Paranasal Sinus Mucosal Regeneration: The Effect of Topical Retinoic Acid

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ABSTRACT

Background: *Paranasal sinus mucosa may suffer morphological and functional alterations as a result of surgical trauma. Mucosal stripping typically yields regenerated mucosa characterized by fibrosis, inflammatory infiltrate, and dysmorphic or absent cilia. The aim of this study was to determine the effect of topical retinoic acid (vitamin A) on regeneration of paranasal sinus mucosa.*

Methods: *Both maxillary sinuses of 12 New Zealand white rabbits were surgically opened and stripped of mucosa. Six rabbits received 0.01% topical retinoic acid gel treatment to the stripped left maxillary sinus (low concentration group). The remaining six rabbits received 0.025% topical retinoic acid gel to the stripped left maxillary sinus (high concentration group). The stripped right maxillary sinus of all 12 rabbits served as the operated, untreated control to reflect the normal healing process. Six other animals served as unoperated controls. The sinus mucosa was examined by light microscopy after 14 days.*

Results: *Untreated regenerated mucosa showed expected changes of submucosal gland loss, basal lamina and lamina propria fibrosis, cellular atypia, and loss of cilia. Topical retinoic acid treatment appeared to result in better mucosal regeneration marked by less cellular atypia and fibrosis. Although the regenerated mucosa was still grossly abnormal, the degree of ciliary loss and cellular derangement was reduced. The lower-concentration retinoic acid group had more favorable morphology than the higher-concentration retinoic acid group, and both were improved when compared with no treatment.*

Conclusions: *In a rabbit model, topical vitamin A in the form of retinoic acid gel appears to enhance regeneration of ciliated paranasal sinus mucosa. This preliminary study suggests that topical retinoids may have applicability in promoting sinus wound healing.* (American Journal of Rhinology 17, 133–137, 2003)

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Paranasal sinus mucosa may suffer morphological and functional alterations as a result of surgical trauma. Despite recent refinements in mucosal-preserving surgical technique, the inadvertent stripping of sinus mucosa is at times unavoidable. Mucosal stripping typically yields regenerated mucosa characterized by fibrosis, inflammatory infiltrate, dysmorphic or absent cilia, and compromised mucociliary function. The improved healing of stripped ciliated epithelium could lead to better function and reduced morbidity.

Vitamin A is believed to regulate cellular proliferation and differentiation of epithelial tissues. Systemic deficiency of vitamin A in experimental animals leads to the development of squamous metaplasia. Metaplasia in respiratory tract epithelium results from proliferation of basal cells and their subsequent transformation into squamous keratinizing cells instead of goblet and ciliated cells.[1–3] Systemic administration of vitamin A has been shown to aid in regeneration of normal ciliated respiratory epithelium in systemically deprived hamsters.[1–4] It is not known if vitamin A treatment would improve sinus mucosa healing and function after chronic sinus disease or surgery because the effect vitamin A has on the paranasal sinus mucosa has never been examined.

The aim of this pilot study was to determine the effect of

topical retinoic acid, an analog of vitamin A, on regeneration of paranasal sinus mucosa in mechanically denuded rabbit sinuses. The New Zealand white rabbit is a well-established sinus model because its maxillary sinuses are large and easily accessible and the immune and healing response closely mimics that observed in humans.[5–13]

MATERIALS AND METHODS

Both left and right maxillary sinuses were opened and stripped of mucosa in 12 New Zealand white rabbits of either sex and a body weight of 2.5–4.0 kg. Six of the left sinuses were treated with a topical aqueous gel containing 0.01% retinoic acid, the active metabolite of vitamin A; the remaining six sinuses were treated with 0.025% topical retinoic acid gel (DPT Laboratories, San Antonio, TX). The right maxillary sinuses of these 12 rabbits were stripped and no treatment was applied; they served as the untreated control group. Each rabbit served as its own control. Six additional animals had their right maxillary sinuses opened but otherwise unaltered to show normal sinus mucosa morphology. The use of animals in this study was approved by the Institutional Animal Care and Use Committee at the Oregon Health and Science University.

Surgery

The animals were anesthetized with an intramuscular injection of rabbit cocktail, 1 mL/kg (1 mL of acepromazine maleate, 10 mg/mL + 2.5 mL of xylazine, and 20 mg/mL + 5 mL of ketamine, 100 mg/mL). Then, They were intubated and surgical anesthesia was maintained with isoflurane inhalant anesthesia throughout the operative procedure. A midline nasal dorsum incision was made and skin flaps were elevated laterally to expose the face of the maxillary sinus. The anterior wall of the left and right maxillary sinuses was removed with a drill and cutting burr. The opening was enlarged as needed with a Kerrison rongeur until the entire anterior bony wall was removed; all other bony walls were undisturbed. The entire mucosal lining of the maxillary sinus was removed *en bloc* with a Rosen elevator, and any remaining mucosal lining was removed with a curette. The mucosa overlying the natural ostium was intentionally preserved to ensure patency of the sinus outflow tract. After the respective treatment (see the following section), the overlying periosteum was closed with a running 3–0 Vicryl suture (Ethicon, Inc., Somerville, NJ). The skin was closed with a running, subcutaneous 3–0 Vicryl suture and the animals were allowed to heal for 14 days. All animals received 3 days of oral antibiotic therapy with enrofloxacin, 22.7 mg/mL, given intramuscularly at 2.5 mg/kg per day. Postoperative pain was controlled with oral buprenorphine (0.3 mg/mL), 0.3–0.6 mg/kg, intramuscularly every 6 hours as needed for pain. There were no surgical complications and no postoperative wound dehiscences or infections.

Treatment

The left sinus cavity was designated as the treatment side in all 12 rabbits, with the right sinus cavity serving as the stripped but otherwise untreated control. Six of the 12 treatment sides were filled with a topical solution of 0.01% retinoic acid in an aqueous gel (low concentration group). The remaining six treatment sides received 0.025% topical retinoic acid in the identical aqueous gel (high-concentration group). These two concentrations of retinoic acid gel were chosen because they are the two concentrations clinically approved for topical use. Care was taken not to overfill the sinus cavities to prevent cross-contamination between treated and untreated sinuses. Six additional animals had their right maxillary sinuses surgically opened but unaltered; these animals served as the normal, nonstripped control group.

Tissue Preparation

On postoperative day 14 the animals were killed with intracardiac pentobarbital (85 mg/kg) in a commercial euthanasia solution (Euthasol). The maxillary sinuses were reopened through the prior incisions and the medial wall of the sinus cavities was harvested carefully to preserve the overlying regenerated mucosa. The underlying bone and mucosa were fixed immediately in 3% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M of phosphate buffer for 24 hours. The tissue was decalcified in 10% ethylenediaminetetraacetic acid-Tris buffer for 14 days, embedded in glycol methacrylate plastic, sectioned at 3 μm, stained with methylene blue and basic fuchsin, and examined with the light microscope. Individual sections were evaluated for gross ciliary morphology, degree of ciliary loss, fibrosis of the basal lamina and lamina propria, overall goblet cell-to-ciliated cell ratio, serous gland presence, and neo-osteogenesis of the underlying bone. The initial tissue evaluation was performed and recorded by two separate, unbiased observers. Multiple sources were used for histological and pathological reference.[12]

RESULTS

Normal Nonstripped Controls

Normal control tissue specimens showed histology comparable with that of normal rabbit maxillary sinus as reported by other authors (Fig. 1, *A*).[7] Two or three layers of pseudostratified ciliated epithelium were identified, with ciliated, goblet, and basal cells on the basal lamina membrane. Ciliated cells outnumbered other cell types. The lamina propria, located below the basal membrane, contained numerous serous glands and vessels.

Stripped Untreated Controls

Compared with normals, the stripped control group showed loss of the submucosal serous gland layer but significant fibrosis of the basal lamina and lamina propria.

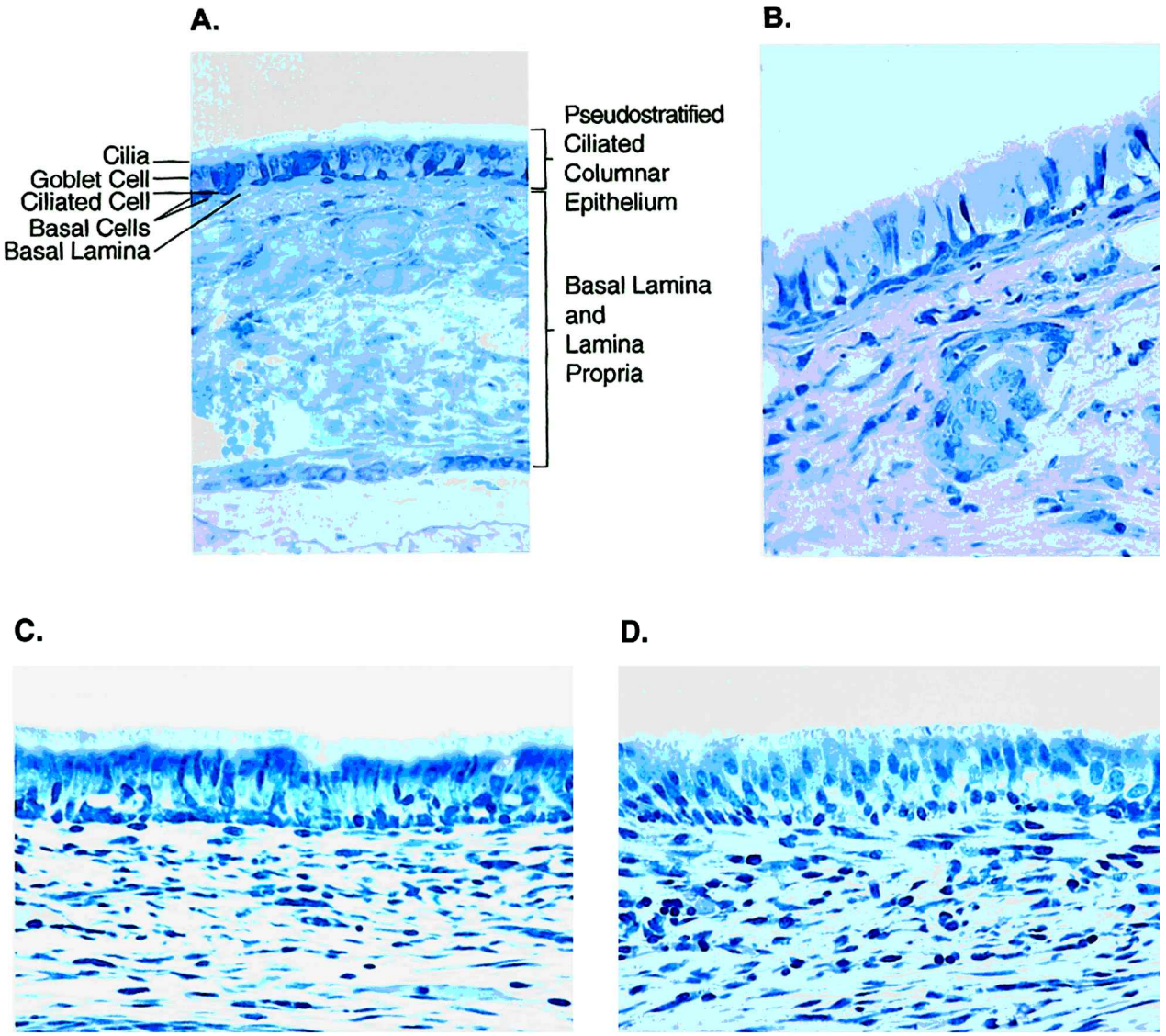

Figure 1. *(A) Normal mucosa (10× methylene blue and basic fuchsin stain). (B) Stripped maxillary sinus mucosa (40× methylene blue percent basic fuchsin stain) with goblet cell hyperplasia, basal lamina and lamina propria fibrosis, and epithelial surface with a few tufts of cilia. There is almost complete absence of ciliated cells. (C) Low-concentration retinoic acid treatment mucosa (40× methylene blue and basic fuchsin stain) with near normal density mucociliary blanket density, loss of submucosal serous glands, and fibrosis of basal lamina/lamina propria. (D) High-concentration retinoic acid treatment mucosa (40× methylene blue and basic fuchsin stain) with less dense mucociliary blanket than the low-concentration group, similar loss of submucosal serous glands, and fibrosis of basal lamina/lamina propria.*

Ciliary density was markedly diminished with few tufts of cilia surrounded predominantly by denuded mucosal segments (Fig. 1, *B*).

Treated Groups

Overall, topical retinoic acid treatment (both high and low concentrations) of stripped paranasal sinus mucosa appeared to improve mucosal and ciliary regeneration. Treated sinuses were marked by less cellular atypia and fibrosis compared with the stripped, untreated control group. The low-concentration retinoic acid group had basal lamina fibrosis and loss of submucosal serous glands but a near normal mucociliary blanket qualitatively. The high-concentration retinoic acid group showed more histological atypia and heterogeneity in the cellular layer when compared with the low concentration and normal groups. However, the high-concentration retinoic acid treatment group had less reactive fibrosis in the basal lamina and lamina propria and more normal mucociliary blanket coverage when compared with stripped, untreated controls.

In terms of ciliary morphology and density, the retinoic acid-treated groups showed cilia that were grossly normal in appearance but diminished in number compared to the non-stripped controls. There were no frankly denuded segments.

When the high and low concentration groups were compared to each other, the low concentration group appeared to have greater density of regenerated cilia.

In terms of other epithelial features, both retinoic acid groups had an increased goblet cell-to-ciliated cell ratio compared with normals. This goblet cell hyperplasia also was noted in the stripped control group. Both retinoic acid groups had increased fibrosis of the basal lamina and lamina propria when compared with normals. However, there was less fibrosis in the retinoic acid treatment groups than in the stripped, untreated controls. Both retinoic acid groups and the stripped control group had a relative loss of the serous gland layer. Morphologically, the regenerated mucosa in the retinoic acid treatment groups was still grossly abnormal, although the degree of cellular and ciliary abnormality was markedly diminished (Fig. 1, *C* and *D*) when compared with stripped controls (Fig. 1, *A* and *B*).

Qualitatively, both retinoic acid groups and the stripped control group had areas of neo-osteogenesis, mainly in areas of denuded mucosa where only a fibrous layer remained. This was an incidental finding and no further quantitative conclusions could be drawn because bone was not labeled before treatment.

Changes within each treatment group, as well as the control groups, were uniform across each group and were consistently seen in all samples within each treatment group. In summary, the lower-concentration retinoic acid group qualitatively had more favorable morphology than the higher-concentration retinoic acid group. However, the higher-concentration group was still dramatically improved when compared with the stripped, untreated control group.

DISCUSSION

The paranasal sinuses are lined by pseudostratified columnar ciliated epithelium that is responsible for clearing normal and infected sinus secretions. Ciliary transport is dependent on a number of complex interactions between the cilia and the sinonasal environment including nasal airflow, ostial patency, intranasal Po_2, humidity, temperature, mucous viscosity, and mucociliary structure and clearance rate.[14–16] The speed of mucociliary transport averages 6 mm/minute in humans and 10–15 mm/minute in rabbits.[17] Mucociliary clearance can be impaired by both infection and surgical trauma. For example, acute and chronic sinus disease may diminish ciliary function and regeneration because of increased fibrosis, decreased numbers of submucosal glands, and marked inflammatory changes.[14,18,19] Additionally, regenerated mucosa from surgically stripped sinuses has shown ultrastructural changes such as compound cilia, ciliary edema and bleb formation, abnormal microtubule formation, and mucosal segments completely devoid of cilia.[20,21] Thus, in the patient undergoing surgery for chronic infection, preservation of mucosal integrity and function are of primary concern.

Many investigators have found paranasal sinus wound healing to be highly complex, occurring in a few well-defined phases. Mucous membrane healing occurs by migration of cells from normal adjacent epithelium, followed by multiplication and differentiation of progenitor cells.[4,20,22] Epithelial regeneration begins within a few hours of the insult at an estimated velocity of 4–20 μm/hour.[2,20,23] In 1979 Bang and Bang found that sinus mucosa basal cells are multipotent with the ability to differentiate into squamous, ciliated, and goblet cells.[22] Inyama *et al.* established that undifferentiated basal cells appear to be the main source of new progenitor cells in paranasal sinus mucosa.[24] Evans *et al.* later confirmed this finding.[25] Cofactors influencing the differentiation of ciliated cells have not been well defined.

Previously, vitamin A has been shown to lead to regeneration of normal ciliated tracheal epithelium in systemically deprived hamsters. Vitamin A is thought to regulate replication of basal cells and therefore ciliated progenitor cells. It also modulates the replication of mucous cells, which are essential for generation of the mucus layer necessary for proper mucociliary transport function. In McDowell's study, preciliated cells were virtually absent in the systemically deprived vitamin A group.[4] With restoration of systemic vitamin A levels, ciliated progenitor cells rapidly developed cilia and further matured into functional ciliated epithelium. Additionally, in a study by Edmondson *et al.*, systemic vitamin A deprivation in hamsters resulted in squamous metaplasia of pseudostratified ciliated tracheal epithelium with loss of goblet cells, resulting in loss of mucus-secreting capability and an overall disruption of the mucociliary microenvironment.[6]

In this pilot study, we examined the effect of topical vitamin A (retinoic acid) on healing sinus mucosa. Patients with chronic sinus disease often have inflamed polypoid mucosa that can be stripped away easily during functional endoscopic sinus surgery, even when great care is taken to preserve the mucosal lining. It appears that topical retinoids may help to restore ciliated paranasal sinus epithelium.

Given that this was a preliminary study that focused on qualitative morphological findings, further studies are necessary to address the limitations of this study. First, our study did not evaluate the functional status of regenerated mucosa. Future studies would benefit from measurements of mucociliary clearance times pre- and posttreatment to quantify mucosal functionality. Second, quantitative analysis of histological changes was not performed. Scanning and transmission electron microscopy could be performed in future studies to quantify submucosal serous gland loss, basal lamina and lamina propria fibrosis, and mucociliary density changes. Bone morphometry studies could also provide quantitative insights into the physiology of sinus bone healing and its relationship to mucosal healing. Third, because we did not include a control group receiving nonmedicated aqueous gel, the observed changes associated with retinoic acid are potentially confounded by possible beneficial moisturizing effects of the gel vehicle. The observed dose-response sensitivity of mucosa to varying con-

centrations of retinoic acid suggests that the positive effects of vitamin A are true. However, we plan to confirm our hypothesis with a follow-up study that will include a control group receiving only a nonmedicated aqueous gel component. Last, future studies would benefit a longer postoperative follow-up period to assess the fate of postsurgical changes observed at the 14-day mark.

CONCLUSION

In a rabbit model, topical vitamin A, in the form of retinoic acid gel, appears to enhance regeneration of ciliated paranasal sinus mucosa after mechanical injury. There appears to be a concentration-dependent effect, with lower concentrations of vitamin A being more beneficial than higher concentrations. Further investigation is required to evaluate the functionality of the regenerated mucosa and to determine the clinical significance for human subjects.

REFERENCES

1. Chopra DP, Cooney RA, and Taylor GW. Effects of vitamin A deficiency on cell proliferation and morphology of trachea of the hamster. Cell Tissue Kinet 23:575–586, 1990.
2. Wong Y, and Buck R. An electron microscopic study of metaplasia of the rat tracheal epithelium in vitamin A deficiency. Lab Invest 24:55, 1971.
3. Chopra DP. Squamous metaplasia in organ cultures of vitamin A deficient hamster trachea. J Natl Cancer Inst 69:895, 1982.
4. McDowell EM, Keenan KP, and Huang M. Restoration of mucociliary tracheal epithelium following deprivation of vitamin A. A quantitative morphologic study. Virchows Arch B Cell Pathol 45:221–240, 1984.
5. Marks SC. Acute sinusitis in the rabbit: a new rhinogenic model. Laryngoscope 107:1579–1585, 1997.
6. Edmondson SW, Reen W, and Mossman BT. Regulation of differentiation and keratin protein expression by vitamin a in primary cultures of hamster tracheal epithelial cells. J Cell Physiol 142:21–30, 1990.
7. Kumlien J, and Schiratzki H. Blood flow in the rabbit sinus mucosa during experimentally induced chronic sinusitis. Measurement with a diffusible and with a non-diffusible tracer. Acta Otolaryngol 99:630–636, 1985.
8. Drettner B, Johansson P, and Kumlien J. Experimental acute sinusitis in rabbits. A study of mucosal blood flow. Acta Otolaryngol 103:432–434, 1987.
9. Drettner B, Johansson P, and Kumlien J. Experimentally induced sinusitis: the importance of vasomotor regulation. Arch Otorhinolaryngol 246:315–317, 1989.
10. Johansson P, and Kumlien J. Blood flow in the rabbit maxillary sinus mucosa during experimentally induced sinusitis. Acta Otolaryngol 106:299–305, 1988.
11. Johansson P, Kumlien J, Carlsoo B, et al. Experimental acute sinusitis in rabbits. A bacteriological and histological study. Acta Otolaryngol 105:357–66, 1988.
12. Johansson P, Kumlien J, Soderlund K, Hultman E. Experimentally acute sinusitis in rabbits. Energy metabolism in sinus mucosa and secretion. Acta Otolaryngol 106:460–467, 1988.
13. Popesko P, Rajtova V, and Horak J. (Eds.) Rabbit. In A Color Atlas of Anatomy of Small Laboratory Animals, Vol. I. Wolfe Publishing, London, 1990, 1–134.
14. Yang-Gi M, Ic-Tae K, and Sang-Hoo P. Mucociliary activity and untrastructural abnormalities of regenerated sinus mucosa in rabbits. Laryngoscope 104:1482–1486, 1994.
15. Min YG, Lee YM, Jung HW, et al. The effect of ostial opening on experimental maxillary sinusitis in rabbits. Rhinology 31: 101–105, 1993.
16. Min YG, Shin JS, and Kim HK. Bacteriological examinations in empyema of the maxillary sinus. Nippon Jibiinokoka Gakkai Kaiho 31:186–189, 1987.
17. Ballenger JJ. The Clinical Anatomy and Physiology of the Nose and Accessory Sinuses. In: Diseases of the Nose, Throat, Ear, Head and Neck (14^{th} ed.). Lea & Febiger, Philadelphia 1–23, 1991.
18. Brownell DH. Postoperative regeneration of the mucous membrane of the paranasal sinuses. A summary of the published investigations. Arch Otolaryngol 24:582–588, 1936.
19. Hilding A. Experimental surgery of the nose and sinuses. III. Results following partial and complete removal of the lining mucous membrane from the frontal sinus of the dog. Arch Otolaryngol 17:760–778, 1933.
20. Forsgren KI, Stierna P, Kumlien J, et al. Regeneration of maxillary sinus mucosa following surgical removal: experimental study in rabbits. Ann Otol Rhinol Laryngol 102:459–466, 1993.
21. Tos M. Goblet cells and glands in the nose and paranasal sinuses. In The Nose. Upper Airway Physiology and the Atmospheric Environment. Proctor DF, and Andersen I (Eds). Amsterdam, the Netherlands: Elsevier Biomedical Press, 99–144, 1982.
22. Bang F, and Bang B. Mucous membrane injury and repair. In Respiratory Defense Mechanisms. Brain P (Ed). New York: Marcel Dekker, 453–488, 1979.
23. Wilhelm DL. Regeneration of tracheal epithelium. J Pathol Bacteriol 65:543–550, 1953.
24. Inayama Y, Hook G, Brody A, et al. The differentiation potential of tracheal basal cells. Lab Invest 58:706–717, 1988.
25. Evans M, Shami S, Cabral-Anderson, et al. Role of nonciliated cells in renewal of the bronchial epithelium of rants exposed to NO_2. Am J Pathol 123:126–133, 1986. □