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

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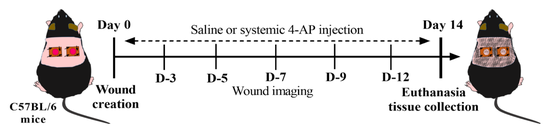
Introduction

- Wound healing involves inflammation, proliferation, regeneration, and 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

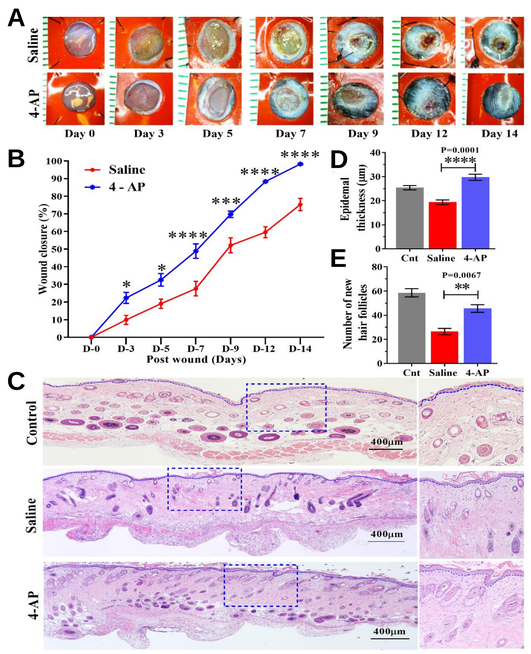


Figure 1. 4-AP expedites wound closure and enhances skin regeneration. Images of wound healing **(A)** and percent wound closure **(B)** relative to the initial wound area, H&E-stained section of skin **(C)**, quantification of epidermal thickness **(D)**, and number of hair follicles **(E)** of saline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

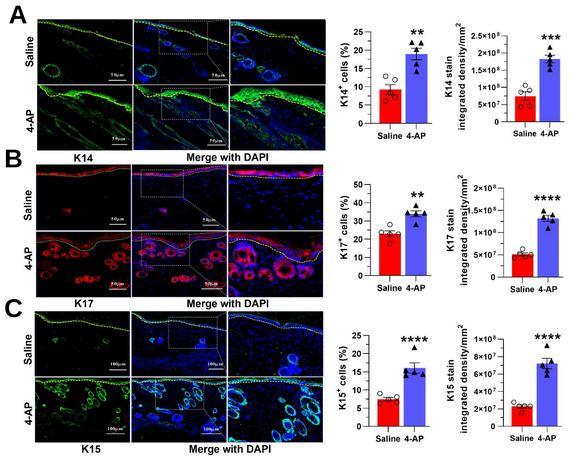


Figure 2. 4-AP increases keratinocyte number and epithelial stem-cell markers in wound healing. Quantitative immunostaining reveals the levels of Keratin 14, 17, and 15 **(A-C)**. Data represent 20 images from 5 different mouse wounds. mean ± SEM, n = 5/ group.

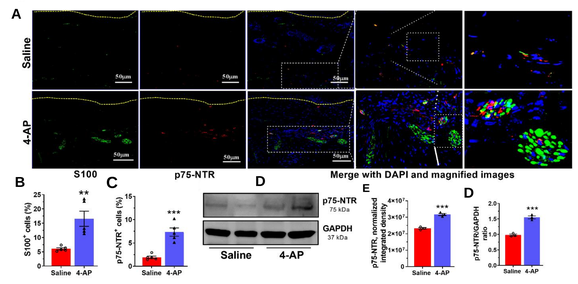


Figure 3. 4-AP increases the number of Schwann cells and the expression of an early SC differentiation state. Quantitative immunostaining expressions of S100 and p75-NTR **(A-C)** and Western blotting of p75-NTR **(D-F)** in skin wounds. mean ± SEM, n = 5/ group.

Results

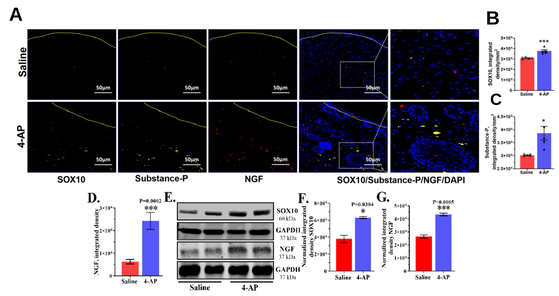


Figure 4. 4-AP enhanced expression of transcription factors, neurotrophic factors, and neuropeptides associated with reinnervation. (A-D). Quantification of immunostaining expression of wound skin for the transcription factor SOX10, neuropeptide substance-P, nerve growth factor (NGF), and nuclear stain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

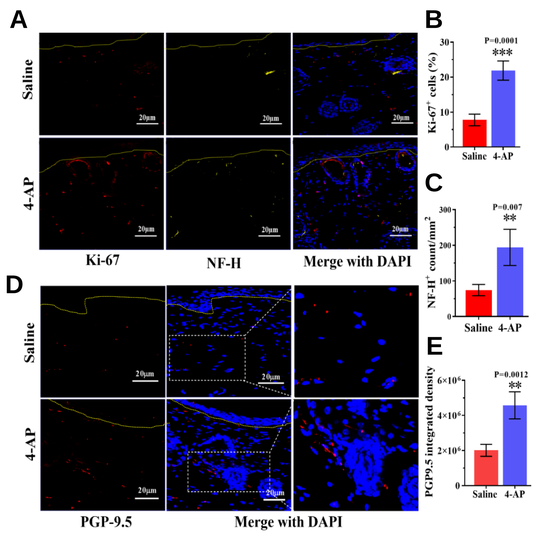


Figure 5. 4-AP facilitates nerve regrowth and increases the expression of PGP-9.5. Results of Ki-67+ and NF-H+ cells **(A-C)** and PGP-9.5 **(D, E)** surrounding de novo hair follicles in the healed wound. Data represent 20 images from 5 different mouse wounds. mean ± SEM, n = 5/ group.

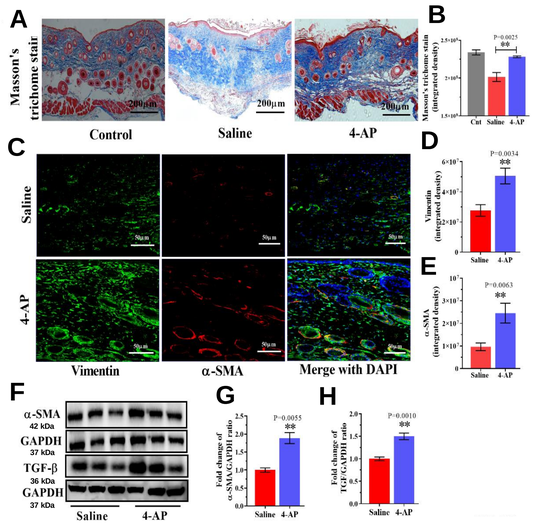


Figure 6. 4-AP treatment increases collagen (A, B), fibroblast, and myofibroblast numbers (C-E) and the levels of alpha-smooth muscle actin (α-SMA) and transforming growth factor-β (F-H). mean ± SEM, n = 5 or 3/ group.

Results

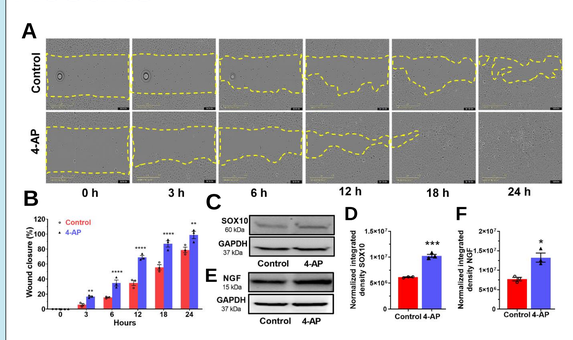


Figure 7. 4-AP accelerates wound healing by activating transcription and neurotrophic factors. The results of the keratinocyte scratch assays **(A, B)** and Western blotting of SOX10 and NGF **(C-F)** are presented. n = 3/ group; *0.05,**0.001, ***0.0002, ****0.0001 vs. control.

Conclusions

- We discovered that systemic administration of 4-AP to wounded mice promoted 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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