GENERAL THORACIC SURGERY

AN EXPERIMENTAL MODEL FOR THE PREVENTION OF POSTANASTOMOTIC TRACHEAL STENOSIS

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Objective: The aim of the current study is to determine the efficiency of an external prosthesis made of expanded polytetrafluoroethylene reinforced with a continuous silicone spiral to prevent postanastomotic stenosis after surgical correction of extensive tracheal defects in rabbits. **Methods:** Forty-five rabbits were used, divided into three groups of 15 animals each. Group A was the control group. Group B animals underwent resection of six-ring segments of the cervical trachea and primary anastomosis. The procedure used in group C was similar to that used in group B, but the tracheal anastomosis was supported by an external expanded polytetrafluoroethylene prosthesis. **Results:** Direct anastomosis after resection of six tracheal rings caused anastomotic stenosis in 100% of the animals. We did not observe tracheal stenosis in any rabbit when we applied an expanded polytetrafluoroethylene tube as an external stent for the tracheotracheal suture. **Conclusion:** We conclude that an external stent can be used to prevent tracheal stenosis resulting from the resection of six cervical tracheal rings in rabbits. (J Thorac Cardiovasc Surg 1997;114:76-83)

Since the first tracheal transection with reanastomosis was successfully carried out on a human being in 1884,¹ a variety of procedures have been put forward for tracheal reconstruction, both in experimental animals and in human subjects. The best surgical technique—resection of the affected segment and primary anastomosis—can be achieved in 90% of patients.² Grillo³ established that the maximum length of resection for primary anastomosis was 6 cm. The maximum length of tracheal resection in pediatric and neonatal patients has not been as clearly identified as it has been in adult patients.⁴⁻⁶ Furthermore, these limits cannot be

exceeded with any guarantee of success. Thus, in the case of extensive resection, alternatives to primary anastomosis must be sought.^{7,8}

Almost all biologic materials have been used in tracheal reconstruction: autogenous grafts of pericardium,⁹ periosteum,¹⁰ rib cartilage,¹¹ esophageal wall,¹² homogeneous grafts of dura mater,¹³ and others. Research on prosthetic materials for the trachea goes back more than 100 years. Long forgotten are the first hazardous tracheal prostheses made of nonporous substances such as glass, stainless steel, vitalio, tantalum, and a long list of other metals. Research soon centered on porous materials, which, in addition to their inert properties, permitted the migration of fibroblasts, encouraging internal growth of fibrous tissue and sealing the inside of the graft.¹⁴⁻¹⁶

The objects of this study are as follows: (1) to identify the normal tracheal parameters of a control group of rabbits, (2) to create an experimental model of a tracheal postanastomotic stenosis resulting from tension, and (3) to identify the effect of an external stent of expanded polytetrafluoroethylene (ePTFE) on the aforementioned anastomosis.

Material and methods

New Zealand White rabbits ($n = 45$) were used. The average weight was 2.876 ± 403 gm and the average age,

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94 ± 9 days. The rabbits were divided into three groups of 15. Group A was the control group, group B was subjected to resection of six cervical tracheal rings with tracheal reconstruction through end-to-end anastomosis, and group C was subjected to the same resection of six tracheal rings as in group B, but with the tracheal anastomosis being supported by an small external ePTFE tube.

The bioprosthetic material used was ePTFE IMPRA Flex ThinWall Small Beading (IMPRA Inc., Tempe, Ariz.), made of ePTFE reinforced with a continuous silicone spiral, with an 8 mm internal diameter. The prosthetic segments were sterilized with ethylene oxide and packaged individually. The experimental study was done in accordance with European Community legislation in force regarding the use of vertebrate animals in experiments and for other scientific purposes (Consejo de Europa. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, 1985).

Anesthetic and monitoring methods. No premedication was administered to the animal. A transcutaneous pulse oximeter was applied and anesthesia was induced through one intramuscular injection of ketamine (50 mg/kg). An intravenous line was set up via the auricular marginal vein and a bolus of 3 mg/kg of propofol was administered. Anesthesia was maintained with a propofol infusion pump (0.9 mg/kg per minute). The lungs were not ventilated with endotracheal tubes.

Surgical method. In group A, the trachea was dissected (without performing tracheotomy), the pretracheal muscle was sutured with a 5-0 Vicryl polyglactin 910 suture (Ethicon, Inc., Somerville, N.J.) and a 3-0 suture was used to close the skin. The cervicotomy was closed in an identical manner in all three groups. In group B, the trachea was totally sectioned with a number 11 scalpel between tracheal rings 4 and 5 and between rings 10 and 11 to resect a segment of trachea containing six tracheal rings. Traction points were applied to both ends of the trachea to prevent retraction of the ends, especially at the distal end, which quickly retracts toward the mediastinum on sectioning. Surgical diathermy was not used, but both ends of the tracheal section were frequently aspirated. The end-to-end anastomosis was achieved with interrupted sutures through the tracheal wall, knotting extramucosally with 5-0 polyglactin 910 suture. In group C, the procedure used was similar to that used in group B, but an external ePTFE prosthesis in the form of a small tube, sectioned lengthwise and measuring 2 cm in length and 8 mm in diameter, was positioned as shown in Figs. 1 and 2. This prosthesis retains its shape even after the tubal wall has been sectioned lengthwise. Each suture was then introduced first under a ring of the prosthesis, then through the lower tracheal segment, through the upper segment, and finally through the upper part of the prosthetic ring. The suture is thus forced to maintain the diameter of the prosthesis, as well as joining the tracheal segments (Fig. 3).

Postoperative studies

Observations. All the animals were examined every day by the veterinary surgeon (D.G.O.) of the Experimental Surgery Unit and by the principal researcher. Special attention was paid to the observation of respiratory dis-

Fig. 1. Diagram of group B (primary anastomosis) and group C (external stent of ePTFE).

tress and stridor at rest and during effort and to local and systemic infection, in accordance with the experiment's protocol.

Endoscopy. A bronchoscopic examination was done during the same period of anesthesia described earlier, with the use of a rigid 2.7 mm pediatric telescope connected to a light source, and note was made of the degree and localization of stenosis, presence of granulation tissue, and progress.

The method of euthanasia chosen for the animals was an intravenous administration of a thiopental sodium bolus (120 mg/kg), which causes death instantaneously and without suffering. Each rabbit was put to death 60 days after surgical intervention or earlier if it showed signs of imminent death from asphyxia.

Morphometry. A camera light sketch was drawn of the anatopathologic preparations under the microscope. Enlargements magnified 20 times were obtained of the tracheal sections and these were used for the morphometric calculations made by the Summa Sketch computer (Summa Graphics, Austin, Tex.) and SigmaScan 3.94 program (Jandel Corporation, San Rafael, Calif.). The following measurements were obtained: median and transverse inner diameters of the tracheal lumen (millimeters); tracheal wall thickness (millimeters), that is, six measurements at different standard points; area (square millimeters); and perimeter (millimeters) of the tracheal lumen. Stenosis was defined as any value of the area of the tracheal lumen of less than mean - 2 standard deviations. With reference to the percentage of stenosis observed, figures were classified into the following groups: less than 25%, between 25% and 50%, between 51% and 75%, and more than 75%.

Anatopathology. The microscopic study took the following parameters into consideration: inflammatory reaction, characteristics of the epithelium, cartilaginous changes, and behaviour of the prosthesis. All the parameters were quantified (0, +, and ++) as a result of the findings and in accordance with the experimental protocol.

Statistics. The size of the sample required was calculated with the use of a type 1 error of 5% and a power of 80%. A simple descriptive analysis was made of the data

Fig. 2. Surgical procedure. *Left*, Resection of six cervical tracheal rings. *Center*, Relation between the prosthesis (2 cm length, 8 mm internal diameter) and the segment resected. *Right*, Technique of insertion of the prosthesis.

Fig. 3. *Left*, Group C. Lengthwise section (a) and cross section (b). Note that the sutures are pulling centrifugally. *Right*, Detail of the knot on the spiral of the prosthesis.

calculating the mean and the standard deviation. The χ^2 test, Fisher's exact test, the t test, and Mann-Whitney's U test were used as appropriate. Statistical analyses were performed on the SPSS/PC+ statistical package (SPSS/PC+ 4.01; SPSS, Inc., Chicago, Ill.). Confidence limits (CL) for proportion failing in each group were calculated by means of the exact binomial 95% by Epi Info, version 6.04a (Centers for Disease Control and Prevention, Atlanta, Ga.).

Results

No animals in group A had stridor, in comparison with seven in group B (46.6%, 95% CL 21.2% to 73.4%) and one in group C (6.6%, 95% CL 0.16% to 31.9%). We found a difference between group B and the control group ($p = 0.006$) and no difference between group C and the control group ($p = 1$). No respiratory distress was observed in animals in the control group. In group B, eight (53.3%, 95% CL 26.5% to 78.7%) had respiratory distress, in com-

Fig. 4. Distribution of the groups according to clinical parameters.

parison with two in group C (13.3%, 95% CL 1.6% to 40.4%). Differences were found between group B and the control group ($p = 0.002$), whereas no differences were found between group C and the control group ($p = 0.48$).

No animals died in groups A and C. Seven animals died in group B (46.6%, 95% CL 21.2% to 73.4%), and all of these deaths were due to symptoms of asphyxia and respiratory insufficiency causing congestive cardiac failure. There were differences between group B and the control group A ($p = 0.006$) but none between group C and the control group ($p = 1$). The average survival time of the animals that died in group B was 30 days. None of the animals studied had anastomotic dehiscences with escape of air, cervical or mediastinal emphysemas, or abscesses of the cervicotomy (Fig. 4).

No stenosis was observed on endoscopic exami-

Fig. 5. Distribution of the degree of stenosis among the groups.

Table I. Results of the morphometric study in the different groups

	Group A	Group B	Group C	p Values	
				A/B	A/C
Anteroposterior diameter	3.99 ± 0.40	2.81 ± 0.90	4.28 ± 0.35	0.00006	0.04
Transverse diameter	5 ± 0.32	3.72 ± 1.13	4.61 ± 0.43	0.00003	0.008
Area	16.7 ± 1.36	9.74 ± 4.33	15.76 ± 1.40	0.000003	0.07
Perimeter	15.38 ± 1	12.23 ± 3.11	15.12 ± 1.02	0.00009	0.38
Average thickness of wall	0.71 ± 0.08	1.63 ± 0.68	1.29 ± 0.20	0.000008	0.000003

Values are mean ± standard deviation.

nation in any animal in the control group or in group C, but 13 animals in group B (86.7%, 95% CL 59.5% to 98.3%) that underwent anastomosis under tension had a certain degree of stenosis: In six the stenosis was less than 25% of the tracheal lumen, in three it was between 25% and 50%, in one between 51% and 75%, and in three the lumen was more than 75% stenotic. Differences were found between the degree of stenosis in group B and in the control group ($p = 0.001$), but no differences were observed between group C and the control group.

Table I shows the most significant data resulting from the morphometric study. With reference to the morphometric values of the average value of the tracheal area of control group A, intervals (mean ± 2 standard deviations) were set to include 95% of the normal values (13.98 and 19.42 mm², respectively). Considering that stenosis occurred in all values under 13.98 mm, the quartiles were defined as less than 25% stenosis (between 13.98 and 10.49 mm²), 25% to 50% stenosis (10.48 to 7.0 mm²), 51% to 75% (6.99 to 3.51 mm²), and more than 75% (under 3.5 mm²). All the animals (100%, 95% CL 78.1% to 100%) in group B had stenosis: in seven (46.6%, 95% CL 21.2% to 73.4%) the stenosis was less than 25%, in four (26.6%, 95% CL 21.2% to 73.4%) it was between 25% and 50%, in one (6.7%, 95% CL

0.16% to 31.9%) it was between 51% and 75%, and in three (20%, 95% CL 4.3% to 48%) the area of tracheal lumen was greater than 75% stenotic. Only one animal (6.7%, 95% CL 0.16% to 31.9%) in group C had stenosis, and this was less than 25% of the area of the lumen. The stenosis in all cases was centrally positioned. Differences were found in the comparison of data from group B with the control group ($p < 0.001$), but no differences were seen between groups A and C ($p = 0.3$). The correlation between the degree of stenosis observed by tracheoscopic and morphometric studies was 85%, as shown in Fig. 5. Fig. 6 illustrates the most representative morphometric pictures of the different groups.

The remaining anatomicopathologic parameters were studied microscopically. The results of the study on control group A gave normal values in all cases: no inflammatory reaction in the tracheal lumen, submucosa, or peritracheal tissue, normal respiratory epithelium, and no cartilaginous changes. The results of groups B and C are shown in Table II.

Discussion

The use of prosthetic materials in tracheal reconstructive surgery is currently a subject of great discussion. The range of materials considered as ideal substitutes for tracheal tissue is limited be-

Fig. 6. Reduced diagram of the morphometric images obtained from the histologic preparations of the different groups. Note the marked reduction in tracheal lumen in group B and absence of stenosis in group C where the ePTFE prosthesis surrounds the tracheal wall.

Table II. Results of the anatomopathologic study

Parameters studied	Group	Grade of wound		
		0	+	++
Neutrophils in lumen	B	13 (86.6%)	1 (6.6%)	1 (6.6%)
	C	15 (100%)	—	—
Leukocytes in submucosa	B	4 (26.6%)	8 (53.5%)	3 (20%)
	C	9 (60%)	6 (40%)	—
Lymphocytes/monocytes/plasma cells in submucosa	B	3 (20%)	8 (53.3%)	4 (26.6%)
	C	1 (6.6%)	14 (93.3%)	—
Fibrovascular proliferation	B	4 (26.6%)	4 (26.6%)	7 (46.6%)
	C	6 (40%)	9 (60%)	—
Granuloma on foreign body	B	6 (40%)	4 (26.6%)	5 (33.3%)
	C	6 (40%)	9 (60%)	—
Necrosis of cartilage	B	13 (86.6%)	2 (13.3%)	—
	C	10 (66.6%)	4 (26.6%)	1 (6.6%)
Epithelial continuity		No	Yes	
	B	11 (73.3%)	4 (26.6%)	
Epithelial metaplasia	C	8 (53.3%)	7 (46.6%)	
	B	10 (66.8%)	5 (33.3%)	
Abscess from prosthesis	C	15 (100%)	—	
	C	15 (100%)	—	

cause the demands are so great. The material must be easy to handle and cut to size. It must be packaged, sterilized, and immediately available. It must also be highly biocompatible, rigid enough to prevent it from collapsing, and impermeable to air. Several authors^{15, 16} have suggested that ePTFE could be an optimum material for such a use. If its applicability were clinically proved,¹⁷ it

would offer the advantages of rigidity (avoiding collapse of the prosthesis as observed in pericardial, periosteal, and dura mater implants¹⁸) and would not be difficult to prepare and shape, as rib cartilage grafts are.

This prosthetic material had two particularly useful characteristics for the purposes of this study: Because it has one wall, when the prosthesis was cut

it did not fray and the desired shape could be easily achieved; because the silicone spiral is attached to the prosthetic wall, the form of the prosthesis was maintained after being cut and the tube did not collapse. With regard to the porosity of the prosthesis, it has been shown that microporous prostheses, with a pore diameter of 30 μm , are more efficient than macroporous ones.^{15, 19} If less porous materials are used, the diameters of the pores do not allow fibroblasts to pass through the wall, and the prosthetic material is poorly incorporated into the tissue.

The normal parameters were taken from analysis of the control group. We found a mean of 37 tracheal rings and an average length of 4.66 cm in the isolated trachea measured immediately after resection. The segment of the cervical trachea for resection measured 2 cm (a mean of 0.97 cm for segments that had already been resected, as clearly seen in Fig. 2). The trachea of a rabbit has a classic C shape, its median diameter is slightly less than the transverse diameter (4 and 5 mm, respectively), and it has a similar area and perimeter (16.7 mm² and 15.4 mm). The thin wall of the trachea is of note, especially in the membranous part, where it measures only 0.5 mm. This complicates the dissection of the posterior side of the trachea and requires a meticulous surgical technique to avoid surgical trauma. Ischemic tracheal necrosis from interruption of the segmental blood supply to the trachea after surgical exposure has been reported.²⁰ In the histologic study, we did not observe tracheal necrosis.

The correlation between the endoscopic and morphometric findings is high (85%), which corroborates the fact that endoscopy is one of the most reliable and precise tests in the evaluation of laryngotracheal wounds. This opinion is widely shared by many authors. However, some authors²⁰ have not found linear correlation between tracheoscopy and histologic studies, and they believe that tracheoscopy is not a reliable method of evaluating mucosal changes in the trachea.

The morphometric study was fundamental in setting the normal parameters. The minimum value of the normal area of the trachea was set to 13.98 mm² and the values of stenosis were divided into quartiles. Other studies²¹ classify these values differently: grade 1, stenosis less than 70% of luminal diameter; grade 2, 70% to 90%; grade 3, more than 90% (tracheal lumen is still patent, or only the subglottis is completely obstructed); grade 4, no tracheal lumen can be seen and the vocal chords cannot be

identified. We believe that this classification can be useful, but we chose to classify the degree of stenosis in relation to the quartile percentage of tracheal lumen narrowing when examined endoscopically and morphometrically.

With regard to the direct anastomosis used in group B, the results wholly corroborate the fact that all tracheal sutures applied under tension are doomed to failure.²² In clinical practice the rate of anastomotic stenosis is high when more than five or six tracheal rings are resected, especially when no tension-freeing technique is used.^{13, 23} However, no study of this type has been made on rabbits. The animals with a higher degree of stenosis also had more inflammation of both the submucosa and peritracheal tissue. This observation confirms that the principal causes of stenosis are fibrovascular proliferation and inflammatory submucosal and peritracheal granulomas. This was also found in other studies,²⁴ which describe infiltration of granulation tissue in the tracheal wall at the anastomosis site and the presence of polymorphonuclear leukocytes.

The principle at stake in group C is simple: if a suture has to be applied with a certain degree of tension, the anastomosis will remain patent if something is positioned to enforce this opening. This principle was first applied in 1960,²⁵ when an external wire mesh was wrapped around the trachea of dogs after resection of eight cartilage rings. The technique failed in all the animals. Other materials have been used, such as external tubes, either to protect the anastomotic suture or to make the flaccid trachea rigid in the case of acquired or congenital tracheomalacia.^{20, 26, 27} Hanawa and associates²⁸ found that the feeding vessels of the trachea might be occluded when an external Marlex mesh prosthesis (Bard Implants, Billerica, Mass.) was sutured to the tracheal wall, and as a result ischemia and then necrosis might develop. Therefore they used adhesives (fibrin glue, polyurethane-prepolymer, lyophilized human dura mater [Lyo-dura; B. Braun Melsungen AG]) instead of sutures. We observed epithelial metaplasia but no ischemia in the mucosa. We believe that in our study it is the suture on the spiral of the prosthesis that prevents recurrence of stenosis. However, a more reliable method would be to use adhesives instead of sutures to fix the prosthesis to the tracheal wall.

The results obtained in our study show that the rigidity of ePTFE prevents the trachea from collapsing and that the prosthesis is well incorporated into

the surrounding tissue. No rabbits died after resection of the six tracheal rings and insertion of an external stent of ePTFE, and only two had associated symptoms: respiratory distress and stridor in one rabbit and distress alone in the other. Stenosis was not observed on endoscopic examination in any of these rabbits. When studied morphometrically, one of these rabbits was classified as having grade 1 stenosis (<25%), but the area of tracheal lumen in this case was 13.48 mm² (only 0.5 mm² less than the minimum normal value). No collapse of the trachea was noted and no prosthetic intrusion into the lumen. The anastomosis site showed complete tracheal epithelialization. The material was extremely well incorporated by this group despite lymphocytes, monocytes, and plasma cells being found in 90% of the animals. The prosthesis did not cause the formation of abscesses in any of the animals when examined microscopically. Fibroblasts migrated through the micropores of the prosthesis in all the animals. Although more than 50% of the animals had granulomas in the submucosa, produced by the foreign prosthetic material or by suture remains, granulomas were not observed in the tracheal lumen of any animal. Some studies²⁹ demonstrate that the polyglactin 910 (Vicryl) sutures produce an intense inflammatory reaction and late fibrosis. In contrast, the monofilament nature of PDS polydioxanone suture (Ethicon, Inc.) results in less intense inflammatory reaction than the multifilament Vicryl suture. Friedman and associates²⁹ suggest that the use of PDS suture results in a larger luminal area at the anastomotic site than Vicryl suture. We agree that the PDS suture probably fares better than Vicryl suture in the trachea, and we plan to use this suture material for the new long-term research that we are initiating.

Two animals that were not included in this study were observed for 18 months after positioning of an external ePTFE stent. They were free of symptoms and had normal tracheal lumina. They were put to death at the end of this observation period.

Results are encouraging from this and other studies that have used ePTFE as a prosthetic material to patch the trachea, although further experimental work must be undertaken before ePTFE can be applied in operations on the human trachea. The new lines of research using porous prostheses coated inside with respiratory epithelial cellular cultures,³⁰ and other lines such as revascularized tracheal transplants,³¹ suggest that the majority of

tracheal surgical problems will be solved in the near future.

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