INTRODUCTION

Subglottic stenosis is the result of abnormal wound healing processes leading to hypertrophic scar formation and obstruction of the airway lumen by excess granulation tissue. Furthermore, wound healing is a dynamic and complex process mediated by a wide range of coordinated cellular reactions, which can be influenced by both local and systemic parameters, such as infection, pressure, tissue necrosis, age, and other patient comorbidities. The most widely practiced treatments include systemic antibiotics, systemic steroids, hyperbaric oxygen, anti-inflammatory agents, antireflux therapy and finally, open or endoscopic surgical repair. Understanding the pathologic wound healing process may help find methods to treat and prevent subglottic stenosis.

Precise cellular and molecular processes underlying fundamental aspects of upper airway injury, inflammation, and fibrosis remains poorly defined. To understand the wound healing pathways, it is necessary to comprehend the temporal pattern of inflammatory mediators which lead to migration of fibroblasts and ultimate granulation tissue formation. Several types of mucosal injury have demonstrated the clinical utility of measuring biochemical markers in secretions. Tissues of the intestine have been analyzed for the presence of factors affecting healing and fibrosis to document the wound healing process, further confirming the potential of such assays. Furthermore, secretion analysis has been extended to wound healing in the upper airway in order to understand the process of wound healing in subglottic stenosis. A number of inflammatory markers (i.e. cytokines) have been implicated in the development of airway stenosis. It has become imperative to characterize the role of such mediators in the development of granulation tissue. The major cytokines observed in chronic inflammatory tissue have been Transforming Growth Factor Beta (TGF-β); Smad markers such as MMP-9 have been linked to the TGF-β granulation pathway. Similarly, Interleukin-1 (IL-1) has been shown to be present in high concentrations in patients with subglottic stenosis. Given the previous implications, we chose to focus our research mainly on these markers.

This study was designed to address the following question: Will injecting MMP-9 topically change histopathology and levels of inflammatory markers compared to the untreated control subjects?

The current study is an extension of previous work, using the ex vivo murine model of subglottic granulation developed by our lab to study mRNA expression levels of an array of inflammatory markers through RT-PCR and to further understand the inflammatory response that leads to the development of subglottic stenosis. We expect to find that topical MMP-9 therapy yields statistically significant differences in the mRNA expression of inflammatory markers in the treated mice compared to untreated mice. With this information, we will be able to understand the inflammatory process behind treatments were given and develop therapeutic regimens aimed specifically at inhibiting the inflammatory markers which lead to the granulation tissue causing the pathology.

MATERIALS/METHODS

Thirty donor C57BL/6 mice were euthanized using a compressed carbon dioxide (CO₂) chamber. A vertical incision from the mentum to the sternum was made, and the laryngotraheal complex (LTC) was exposed through careful dissection and kept in situ. Half of the LTCs underwent epithelial injury as described below.

Hydrochloric Acid: Application of 0.5mL hydrochloric acid (HCl) solution, titrated to pH4, to the subglottic mucosa via a 1mL tuberculin syringe at the future inferior tracheal incision. After 5 minutes, the HCl solution was irrigated away from the airway using 0.5mL normal saline solution. Control: No airway injury

LTCs from the injured group and the control group were harvested and placed into normal saline solution for transplantation into syngeneic recipient mice. Recipient C57BL/6 mice were anesthetized, and using sterile technique, 1cm incisions were made on the dorsum. Two separate subcutaneous pockets were created into which an LTC from each group was transplanted. The incisions were closed with Vetbond.

RESULTS

The mice were then placed into two arms: the treatment arm and the control arm. In the treatment arm, daily subcutaneous injections of MMP-9 compound 0.5 cc were injected into the transplant pockets of the recipient mice for 3 weeks. In the control arm, no treatments were given. All recipient mice were euthanized at 3 weeks post-transplantation using a compressed CO₂ chamber. Transplanted LTCs were harvested via the previously placed incisions. These were either used for RT-PCR or carefully fixed in formalin, and blocked in paraffin.

Figure 2. Laryngotraheal complexes were harvested 3 weeks post-transplantation from their subcutaneous pockets.

Four slides of 5 micrometer thickness were made per millimeter of tissue and stained with H and E. The remaining LTCs underwent RT-PCR using primers for IL-1 and MMP-9.

Figure 3. A. Injured group at 4x and 40x magnifications harvested 3 weeks transplantation shows significant granulation, angiogenesis and an attenuated epithelium. B. Control Group shows preservation of airway epithelium and no evidence of granulation. C. c-fos, L-1, U-1, E-1, angiogenesis. B. Control treated Group shows preservation of airway epithelium and no evidence of granulation. C. c-fos, L-1, U-1, E-1, angiogenesis.

DISCUSSION

This study represents a novel approach to studying the inflammatory processes leading to subglottic stenosis through tissue analysis rather than secretion analysis. Using an animal model of subglottic granulation, we aimed to test the effects of topical anti-inflammatory treatment with a view towards developing clinical tools for novel treatment to prevent the scarring process.

There was no significant effect found on early granulation tissue after topical anti-inflammatory therapy. This may be due to the limitations of the ex- vivo animal model and the materials used. Firstly, it has been found that the LTCs when harvested at 3 weeks are encased in a fibrous pocket with neovascularization allowing for viability of tissue. As such, the topical treatment may not have permeated the pocket to reach the LTC. This may lead to further therapeutic investigations using systemic rather than topical treatments.

Furthermore, there is little published definitive data regarding the utility of such topical application versus the systemic administration of MMP-9 inhibitor. The optimal timing, route of administration, and dosing are still primarily anecdotal in the literature, and we aim to define such parameters in future studies.

CONCLUSIONS

Using a previously described novel murine model, we begin to delineate inflammatory markers that can be targeted for potential therapy. In response to topical treatment of MMP-9 inhibitor, the levels of inflammatory markers do not change. Further treatment courses including systemic MMP-9 inhibitor or anti-TGF beta will likely lead to a more robust response and reveal novel treatment modalities.

REFERENCES