A Preliminary Report on the Effects of Paclitaxel-Impregnated Stents on Sheep Nasal Mucosa

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ABSTRACT

Introduction: *Traditional frontal sinus stents serve only as mechanical devices. It has been proposed that stents also may serve as drug-delivery systems for the topical application of drugs that minimize postoperative scarring. Paclitaxel (Taxol), which has recognized antiscarring effects, may be incorporated* via *a polymeric formulation into standard rubber stents. The impact of topically applied paclitaxel on the morphology of the nasal mucosa is unknown.*

Methods: *An adult sheep model was used for this study. A modified rubber T-tube stent (incorporating paclitaxel at varying dosages) was secured to each side of the septum in four animals (eight sides). An unmodified T-tube was placed on each side of one animal, a T-tube with the drug carrier (but no paclitaxel) was placed on each side of the second animal, and T-tubes with varying paclitaxel were placed on each side of the final two animals. After 4 weeks, animals were killed and the nasal mucosa was harvested. The nasal mucosa was sectioned and stained with hematoxylin and eosin. A pathologist then assessed the nasal mucosa for vascular congestion, glandular atrophy, chronic inflammation, mucosal metaplasia, and mucosal ulceration.*

Results: *No consistent histopathological differences were noted in the specimens. All specimens showed varying degrees of vascular congestion, glandular atrophy, chronic inflammation, and mucosal metaplasia; the paclitaxel-impregnated stents were not consistently associated with more severe mucosal injury. Finally, mucosal ulceration was noted to be very rare in all specimens.*

Conclusion: *This preliminary report describes the impact of paclitaxel-impregnated stents on sheep nasal mucosa, which tolerated these stents very well. Because paclitaxel minimizes scarring reactions at very low concentrations, paclitaxel-impregnated stents may prove useful in clinical situations in which frontal sinus stenting is deemed necessary. Additional investigations with animal models, as well as clinical trials, may be warranted.* (American Journal of Rhinology 18, 119–124, 2004)

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Over the past 100 years, rhinologic surgeons have devised a variety of procedures for the treatment of refractory chronic frontal sinusitis. Many surgeons have used and continue to use frontal ostium stents as a means to ensure frontal patency. Unfortunately, the results have been

mixed, and the ideal stent still has not been developed.[1] Although current frontal stents serve merely as a mechanical means to preserve patency, stents also may provide a substrate for the delivery of compounds that can modulate wound healing.[2]

Paclitaxel (Taxol) (Bristol-Myers Squibb, New York, NY) is an antineoplastic compound isolated from the Pacific yew tree. Stabilizing microtubular arrays, it is a potent inhibitor of cell growth. In addition, paclitaxel also indicates strong antiangiogenic and antifibroblastic effects that persist at concentrations of 1000-fold less than that used for systemic chemotherapy. Recent advances in polymeric technology have enabled the incorporation of paclitaxel into rubberized tubing, providing low-level, sustained, and localized release of the drug.[3] This provides a unique opportunity to potentially modify the postoperative healing process at the frontal ostium, a region predisposed to stenosis.

The impact of paclitaxel on sinonasal mucosa is unknown. This pilot study was designed to establish the histological effects of paclitaxel-impregnated stents on nasal mucosa.

METHODS

This was a prospective, double-blinded, randomized study evaluating the effect of paclitaxel on the septal mucosa of sheep. This animal was chosen for our model because of its unique characteristics in terms of nasal volume and shape. Preliminary work in sheep cadavers showed the feasibility of stent placement. Smaller animals such as rabbits or rodents failed to have adequate nasal volume and larger animals proved cost prohibitive.

All stents were Deaver T-tubes composed of medical grade X-ray opaque rubber with an approximate diameter of 3 mm and length of 2.5 cm. Stents were coated with 5% w/w or 20% w/w paclitaxel in ethylene vinyl acetate (EVA) copolymer (60:40 ethylene/vinyl acetate composition; Polysciences, Warrington, PA), an elastic, biocompatible, nondegradable polymer in the following manner. Paclitaxel and EVA were dissolved in dichloromethane at 5% w/v. The ratio of paclitaxel to EVA in these solutions was either 1:20 (5% paclitaxel) or 1:5 (20% paclitaxel). Weighed stents were plugged at the openings, dipped in the solutions four times, and dried between dips using rotation of the stents in a stream of nitrogen to ensure even coating. Then, stents were dried overnight under vacuum and reweighed to determine the weights of EVA and the drug coating the stent. To determine the rate of release of paclitaxel, the stents were placed in 10 mL of phosphate-buffered saline, pH 7.4, and incubated at 37°C with orbital shaking at 50 rpm. The amount of paclitaxel released from the stent was determined using the high-performance liquid chromatography methods as previously described.[4]The paclitaxel/EVA-coated stents then were sterilized using γ-radiation. In this drug delivery system, $\sim$10% of the total amount of paclitaxel incorporated into the stent coating is released in the first 6 days. Sustained, localized delivery of the drug over a prolonged period occurs while the stent is in place. Each stent contained 200–500 μg of paclitaxel (much less than the standard chemotherapeutic dose of 135–175 mg/m^2 every 3 weeks).

All animals underwent similar procedures by the same surgeon blinded to stent composition. Four animals were included in the study. In the first control animal, a stent with 60/40 EVA was placed on each side, and in the second control animal, untreated stent was placed on each side. A 20% paclitaxel stent was placed on each side of a third animal, and a 5% paclitaxel stent was placed on each side of a fourth animal.

After administration of general anesthesia, the nasal cavity was closely examined with a standard 4-mm, 0° nasal telescope. A 2–0 nylon suture was threaded through the stent, which then was placed along side the right septum. The suture was passed to the contralateral nasal cavity *via* the nasopharynx. A second section of stent was threaded onto the suture. A needle was used to pass the suture through the caudal septum and then tied to itself. The net result was a large mattress-type suture that held a stent in place along each side of the nasal septum.

Stents were left in place for 4 weeks. Over the 1st week, topical nasal saline spray was applied. At the time of animal death, each animal was euthanized with concentrated sodium pentobarbital. Then, the stent sutures were cut, the septum was resected, and mucosal specimens were placed in 10% buffered formalin. After fixation, samples were sectioned and processed for routine light microscopy using hematoxylin and eosin samples.

A pathologist blinded to the stent composition evaluated the nasal mucosa for vascular congestion, glandular atrophy, chronic inflammation, mucosal metaplasia, and mucosal ulceration. Four areas of mucosa in contact with each stent were assessed; eight areas per animal were available for review. Vascular dilation, glandular hypertrophy, and chronic inflammation were characterized as none (score of 0), mild (score of 1), moderate (score of 2), and severe (score of 3); a composite average score for each side was determined. The presence or absence of mucosal metaplasia and ulceration also was evaluated. Finally, the percent of specimen areas with each histological feature was calculated. All animal procedures were performed in accordance with the guidelines of the Saint Louis University Animal Care Committee.

RESULTS

While the stents were in place, no changes were observed in animal behavior. This suggests that the sheep tolerated the stents without any apparent difficulty. No consistent gross or histological differences were noted among the specimens. At death, no gross injury was noted in the nasal septa of any animal. However, on a microscopic level, all histological samples revealed varying degrees of vascular congestion, glandular atrophy, and vascular dilatation; no significant differences in chronic inflammation,

TABLE I

Semiquantitative Assessments of Histological Changes Associated with Stent Placement

	Number of Specimens	Vascular Dilatation	Glandular Atrophy	Chronic Inflammation	Squamous Metaplasia (%)	Mucosal Ulceration (%)
Control 1 (60/40 EVA carrier)	8	2	1.6	1.6	75	13
Control 2 (stent only)	8	2.3	2	1.5	50	0
5% Paclitaxel plus 60/40 EVA	8	2.7	2.6	1.4	25	0
20% Paclitaxel plus 60/40 EVA	8	2.3	2.3	1.4	75	0

Vascular dilation, glandular hypertrophy, and chronic inflammation were characterized as none (score of 0), mild (score of 1), moderate (score of 2), and severe (score of 3); a composite average score for each side was determined. The presence or absence of squamous metaplasia and mucosal ulceration was determined in each specimen. The percent of specimens with each histological feature is reported.

mucosal ulceration, or squamous metaplasia was seen with respect to either control group. Interestingly, stents containing 20% paclitaxel achieved values closer to control levels than 5% paclitaxel. Mucosal ulceration was rare in all specimens. The only specimens showing microscopic mucosal ulceration were the specimens from the control animal containing 60/40 EVA rubber; the mucosal ulceration was present in one of eight specimens from this animal. Over all, the paclitaxel-impregnated stents were not consistently associated with more severe mucosal changes than either control material. Data are summarized in the Table I. Representative histological sections are presented in Figs. 1–4.

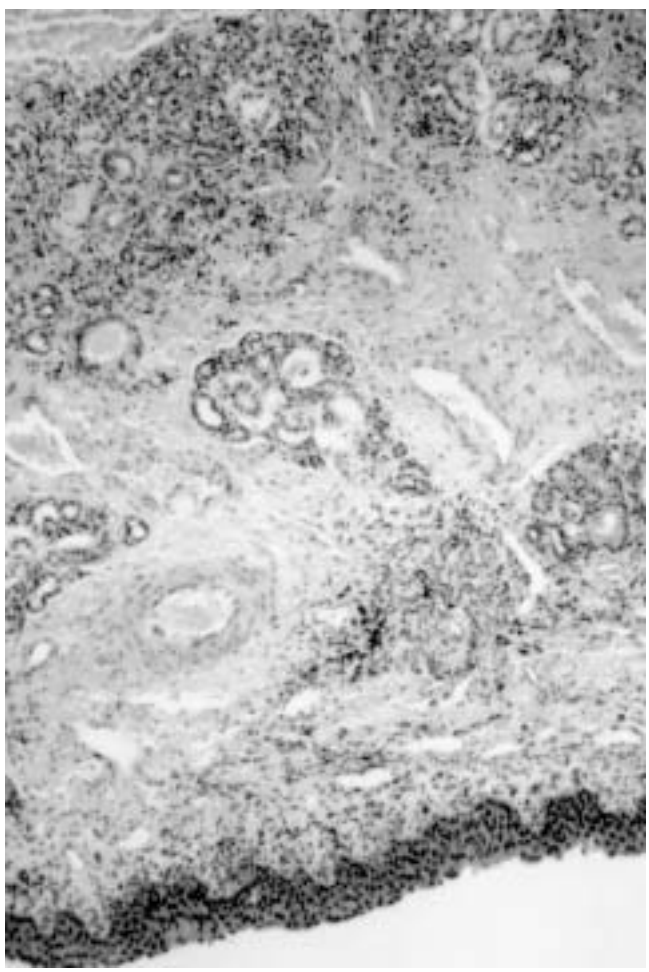

Figure 1. *Mucosal specimen from 20% paclitaxel stenting shows moderate inflammation and no vascular dilatation or glandular atrophy (hematoxylin and eosin, 100× magnification).*

DISCUSSION

This pilot study describes the impact of paclitaxel-impregnated stents on the morphology of sheep nasal mucosa. Although this is a small descriptive study, these initial observations suggest that additional investigations are warranted.

Some rhinologists have used rubber stents to maintain patency of the frontal recess while mucosal healing takes place.[5] A wide number of different stent designs and materials have been tested, but, unfortunately, none have shown consistently good results. Ostial closure from scar formation after stent removal is the most frequent cause for recurrence of symptoms.[6] It also has been proposed that pressure and possible foreign body reaction to the stent may exacerbate any ongoing scarring. It seems reasonable to assume that simply stenting the region does nothing to alter the biology of the healing process itself. That is why stenting often fails. Manipulation of wound healing factors may prevent frontal recess stenosis. In an attempt to modulate the local environment, stents impregnated with dexamethasone have been used.[2]

Over the past several years, attention has focused on topical or injected mitomycin C as a method for reducing the risk of mucosal scarring. Derived from *Streptomyces caespitosus*, mitomycin C is a natural antibiotic that functions as a bifunctional alkylating agent, forming cross-links that prevent DNA synthesis. When applied topically, mitomycin C inhibits fibroblastic activity and thereby impairs the proliferative phase of wound healing. Currently, topical

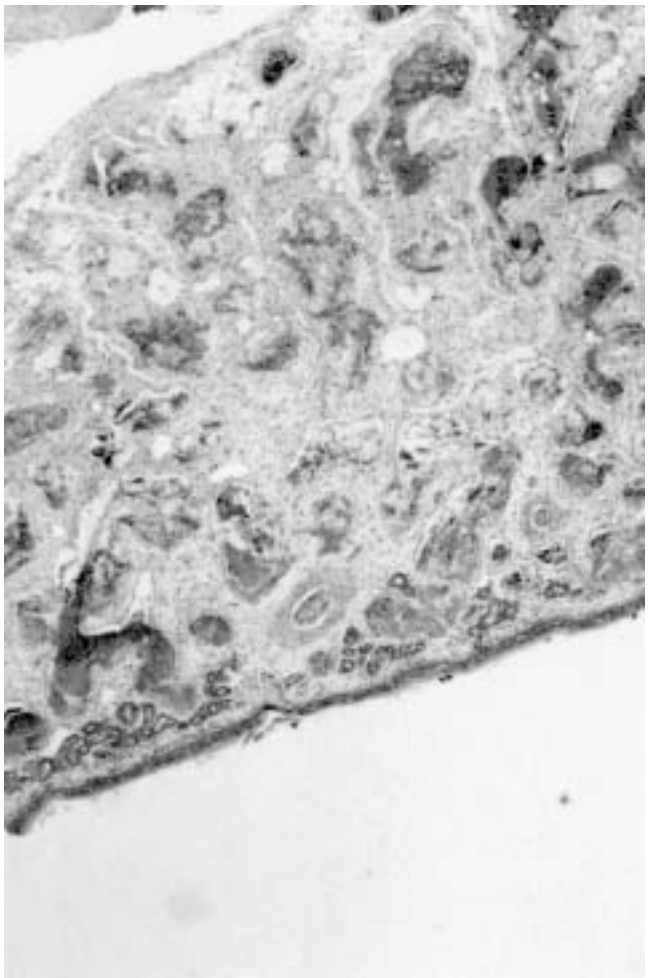

Figure 2. *Mucosal specimen from paclitaxel-free stenting (rubber stent only) shows moderate inflammation and severe vascular dilatation and glandular atrophy (hematoxylin and eosin, 100× magnification).*

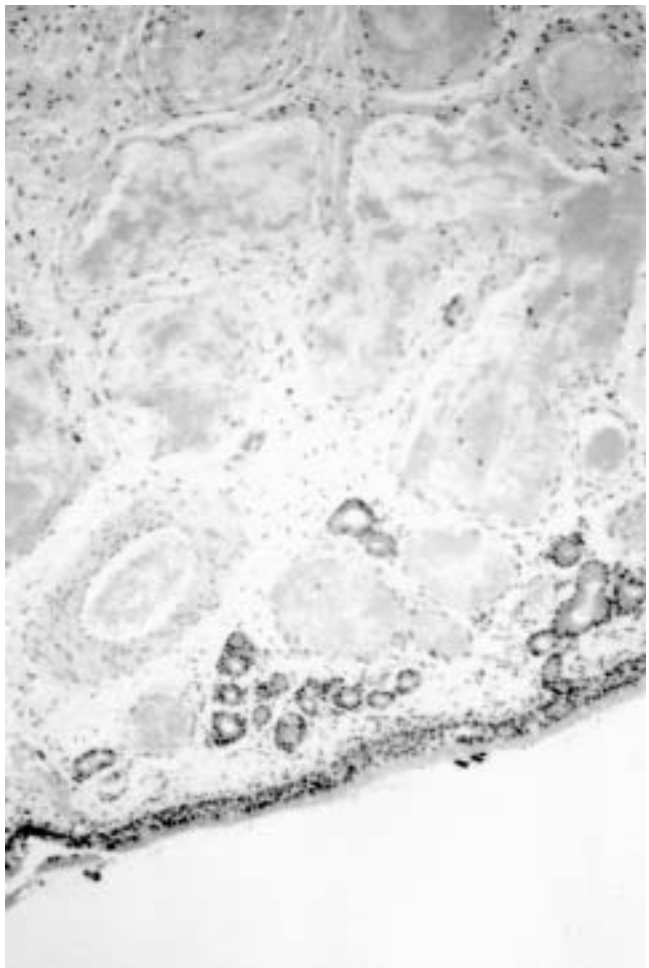

Figure 3. *Mucosal specimen from 5% paclitaxel stenting showing mild inflammation and severe vascular dilatation and glandular atrophy (hematoxylin and eosin, 100× magnification).*

mitomycin C is used to prevent tracheal stenosis and to preserve myringotomy patency.[7,8] Mitomycin C also is being evaluated in functional endoscopic sinus surgery.[9]

One of the unfortunate side effects of alkylating agents like mitomycin C is the induction of secondary malignancies when used systemically at high doses.[10] Although topical applications of mitomycin C are extremely low dose and limited in scope, concerns over this phenomenon have limited its acceptance. Although topical mitomycin C has not been linked to secondary malignancies, the use of a substance with potential mutagenic effects is problematic.

Paclitaxel, which prevents the depolymerization of microtubules, has a wide range of cellular effects, including inhibition of cellular proliferation, limitation of the inflammatory cascade, and induction of apoptosis in hyperplastic cells.[11,12] Furthermore, as a potent inhibitor of transcription factor activator protein 1, paclitaxel markedly reduces angiogenic potential and limits neutrophil activation. Interestingly, these anti-inflammatory and antiangiogenic properties persist at very low concentrations of paclitaxel. The recognized mechanisms of actions of this substance do not have mutagenic potential, and paclitaxel's actions have been studied extensively in the eye, hepatobiliary tree, and cardiovascular system without establishing carcinogenic potential.[13] The antiproliferative properties of paclitaxel also afford it an antibacterial effect, making it an attractive compound to incorporate into any frontal recess stent.[13] Paclitaxel is a hydrophobic compound, and thus its use is ideally suited for topical application with minimal systemic manifestations.[9,11]

Admittedly, this pilot study has limitations. First, only one animal (two sides) was in each study group, and thus meaningful statistical analysis is not feasible. Conceivably, significant mucosal disruption from paclitaxel-impregnated stents may occur, but this study was not powerful enough to detect it. Second, the animals in this project had normal, healthy nasal mucosa. As a result, it may be inappropriate to draw conclusions about the impact of paclitaxel-impregnated stents on inflamed, diseased mucosa and/or injured, denuded mucosa in the clinical setting.

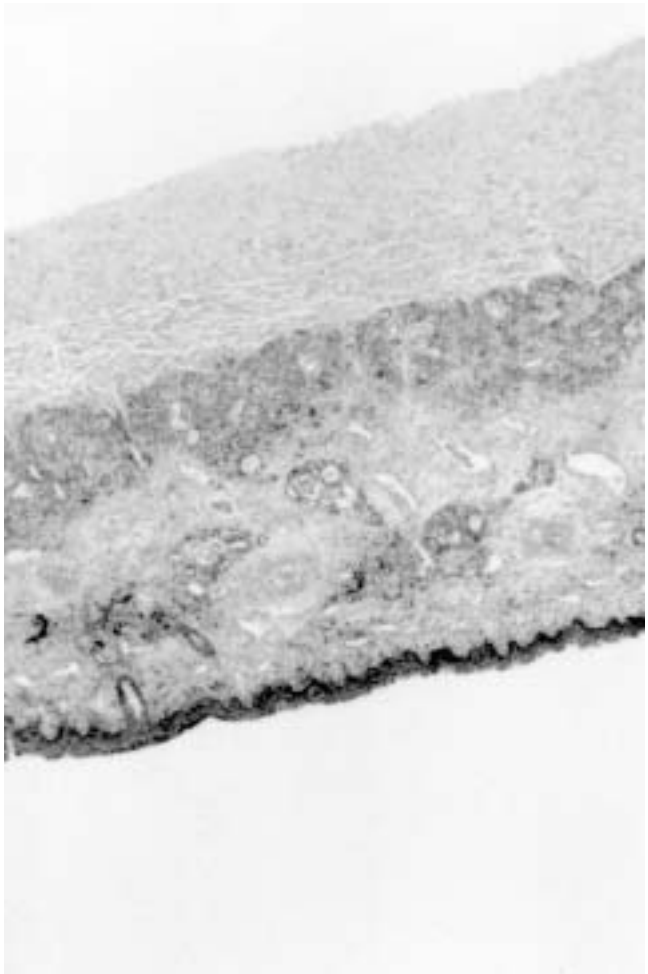

Figure 4. *Mucosal specimen from paclitaxel-free stenting (rubber stent with EVA carrier) showing moderate inflammation, no vascular dilatation, and mild glandular atrophy (hematoxylin and eosin, 100× magnification).*

The work presented in this study suggests that nasal mucosa can tolerate the placement of paclitaxel stents easily. Clearly, this is not a definitive toxicology study; however, this initial success should serve as the foundation for further investigations, which may include determinations of paclitaxel concentrations in adjacent tissues and in the peripheral circulation. In addition, cilia morphology needs to be assessed *via* electron microscopy, and the impact of low-dose paclitaxel on cilia function must be determined. The presence of other potential changes in mucosal architecture, including alterations of the basal lamina, should be investigated also.

It should be noted that the sheep model for surgical rhinologic research has received interest from other investigators.[14–16] A sheep model was selected for this study after consideration of the volume and shape of the nasal cavity. Preliminary work in sheep cadavers showed the feasibility of stent placement. Smaller animals (such as rabbits or rodents) do not have adequate nasal volume, and the use of most larger animals is cost prohibitive. Sheep have favorable nasal anatomy and are relatively inexpensive. This animal model may have other applications in rhinologic research.

Although this study describes a potential new means for frontal stenting, it should not be considered an endorsement of frontal sinus stents for all nonobliterative frontal sinus procedures. Endoscopic frontal sinusotomy, without stenting, is the mainstay of treatment for refractory chronic frontal sinusitis. In contemporary practice, stents should be reserved for selected revision cases. Today, frontal stenting is the rare exception, not the rule.

CONCLUSIONS

According to convention, frontal stents serve a purely mechanical purpose to assure frontal ostium patency; however, such stents also may serve as a vehicle for the delivery of drugs that can modify wound healing. In this study, the effects of paclitaxel-impregnated stents on nasal mucosa in a sheep model are described. The observed mucosal changes were minimal. Additional investigation for the development of paclitaxel-impregnated stents is warranted.

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