Experimental Models of Tracheobronchial Stenoses: A Useful Tool for Evaluating Airway Stents

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Abstract

Background. Stent implantation is a conservative alternative to open operation for treating benign tracheobronchial strictures. Most of the presently available stents were primarily designed for endovascular use. Their respiratory use entails a risk of iatrogenic complications. From a scientific and from an ethical point of view these risks justify preclinical evaluation of new respiratory stents in experimental models of central airway stenoses. Therefore, an attempt was made to develop such models in piglets and adult minipigs.

Methods. Tracheal stenoses were obtained by creating first a segmental tracheomalacia through extramucosal resection of cartilaginous arches. The fibrous component of the stenoses was then obtained through bronchoscopic application of a caustic agent causing progressive deep mucosal and submucosal injury. Stenoses of the main bronchi were created by topical application of the caustic agent only.

Results. These models demonstrated the typical features of benign fibromalacic tracheobronchial stenoses with constant recurrence after mechanical dilation. Preliminary experiments showed that short-term problems of tolerance of stent prototypes are easily demonstrable in these models.
Conclusions. These experimental models, which simulate quite realistically human diseases, offer the opportunity to perfect new tracheobronchial stents specifically designed for respiratory use and to evaluate their long-term tolerance before their use in humans.

• Introduction

See also page 656.

A variety of silicone or metallic stents are currently used to restore airway patency in malignant tracheobronchial stenoses. Stent implantation immediately alleviates dyspnea and usually allows postobstructive pulmonary superinfections to resolve. Thus, in cancer patients, the benefits of stenting the airways largely outweigh the risks inherent to the stents.

More recently temporary or definitive stent implantation has also been proposed as a conservative alternative to open operation to treat benign tracheobronchial strictures such as observed after sleeve resections, pulmonary transplantation, or prolonged endotracheal intubation [1-12]. The metallic stents, which are flexible and expandable, have a great potential for splinting these benign stenoses, which usually combine fibrous scars, airway distortion, and tracheobronchial collapse. There is, however, compelling evidence that metallic stents can be responsible for immediate or delayed iatrogenic complications including development of bulky granulation tissue between the wire struts [2, 3, 5-8, 11, 13], misplacement, migration, unravelling or fracture of the stents [4, 5, 8, 12], bronchial or tracheal wall perforations, and fatal hemoptysis [3, 8]. Because removal of the stents is hardly ever possible [3, 6, 8, 11, 12, 14], their potential risks should be taken into account when considering treatment of benign tracheobronchial strictures.

In fact, metallic stents were primarily designed for endovascular use and not for respiratory use. Although endovascular tolerance of these stents was extensively evaluated in animals and in humans, evaluation of their safety and tolerance in benign tracheobronchial lesions has been very limited. Indeed, the only presently marketed stent that has undergone preclinical evaluation in animals is the Gianturco stainless-steel "zig-zag" stent [3, 15]. From these studies it was obvious that the severe complications now observed with tracheobronchial use of this stent were predictable. We thus believe that from a scientific and from an ethical point of view the iatrogenic risks of tracheobronchial stent implantation justify preclinical evaluation, especially if the indications for stenting the airways are not restricted to salvage therapy in cancer patients.

We therefore considered two different approaches. At first laboratory experiments with stents in excised or artificial airways were conducted [6, 7]. Mechanical properties of silicone and metal stents, including stability against external compression, change of length, cough behavior, and fatigue stability were tested. After these laboratory tests we attempted to develop animal models mimicking the benign tracheobronchial strictures encountered in humans and started evaluation of
Materials and Methods

Animals

Piglets (Largewhite-Landrace) and adult minipigs (Pittman-Moore and Gottingen) weighing 23 ± 6 kg were used. All animals were treated in compliance with the guidelines of the Department of Experimental Research of the Lille University and with the "Guide for the Care and Use of Laboratory Animals" (NIH publication 85-23, revised 1985). In addition, the experimental protocols were reviewed and approved by the Animal Experimental Committee of the French ministry of agriculture.

Pigs of an average weight of 23 kg were chosen because their tracheal size approximates that of a human adult of 45 to 60 kg. This allows rigid bronchoscopy with the same instruments as those used in humans, and thus facilitates further extrapolation of the experience gained with therapeutic bronchoscopy in the pig models of central airway diseases.

Largewhite-Landrace piglets weighing 21 ± 5 kg were used to create the bronchial stenosis model and initially also to create the tracheal stenosis model. Adult minipigs were subsequently used for the tracheal stenosis model development phase.

Anesthesia

All the surgical and bronchoscopic procedures were performed under general anesthesia. The animals were premedicated intramuscularly with ketamine and atropine. Before the surgical procedures they received 1 g cefazolin for antibiotic prophylaxis. Anesthesia was induced with intravenous propofol (2 to 3 mg/kg) followed by a continuous infusion of propofol (10 mg • kg⁻¹ • h⁻¹) for maintenance. Analgesia was provided by repeated intravenous bolus doses of 0.5 mg alfentanil. Neuromuscular blockade was obtained with repeated intravenous bolus doses of suxamethonium (0.1 mg/kg). Ventilation was provided by a high-frequency jet ventilator through a cannula adjusted to the proximal port of the endotracheal tube or to the rigid bronchoscope. Blood pressure, pulse oxymetry, and electrocardiogram were monitored throughout the procedures.

Model development

TRACHEAL STENOSIS DEVELOPMENT PHASE.

According to the technique described by Johnston and associates [18] and Mair and colleagues [15], a limited tracheomalacia was created by extramucosal resection of about 50% of the circumference of three consecutive cartilaginous arches. To obtain extrathoracic tracheomalacia we exposed the trachea just above the thoracic inlet through a low cervical midline incision and then resected the sixth to the eighth tracheal rings. To obtain intrathoracic tracheomalacia we exposed the trachea between the tracheal bronchus and the thoracic inlet through a right thoracotomy incision as described by Vinograd and co-workers [19]. The cartilaginous resection was then performed upward, starting at the third ring above the tracheal bronchus.
Two weeks later and then every 3 weeks the animals underwent rigid bronchoscopy for endotracheal examination and for mucosal application of a 23% solution of NaOH (pH 14) at the level of the flaccid tracheal segment. NaOH was used because this caustic agent is known to induce profound tissue necrosis followed by fibrous scar formation. The solution was carefully applied onto the mucosa with a cotton swab. Circumferential application was performed in the first 2 pigs. Application onto the posterior membranous wall was subsequently avoided.

**BRONCHIAL STENOSIS DEVELOPMENT PHASE.**

Mucosal application of the NaOH solution onto the first centimeter of the left main bronchus was performed with a cotton swab. The left main bronchus was chosen because, in the pig, the right main bronchus is very short. Hence, a stent subsequently placed at this level would have overlapped the middle lobe bronchus and impaired the clearance of secretions from this lobe. The NaOH solution was applied onto the whole circumference of the bronchus in the first 2 pigs and was limited to two thirds of the circumference thereafter. One week later and every other week thereafter bronchoscopic examination was performed to remove obstructive necrotic membranes. Subsequent application of NaOH was repeated as needed.

**Dilation and Stenting of the Experimental Stenoses**

To assess the potential for recurrence of the induced stenoses, mechanical dilation was attempted once the stenoses became clinically and endoscopically evident. An adult rigid bronchoscope (Karl Storz Gmbh, Tuttlingen, Germany) with an external diameter of 11.4 mm was used to dilate the tracheal stenoses. Angioplasty balloons, 8 to 12 mm in diameter (BALT, Montmorency, France), were used for bronchial stenosis dilation.

Preliminary evaluation of the Palmaz steel stent (Johnson & Johnson Corp, Warren, NJ) was carried out in 4 normal pigs and in 2 pigs with an experimental bronchial stenosis. Two of these stents were coated with a Dacron graft sewn to the metallic mesh. Two additional self-expandable metallic-coated stent prototypes were tested in 4 pigs with an experimental tracheal stenosis.

**Follow-up and Evaluation**

Clinical signs were monitored daily with special attention to weight loss, cough, sputum production, wheezing, and dyspnea. Video recordings and photographs were taken at each bronchoscopic examination. Additional bronchoscopies were performed as warranted by clinical signs. Animals showing severe respiratory compromise were euthanized under general anesthesia with intravenous potassium chloride.

The remaining animals were euthanized at various times postoperatively for postmortem examination. Lungs, mediastinum, trachea, and esophagus were removed in one single block through a cervicothoracic midline incision for complete histopathologic examination.

- **Results**

**Tracheal Stenosis**
Tracheal Stenosis

Three Large White-Landrace piglets and 7 minipigs underwent limited extramucosal resection of three consecutive extrathoracic tracheal arches. In 5 additional minipigs the cartilaginous arches were resected from the intrathoracic trachea. No signs of airway obstruction developed postoperatively in any pig. In 1 of the minipigs accidental tracheal mucosal breach was responsible for massive subcutaneous emphysema, which developed within a few hours, and the animal had to be euthanized. Three other minipigs died unexpectedly about 2 weeks postoperatively because of wasting diarrhea. No sign of tracheal obstruction was evidenced on postmortem examination. In the remaining 11 pigs a first mucosal application of the NaOH solution was carried out at the level of the malacic tracheal segment 2 weeks after the surgical procedure. The first Large White-Landrace piglet died 4 days after the bronchoscopy because of a perforation of the posterior wall of the trachea. Thus, posterior application of the caustic agent was subsequently avoided. Follow-up bronchoscopy in the remaining 2 Large White-Landrace piglets showed deep mucosal ulceration with exposure of bare cartilage. Healing of this caustic injury generated progressive scar tissue formation with a resulting stenosis, which became apparent about 3 weeks after the second application of the NaOH solution. The stenosis was limited, however, with a decrease of the tracheal diameter of less than 40%. By this time the animals' weight had nearly doubled so that the concomitant enlargement of the tracheal cross-section had probably hampered the development of a significant stricture. Therefore adult minipigs were used for further studies. For an unexplained reason 1 of the minipigs grew by more than 30% within 2 months and thus, like the Large White-Landrace piglets, had development of only insignificant tracheal stenosis.

In all the other minipigs a tight tracheal stenosis progressively developed after 2 to 4 topical applications of the NaOH solution. These animals progressively experienced exertional dyspnea and wheezing. Bronchoscopic examination showed circumferential contracting scarring, 1 to 3 cm in length, with a 60% to 80% reduction of the tracheal lumen (Fig 1). Mechanical dilation with the rigid bronchoscope was quite easy, but stenosis uniformly recurred within 3 weeks. These features attested to the presence of an ongoing inflammatory scarring process and the presence of tracheal wall damage (malacia). Microscopic examination showed the typical wall alterations of cicatricial tracheal stenoses, including subacute mucosal and submucosal inflammation, chondritis, and progressive degeneration of the cartilaginous support (Fig 2).

Fig 1. Endoscopic view demonstrating a circumferential contracting, scarring stenosis at the level of the intrathoracic trachea.
Bronchial Stenosis

In the first 2 animals, circumferential application of the NaOH solution resulted in complete inflammatory obstruction of the main bronchus with massive postobstructive pneumonia. Thus, for further studies, caustic burning was restricted to two thirds of the bronchial circumference. The following 7 animals demonstrated progressive formation of a circumferential stenosis after one or two applications of the caustic agent (Figs 3, 4033). Mild coughing with retention of secretions distal to the stenosis ensued. As with tracheal stenoses, the bronchial stenoses uniformly recurred after balloon dilation.

Fig 3. (A) Endoscopic view of the main carina. (B) Same view 2 weeks later, once the left main bronchial stenosis has developed.
Preliminary Experiments With Stents

The animals in which an uncovered Palmaz stent was implanted into the left main bronchus showed only moderate granuloma formation at the stent extremities, which was maximal by day 7 and resolved by day 21. Moderate to severe (2:50%) crushing of this noncompliant stent progressively appeared so that by day 21 the stent no longer molded the total circumference of the bronchial wall (Fig 5©). The two Dacron-covered Palmaz stents migrated distally to the left main bronchus and thus partially occluded the left upper lobe bronchus. This resulted in postobstructive left upper lobe pneumonia.

A self-expandable metallic stent prototype completely coated with a silicone sheet was used to treat experimentally induced tracheal stenosis in 2 pigs. One of these pigs expectorated the stent 5 days later without further clinical consequences. The other died of respiratory obstruction 4 days postoperatively. Postmortem examination showed that the stent migrated proximally to the stenosis.

The second self-expandable metallic stent prototype was partly covered with a Dacron graft. Partial fractures of the stents were observed 2 weeks after implantation. Granulomas developed
between the metallic struts at the proximal and distal parts of the stent, which were not covered by the Dacron graft (Fig 6ES). This led to progressive respiratory failure in 2 pigs, whereas in the last pig the inflammatory reaction resolved and was followed by complete reepithelialization of the stent extremities.

**Fig 6.** Internal view of the trachea after longitudinal section at the level that was stented with the Dacron-covered metallic stent prototype. Bulky granulation tissue can be seen where the uncovered proximal and distal stent extremities molded the tracheal wall.

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**Comment**

Therapeutic bronchoscopy has become an accepted conservative approach in patients with benign tracheobronchial stenoses who cannot undergo an open operation. Laser resection and mechanical dilation can be helpful for treating short, weblike fibrous strictures. Unfortunately, restoring the airway caliber is usually of short-term efficacy because most of the cicatricial stenoses comprise segmental deterioration of the cartilaginous support with resulting tracheobronchial wall instability. Thus, splinting these stenoses with a stent is often necessary to maintain airway patency. Silicone rigid stents, which are very effective for palliating neoplastic strictures, have a high rate of secondary migration in benign stenoses. Metallic stents have theoretic advantages in this indication. Their radial expandability and longitudinal flexibility ensure their stability in malacic or distorted airways. In addition, compared with the silicone stents, their high internal to external diameter ratio, their partial collapse during cough, and their secondary reepithelialization allow better clearance of respiratory secretions.

In contrast to silicone stents, the presently marketed metallic stents are hardly removable when complications occur. Thus, the risk of potentially severe complications, which is probably underestimated, is of concern when treating benign lesions with these stents.

Like benign tracheobronchial stenoses in adults, severe tracheomalacia can be a distressing cause of morbidity and mortality in young infants. Because surgical correction has an unacceptably high rate of complication and failure, pediatric surgeons have considered alternative conservative approaches including endotracheal splinting with self-expanding stents. Preclinical testing appeared to be mandatory before using these stents in infants. Therefore, experimental animal models of tracheomalacia have been developed during the past years [15,18-2JJ. Several stent prototypes,
not yet marketed, have been tested in these models, with variable success [5, 8, 20, 21].

In this study we developed experimental models of tracheal and bronchial stenoses to test new stents specifically designed for these indications.

Tracheal stenosis required limited (50% of the circumference) extramucosal resection of three consecutive cartilaginous arches to be performed first, to get the malacic component of the stenosis. The cartilaginous resection was performed at the level of the intrathoracic or at the level of the extrathoracic trachea to get intrathoracic or extrathoracic stenoses, respectively. The fibrous component of the stenoses was obtained after two to four bronchoscopic applications of a caustic agent causing progressive deep mucosal and submucosal injury. The tracheal stenosis resulting from the combination of scar tissue formation and tracheomalacia required about 8 weeks to be clinically significant in adult minipigs. In piglets, which doubled their weight during this period of time with a proportional enlargement of their tracheal cross-section, significant stenoses did not develop. In contrast, bronchial stenosis became clinically significant after only one or two bronchoscopic applications of the caustic agent without prior cartilaginous resection. Thus, piglets could be used instead of adult minipigs, at least if only short-term evaluation of stents is considered, in this experimental model of bronchial stenosis.

These animal models demonstrated the typical features of benign fibromalacic strictures encountered in humans, with constant recurrence after mechanical dilation. Preliminary experiments showed that short-term problems of tolerance of stent prototypes can be easily demonstrable in these models. Further studies are presently ongoing in these experimental models to perfect new tracheobronchial stents and to evaluate their long-term tolerance before their use in humans.

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**Footnotes**

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