Animal model of glottic injury and response to collagen type IA inhibition

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Abstract

Objective
Examine wound healing in the ovine vocal fold and response to a novel type IA collagen inhibitor (halofuginone).

Methods
An ovine laryngeal model was utilized to study right vocal fold and subglottic injury and healing. 24 sheep were divided into four groups. Sheep underwent controlled injury of the right vocal fold and subglottis preceded or followed by administration of halofuginone. Biopsies were taken at commencement, at one month and then sheep were euthanised and larynges explanted at three months. Specimens were examined for elastin, epithelial thickness, and collagen density.

Results
Treatment with halofuginone resulted in significantly less elastin and collagen in the vocal fold at one month following injury. By three months there was a similar density of collagen in treated vs untreated animals’ glottis, but elastin remained significantly lower in treated sheep.

Conclusion
Treatment with halofuginone in an ovine model of laryngeal injury results in lower levels of elastin at one month but a return to normal values of collagen by three months post injury. This suggests that administration of a collagen type IA inhibitor may affect collagen deposition and other extracellular matrix proteins including elastin. This has implications for changes in vocal fold pliability and treatment of vocal fold scar.

Introduction
Vocal fold injury in humans results in severe voice alteration that is often permanent. Insights into mechanisms of vocal fold scar development are needed to identify therapeutic targets. Animal models offer a controlled environment for assessment of tissue behaviour. Sheep were selected as a laryngeal model due to similarities in anatomic characteristics of the larynx1-3. Fibrosis is orchestrated by a cascade of proteins, resulting in deposition of type I collagen (scar). Halofuginone is a specific collagen type IA inhibitor, blocking second messenger intra-nuclear signals4. This study examined the effect of halofuginone therapy following controlled vocal fold and subglottic injury.

Materials and Methods
Ethical approval was received from the University of Auckland Animal Ethics Committee. An ovine laryngeal model was utilized to study vocal fold and subglottic injury response to halofuginone. 24 sheep were divided into four groups with differing doses and routes of administration of halofuginone. Sheep underwent controlled injury of the right vocal fold and right subglottis, under general anaesthesia with spontaneous breathing. Biopsies were taken at commencement, at one month and then sheep were euthanised and larynges explanted at three months. Specimens were examined for elastin, epithelial thickness and collagen density. Elastin and collagen density was estimated by standardized manual grid counting of photomicrographs.

Results
All sheep tolerated surgical procedures. One sheep death occurred due to bacterial pneumonia. All groups demonstrated consistent loss of elastin at the injury site at one and three months compared to prior to injury. To ascertain whether this effect was modified by application of test drug, inter group comparisons were made. Comparing glottic elastin density in the control group to all treated groups combined, there was a significant difference at three months post injury, with a greater decrease in elastin in sheep receiving halofuginone (p=0.006). No difference was noted at baseline or at one month. When comparing collagen density in the control group to all treated groups combined, there was no difference at baseline, a significant decrease in collagen in treated groups at one month (p=0.016) but this was non-significant by three months (p=0.22).

In the subglottis, density of elastin was similar at baseline between control and treated sheep groups. At one month there was a trend toward less elastin in the treated sheep (p=0.059) and at three months a significant decrease in elastin in treated sheep was noted (p=0.0006). Collagen density in the subglottis was similar at baseline between control and experimental groups but a significant change occurred over time. At one month there was a reduction in collagen in the treated sheep (p=0.009) but by three months there was a significant increase in collagen density in treated sheep (p=0.007).

Conclusions
In an ovine model of laryngeal injury treated with a collagen type IA inhibitor, loss of elastin at the injury site was identified but no signs of excessive collagen deposition were seen at either one or three months. This suggests that loss of vocal fold pliability may be related to loss of elastin or degradation of other extracellular matrix components, rather than to excessive collagen deposition and that manipulation with a collagen type IA inhibitor may influence wound healing in the glottis.

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References

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