Effect of Retinoic Acid on *Xenopus laevis* Post-embryonic Wound Healing

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ABSTRACT

Acne vulgaris is a common condition resulting from the obstruction of hair follicles and their sebaceous glands. Various treatment products exist aimed at resolving acne development. Retinoids are a common ingredient in acne and acne scar treatment products. Retinoids are known to have an effect on collagen which is an important protein found in the connective tissues of humans (1) but how tretinoin, a type of retinoid medication, impacts wound healing is not fully understood. Here, using *Xenopus laevis* embryonic frog as a model system, the effect of tretinoin on wound healing and tissue regeneration was evaluated. Aniline blue histological stain was used to observe collagen deposition in relation to the wound location. Additionally, a GFP reporter was used to measure Wnt signaling related to the wound healing response in the presence of retinoids. Low doses of tretinoin resulted in decreased canonical Wnt signaling and decreased collagen proximal to the wounding site. The results of these experiments indicate that tretinoin has a dose-dependent effect on both Wnt signaling and collagen, which are likely to have an impact on regenerative properties of the tissues.

INTRODUCTION

Acne vulgaris is known to result in scarring if left untreated or not properly treated. Various treatment methods are purported to improve acne and acne scars. However, many of these treatment methods pose potentially negative impacts to patients (2). Chemical peels are a common treatment method used to resolve acne vulgaris and improve the appearance of acne scars. Retinoic acid is a common component in skincare treatments, including chemical peels. Retinoic acid is a potent modulator of cell signaling and can have positive effects toward clearing acne and skin scarring. But results can vary dramatically and ongoing safety and efficacy concerns necessitate a better understanding of the full effects of retinoic acid on skin undergoing wound healing processes. Retinoic acid impacts a host of cell signaling pathways, including those associated with the growth factor Wnt. Wnt is an important signaling molecule involved in wound healing and leading to expression of collagen, an extracellular matrix component of skin (3).

Bisected embryos of the African clawed frog, *X. laevis* provide a robust model of wound healing. Using this model, the effects of different concentrations of all-trans retinoic acid (tretinoin) on Wnt signaling and collagen expression during wound healing were examined.

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METHODS

X. laevis Wounding and Tretinoin Exposure

- Bisected Embryos
  - 5 posterior treated (50, 5, 0.5 ng/mL)
  - 5 anterior untreated [vehicle (DMSO) alone]
  - 5 posterior untreated [vehicle (DMSO) alone]
  - 5 anterior treated (50, 5, 0.5 ng/mL)
- Whole Embryos
  - 15 treated (50, 5, 0.5 ng/mL)
  - 5 untreated [vehicle (DMSO) alone]

Wnt Reporter: GFP Fluorescence (Fig. 1)

- 24 hrs post-wounding, image under blue light

Aniline Blue Staining of Collagen

- 1 week post-wounding, fix embryos
- Rinse and immerse in PTA-PMA reagent (10 min)
- Immerse in aniline blue reagent (5 min)
- Rinse and immerse in 1% acetic acid (5 min)
- Rinse and replace with distilled water
- Image whole-mount embryos

RESULTS

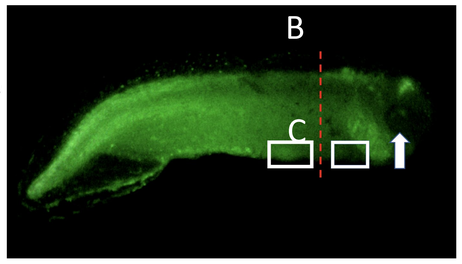

Figure 1. GFP fluorescence in a WntRE-GFP *X. laevis* embryo shows tissues with active Wnt signaling. Dashed red line denotes approximate site where embryos were cut when bisected. White boxes indicate ventral regions of the posterior (left) and anterior (right) halves. White arrow points to the eye (for orientation).

Figure 2. Normalized GFP fluorescence resulting from Wnt signaling in unwounded (blue) and wounded (red) embryos. The initial fluorescence (T0) and 24-hours after the time of wounding (T24) are shown for both the anterior and posterior regions. Unwounded embryos (blue) showed an increase in GFP fluorescence over the 24 hour culture period. Wounded embryos (red) maintained fluorescence intensity only slightly varying from the starting intensity.

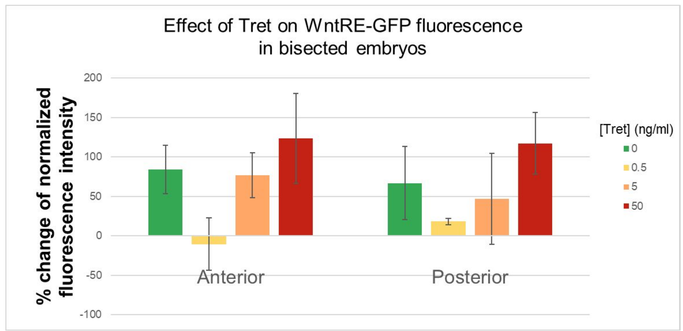

Figure 3. Percent change of normalized fluorescence intensity in the anterior and posterior halves of bisected embryos. Embryos were treated with vehicle alone or varying concentrations of tretinoin. Increased Wnt signaling fluorescence over the initial 24 hour treatment period was suppressed in the bisected embryos as a result of the wounding. Tretinoin-treated embryos that were bisected showed a reduction in Wnt signaling, indicated by GFP fluorescence, when treated with 0.5 ng/mL of tretinoin but not when exposed to higher concentrations.

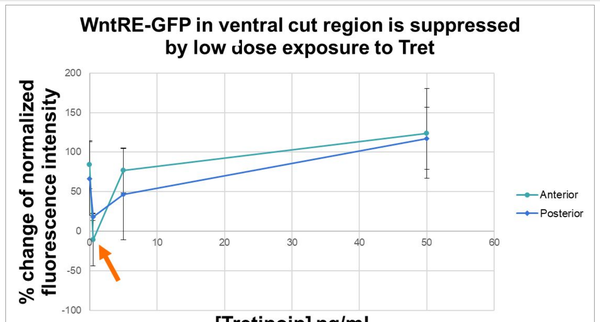

Figure 4. Percent change of normalized fluorescence intensity in the ventral region of bisected embryos treated with three different dosages of tretinoin. Dosages used to treat bisected embryos range from 0.5 ng/mL to 50 ng/mL. Exposure of bisected embryos to 0.5 ng/mL tretinoin uniquely resulted in a further decrease in fluorescence intensity (orange arrow), even less than wounding alone.

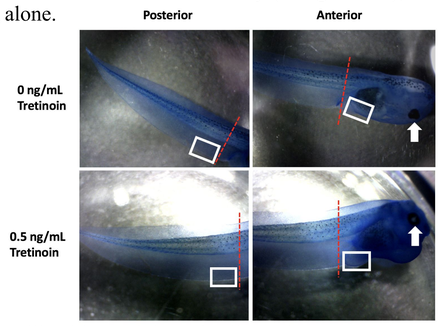

Figure 5. Aniline blue staining of collagen in uncut embryos. Shown are posteriors and anteriors of whole embryos. Dashed line shows the site of where the cut was made in bisected embryos. White arrows indicate the location of the eye on the anterior half of the embryo. Boxes note the ventral portion of the embryo that was analyzed.

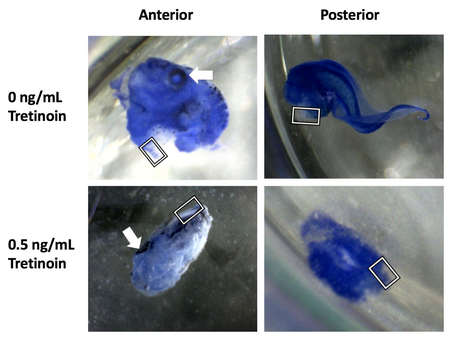

Figure 6. Aniline blue collagen staining of bisected embryos. Anterior and posterior halves of the embryos are complementary to each other. White arrows indicate the location of the eye on the anterior half of the embryo. White boxes represent the ventral cut section of the embryo. The 0.5 ng/mL tretinoin treated portions of the bisected embryos were much more susceptible to lysis while being stained. The anterior portion of the cut embryo treated with 0.5ng/ml tretinoin was more lightly stained, suggesting that collagen deposition was reduced.

CONCLUSION & DISCUSSION

Tretinoin-treated embryos showed decreased expression of the WntRE-GFP reporter, a measure of canonical Wnt signaling, at the wounding site. Retinoic acid can activate non-canonical Wnt pathways, which can consequently antagonize canonical Wnt signaling (4). Differing dosages of retinoic acid can also have opposing outcomes. For example, low doses of retinoic acid, but not high doses, induce cell proliferation in cancer cells by increasing growth factor signaling and activating ERK1 (5).

Wnt is an important signaling molecule involved in wound healing. Monitoring Wnt signaling in response to the retinoid treatment provided deeper insight to the possible upstream signaling leading to collagen expression during healing. A decrease in both Wnt signaling reporting and collagen suggests that they may indeed be linked.

This is the first study to observe collagen in whole-mount *Xenopus* embryos utilizing aniline blue staining. Although generally successful, the acid wash steps required to permeabilize the embryos and prime the collagen also compromised the integrity of a ventral edema induced by wound healing. Further refinement of the procedure may allow for better assessment of collagen in fragile embryos such as these.

Dynamic regulation of Wnt signaling and collagen deposition are reflective of the many functions associated with them. Canonical Wnt signaling is responsible for cell proliferation, differentiation and migration, and developmental patterning. Collagen provides structural support to connective tissues such as skin. Both Wnt and collagen are critical for normal development of *Xenopus* embryos, and the wounding method was performed in early stages of development. Wounding the embryos at a later stage of development, when Wnt signaling is less developmentally important, may produce different results. In addition to exhibiting clear dose-dependent differences, tretinoin could have differential effects on Wnt signaling and collagen deposition depending on the differentiation state of the tissues.

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