CHONDROITIN SULFATE IN NORMAL AND SCARRED RAT VOCAL FOLDS

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ABSTRACT

Introduction: Chondroitin sulfate (CS) is a glycosaminoglycan (GAG) known to work in various signal transduction pathways. Recent studies showed that CS is upregulated in keloid tissue, and it was suggested that CS acts in wound healing by regulating cell adhesion and cell proliferation. We hypothesized that CS plays an important role in the wound healing process of vocal folds. Here we examined the expression of CS in normal and scarred rat vocal folds.

Materials and methods: The vocal folds of 24 female 13-wk-old Sprague-Dawley rats were unilaterally stripped by micro-scissors. After sacrifice, the rats’ larynges were harvested at 1 day, 3 days, 1 week, 1 month, and 3 months after stripping. The CS expressions in the untouched and scarred vocal folds were examined by immunohistochemistry, and a quantitative measurement was performed by image analysis.

Results: CS was expressed in both the scarred and normal vocal folds. The ratio of the areas expressing CS in the scarred vocal folds were significantly lower than those in the normal vocal folds at day 3 (p<0.005), day 7 (p<0.01), 1 month (p<0.01) and 3 months (p<0.05), and the ratio was lower but not significantly on day 1 (p=0.1) (Chart.1).

Conclusions: The expression pattern of CS in the scarred rat vocal folds was different from that in human keloid tissue. The results suggest that the wound healing process of the vocal folds is different from that of keloid tissue.

INTRODUCTION

Vocal fold scar is one of the challenging diseases. Several prior studies have focused on the mechanisms of the development of vocal fold scarring and the healing process.1 A chondroitin sulfate (CS) is a sulfated glycosaminoglycan (GAG). It can be found in intracellular organelles, on the cell surface, and in the extracellular matrix (ECM).2 For neuroscientists, CSs were increased after CNS injury. They acted in the inhibition of neurite outgrowth.3 In dermatology, a keloid is one of the dermal fibrotic growths. It lacks plasticity and contains an excessive accumulation of ECM and a decreased amount of elastic fiber. The level of CS in the keloids was found to be 6.9-fold higher than in normal skin.4 We hypothesized that CS may play one or more important roles in the healing process of vocal fold scars, and we conducted this study to identify the expression of CS in normal and scarred rat vocal folds.

METHODS AND MATERIALS

24 female 13-wk-old Sprague-Dawley rats were anesthetized. Scar models were performed at 5 time-points 1 day, 3 days, 1 week, 1 month, and 3 months with 4-5 rats at each time point. Rat videolaryngoscopic surgery was visualized. Unilateral vocal fold stripping was performed using micro-scissors. The other side was left intact. The larynges were harvested. Coronal sections were sliced and stained.

Immunohistochemistry

The sections were blocked by 5% skim milk. The primary antibody: mouse anti-CS56 (1:200; Abcam, Cambridge, MA). The secondary antibody: goat anti-mouse Alexa 488 (1:500; Invitrogen, Carlsbad, CA).

Images were captured and analyzed by software CS immunopositive areas and pixels were measured and calculated

Ratio of CS positive : CS positive area Total vocal fold area

Statistical analysis used paired t-tests to compare the differences between the normal side and the scarred sides within each group.

RESULTS

CS was expressed in both scarred and normal vocal folds. It was found in the lamina propria. (Fig.1)

The ratio of the areas expressing CS in the scarred vocal folds were significantly lower than those in the normal vocal folds at day 3 (p<0.005), day 7 (p<0.01), 1 month (p<0.01) and 3 months (p<0.05), and the ratio was lower but not significantly on day 1 (p=0.1) (Chart.1)

DISCUSSION

This is the first report describing the immunological staining of CS in vocal folds. After the injury, a significant decrease in CS with scarring was observed. This reduction consistent with previous study that measured CS by fluorophore-assisted carbohydrate electrophoresis (FACE) but different from in normal skin tissue and keloids that the level of CS was increased after the injury.4 We hypothesized that vocal folds may undergo a unique scar remodelling process.

CONCLUSIONS

CS is present in both normal and scarred vocal folds. The expression pattern of CS in the scarred rat vocal folds was different from that reported in keloid and normal skin tissue.

Our present findings suggest that the wound healing process of vocal folds is different from that of normal skin and keloid tissue.

REFERENCES


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