopaedic Surgery and Sports Medicine, College of Medicine, University of Arizona, Tucson, AZ, 85724, USA.

Introduction
- Wound healing involves inflammation, proliferation, regeneration, remodeling, each of which is regulated by cutaneous innervation.
- Discovering key molecular targets that trigger accelerated wound healing is a critical therapeutic strategy.
- We discovered that 4-aminopyridine (4-AP), a potassium channel blocker, greatly enhances skin wound healing.
- 4-AP treatment promotes healing-related changes in multiple cell types, including keratinocytes, fibroblasts, myofibroblasts, Schwann cells, neurons and blood vessels.
- As 4-AP is already approved for human use by the FDA, our discoveries enable rapid development of a new approach to enhance the skin wound healing process. The FDA recently approved our first trial for wound healing.
- We hypothesized that 4-AP treatment enhances tissue repair and regeneration via cutaneous nerve regeneration in full-thickness skin wounds.

Methods
- In 10-week-old male C57BL/6 mice, paired 5-mm dorsal excisional wounds were created and splinted to prevent wound contraction.
- Mice were randomly divided into two groups and given either saline or systemic 4-AP (1.6 mg/kg body weight at human dosages) daily for 14 days.
- Wounds were monitored by digital imaging for morphometry, percentage of wound healing, and tissue regeneration on days 3, 5, 7, 9, 12, and 14 post-wound.
- Skin tissue was harvested on day 14 for histo- and immunofluorescence staining, and western blot analysis.

Results

Figure 1. 4-AP expedites wound closure and enhances skin regeneration. Images of wound healing (A) and percent wound closure (B) relative to the initial wound area, H&E-stained section of skin (C), quantification of epidermal thickness (D), and number of hair follicles (E) of saline and 4-AP treated groups. Mean ± SEM, n = 5/group.

Figure 2. 4-AP increases keratinocyte number and epithelial stem-cell markers in wound healing. Quantitative immunostaining reveals the levels of Keratin 14, 17, and 15 (A-C). Data represent 20 images from 5 different mouse wounds. mean ± SEM, n = 5/group.

Figure 3. 4-AP increases the number of Schwann cells and the expression of an early SC differentiation state. Quantitative immunostaining expressions of SOX10 and p75-NTR (A-C) and Western blotting of p75-NTR (D-F) in skin wounds. mean ± SEM, n = 5/group.

Figure 4. 4-AP enhanced expression of transcription factors, neurotrophic factors, and neuropeptides associated with reinnervation. (A-D). Quantification of immunostaining expression of wound skin for the transcription factor SOX10, neuropeptide substance-P, nerve growth factor (NGF), and nuclear stain DAPI. Data represent 20 images from 5 different mouse wounds, mean ± SEM, n = 5/group (E-G). Quantification of Western blot expression of SOX10, NGF, and GAPDH. mean ± SEM, n = 3/group.

Figure 5. 4-AP facilitates nerve regrowth and increases the expression of PGP-9.5. Results of Ki-67+ and NF-H+ cells (A-C) and PGP-9.5 (D, E) surrounding de novo hair follicles in the healed wound. Data represent 20 images from 5 different mouse wounds. mean ± SEM, n = 5/group.

Figure 6. 4-AP treatment increases collagen (A, B), fibroblast, and myofibroblast numbers (C-E) and the levels of alpha-smooth muscle actin (α-SMA) and transforming growth factor-β (F-H). mean ± SEM, n = 5 or 3/group.

Figure 7. 4-AP accelerates wound healing by activating transcription and neurotrophic factors. The results of the keratinocyte scratch assays (A, B) and Western blotting of SOX10 and NGF (C-F) are presented. n = 3/ group; *p<0.05, **p<0.001, ***p<0.0001 vs. control.

Conclusions
- We discovered that systemic administration of 4-AP to wounded mice promotes reinnervation, fibroblast de-differentiation, wound healing and tissue regeneration.
- 4-AP enhanced many of the key features required for successful wound healing and is a promising therapeutic adjuvant for skin wound healing and tissue regeneration.
- 4-AP is already FDA approved for treatment of multiple sclerosis and other chronic neurological syndromes.
- 4-AP has strong potential for rapid translational development for skin wound healing and tissue regeneration.

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Reference
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Contact
- J. Elfar; Tenured Professor & Chair; email: openelfar@gmail.com
- P. Govindappa; Assistant Research Professor; email: premg@arizona.edu