Title

Teflon Injection into the Trachea Causes Predictable Fibroblastic Response and Collagen Deposition

Journal

Journal of Bronchology & Interventional Pulmonology, 23(4)

ISSN

1070-8030

Authors

Longoria, Javier A
Fujiwara, Miwa
Guerra, Catalina
et al.

Publication Date

2016-10-01

DOI

10.1097/lbr.0000000000000290

Peer reviewed

Teflon Injection into the Trachea Causes Predictable Fibroblastic Response and Collagen Deposition

A Pilot Study

Javier A. Longoria, MD, Miwa Fujiwara, MD,† Catalina Guerra, DVM,‡ Jeffrey L. Lee, DVM,‡ Catherine S.H. Sassoon, MD,† and Farhad Mazdisnian, MD†*

Background: **Expiratory central airway collapse is an increasingly recognized abnormality of the central airways and may be present in as many as 22% of patients evaluated for chronic obstructive pulmonary disease and/or asthma. Many current treatment options require invasive procedures that have been shown to cause significant morbidity and mortality. To test the hypothesis that Teflon injection will induce sufficient fibroblast proliferation and collagen deposition, we evaluated the time course on the effect of Teflon injection in the posterior membranous trachea on the histopathology of the tracheobronchial tree.**

Methods: **Six Yucatan Pigs were assigned to undergo general anesthesia and injection of 0.3 to 0.5 mL of sterile Teflon paste in 50% glycerin into the posterior membranous tracheal wall. A control pig received an equivalent volume of glycerin. Animals were euthanized in predefined intervals and tracheas were excised and examined under light microscopy for identifying fibroblast proliferation and collagen deposition.**

Results: **Compared with the control pig, the Teflon injection site showed tissue reaction of fibrohistiocytic proliferation and subsequent collagen deposition in all animals. Furthermore, the increased fibroblast proliferation and collagen deposition were time dependent ($P < 0.01$).**

Conclusion: **This pilot study demonstrates histopathologic changes in the trachea after Teflon injection, comprised of increased fibroblast activity and collagen deposition that could be of potential use in creating greater airway rigidity in patients with sever diffuse excessive dynamic airway collapse.**

Key Words: expiratory central airway collapse (ECAC), Teflon, bronchoscopy

(*J Bronchol Intervent Pulmonol* 2016;23:283–287)

Expiratory central airway collapse (ECAC) is a syndrome comprising 2 different pathophysiologic entities. Excessive dynamic airway collapse (EDAC) and tracheobronchomalacia (TBM). EDAC defines the pathologic collapse and narrowing of the airway lumen which is entirely due to the laxity of the posterior wall membrane with structurally intact airway cartilage. TBM is a disease in which the central airways contain areas of weakness due to softening of the supporting cartilage and hypotonia of myoelastic elements, resulting in central airways collapse during exhalation.[1–5] This pathologic weakening of airways can produce dynamic airflow obstruction with symptoms of dyspnea, orthopnea, intractable cough, inability to clear secretions, and even respiratory failure. EDAC and TBM have been reported to be present in 4% to 23% of patients undergoing bronchoscopy for various indications.[6–8] The incidence of EDAC is 22% in patients with chronic obstructive pulmonary disease and/or asthma.[9] Acquired TBM can be localized or diffuse and has been associated in patients with prolonged endotracheal intubation, tracheostomy, vascular

Received for publication December 4, 2015; accepted April 13, 2016.
From the *Department of Medicine, University of California, Irvine; †Department of Medicine, VA Long Beach Healthcare System, Long Beach; and ‡Steers Biological Resources Center, Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA.
JA.L.: collected data, analyzed/interpreted data and drafted manuscript. M.F.: analyzed/interpreted data, assisted in writing the article, assisted in revision. C.G.: assisted in conception/design and acquisition of data. J.L.L.: assisted in conception/design and acquisition of data. C.S.H.S.: assisted in design of study, analysis/interpretation of data, and revision of manuscript. F.M.: involved in conception and design of study, acquisition of data, interpretation of data, and revision of manuscript. F.M., C.S.H.S., C.G., and J.L.L.: conception and design. J.A.L., C.S.H.S., M.F., and F.M.: analysis and interpretation. J.A.L., M.F., C.S.H.S., and F.M.: drafting of manuscript for important intellectual content.
Supported by Southern California Institute for Research and Education, Long Beach, CA.
Disclosure: There is no conflict of interest or other disclosures.
Reprints: Farhad Mazdisnian, MD, VA Long Beach Healthcare System, 5901 E. 7th Street 111P, Long Beach, CA 90822 (e-mail: farhad.mazdisnian@va.gov).

DOI: 10.1097/LBR.0000000000000290

rings, chronic irritation of the airway, chronic obstructive pulmonary disease/asthma, and chronic infections of the airway.[10–12]

Current treatments for ECAC include a range from conservative approaches to more invasive procedures, all of which serve to splint the central airways. Conservative treatments include inhaled bronchodilators and continuous positive pressure ventilation (CPAP) which can be used as a pneumatic stent and is particularly useful for diffuse disease.[13] Silicon endoluminal stents deployed through rigid bronchoscopy are a minimally invasive approach which have been shown to improve pulmonary function test, symptoms, quality of life, and functional status in patients with severe symptomatic ECAC. However, they are fraught with complications. Approximately 90% of these patients experience complications such as migration, mucus impaction, and stent fracture.[14–20] The most invasive, yet perhaps the most durable, is surgical tracheobronchoplasty (TBP) which stabilizes the membranous portion of the trachea and mainstem bronchi through fixing the posterior membranous wall to synthetic mesh.[21–23] Although TBP does improve quality of life and functional capacity, the complication rate including prolonged mechanical ventilation, respiratory infections, pulmonary embolism, and atrial fibrillation is very high, approaching 43%, and requires prolonged hospital stay.[21,22,24]

Teflon (polytetrafluoroethylene) is an inert chemical that has been successfully used in vocal cord dysphonias and vesicoureteral reflux in children. In the area of injection, there is a marked fibroblastic proliferation, resulting in augmentation and stability of tissues. These effects appear to be long lasting with minimal side effects.[25–28] However, it is unknown if a similar reaction occurs when Teflon is injected in the tracheobronchial tree. The purpose of this pilot study is to evaluate the effect of Teflon injection on the histopathology of the pig's tracheobronchial tree. Some of these results have been previously reported in the form of an abstract.[29]

METHODS

The study was approved by the Research and Development Subcommittee on Animal Studies of VA Long Beach Healthcare System, and was conducted and approved in conjunction with LA Biomedical Research Institute at Harbor-UCLA Medical Center. A total of 7 Yucatan Pigs were studied. Six of the animals were assigned into the treatment arm to receive Teflon injection, and 1 animal served as control. The duration of study after injection was 12 weeks. General anesthesia was administered (Ketamine 20 mg/kg IM and Xylazine 2 mg/kg IM), and after administration of antisialagogues (Atropine 0.04 to 0.4 mg/kg IM), the animals were intubated with a 10 mm external diameter Efer-Dumon Rigid Bronchoscope. An intravenous catheter was placed in the ventrolateral ear vein for administration of 0.9% NaCl at 500 mL/h for the duration of the procedure. During surgery, general anesthesia was maintained with 1% to 2% isoflurane. All pigs received 3 to 5 mg/kg intravenous cefazolin. A flexible bronchoscope was used to survey the pig's tracheobronchial tree for any endobronchial lesions or evidence of obstruction both before and after injection of Teflon. Teflon that had been sterilized using an autoclave was mixed with sterilized 50% glycerin to create a paste to a volume of 0.3 to 0.5 mL. The Teflon paste was injected through a 15-G injection and puncture cannula (Richard Wolf Medical Instruments) into the submucosa of the tracheal posterior membrane approximately 2 cm above the main carina. The control pig received an equivalent volume of glycerin. The animals were then extubated and recovered from general anesthesia. Postoperatively, all animals received buprenorphine 0.01 mg/kg intramuscular (IM) every 6 hours as needed for pain and Penicillin G (10,000 to 40,000 U/kg IM every 3 d). Postsurgical animal care was monitored every 15 minutes until the animals regained normal posture and then at least once per day until euthanized. One animal each was euthanized at weekly interval after 1 to 4 weeks, 1 animal after 8 weeks, and 1 animal after 12 weeks of Teflon injection. The control pig was euthanized after 8 weeks of injection. Euthanasia was performed using intravenous pentobarbital (100 mg/kg) injection into the ventrolateral ear. After euthanasia, the trachea was immediately excised and placed in formalin solution. The tissue was then dehydrated in graded series of alcohols and embedded in paraffin. A rotatory microtome was used for serial sections of 6 μm thickness and 8 mm in diameter. The tissue was then stained with hematoxylin and eosin and examined by a pathologist for presence of fibroblast proliferation, exuberant formation of giant cells/granulomas, and fibrosis. The fibroblast numbers for each section were counted in a calibrated grid with an area of 0.01 mm^2 at

×1000 magnification. Grids in 10 different locations of fibrosis per animal were counted.

Statistical Analysis

Values were expressed as mean ± SD. Comparison on the number of fibroblasts among animals was performed using one-way analysis of variance (SigmaPlot 2001; SPSS Inc., Chicago, IL).[30]

RESULTS

Five of 6 animals survived the procedure and were euthanized according to the scheduled time. The animal scheduled for euthanasia after 3 weeks post-Teflon injection bled intraoperatively as a result from rigid bronchoscope trauma, before Teflon injection, and had to be euthanized immediately. Tissue reaction was observed in all animals with some degree of variation. The trachea killed at 8 weeks had the most prominent reaction, showing a marked fibrohistiocytic and chronic inflammatory reaction to the Teflon particles. This resulted in a robust response of mature production of collagen by fibroblasts (Fig. 1). The control (glycerin only) section shows the area around the injection site with only few extravasated red blood cells and iron containing hemosiderin granules in macrophages (Fig. 2).

The number of fibroblasts within the specified area was counted and shown in Table 1. Fibroblast proliferation started within 1 week and increased significantly in each subsequent week (Table 1, $P \leq 0.01$). Fibroblast proliferation increased until after week 8 of Teflon injection when fibroblast proliferation subsided and was replaced by mature collagen deposition (Table 1). In all of the sections, the cartilage and mucosa were intact and there was no tracheal endoluminal granulation.

DISCUSSION

The reported incidence of EDAC and TBM in patients undergoing bronchoscopy is between 4% and 23%.[6–9] This pathologic weakening and collapse of the airway can produce dynamic airflow obstruction with symptoms of dyspnea, orthopnea, intractable cough, and inability to clear secretions, predisposing the patient to recurrent infections.[10–12] Some patients with severe, diffuse ECAC may present with respiratory failure requiring mechanical ventilation while others may have unexplained extubation failure.[31] Our proposed study using Teflon injections into the posterior membranous trachea demonstrated histopathologic changes consistent with predictable, marked fibroblast proliferation in the immediate postinjection time. This was then followed by slowed proliferation of fibroblasts and collagen deposition in injection sites presumably through previously described foreign body granulomatous reaction.[25]

Limitations of this pilot study include the small sample size and relatively short follow-up period of observation of post-Teflon injection. A study with a large sample size and the long-lasting effects of tracheal Teflon injection will

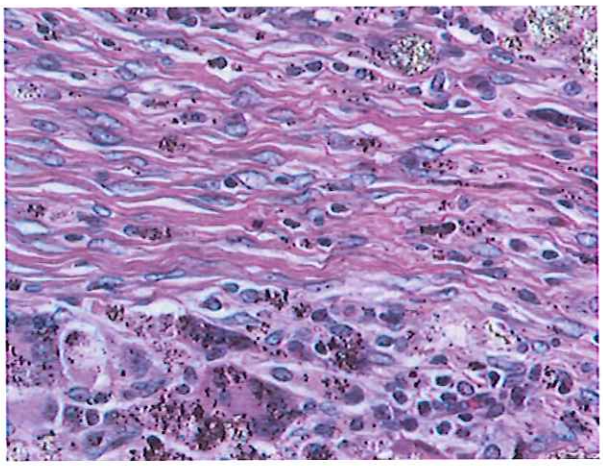

FIGURE 1. Posterior membrane of pig trachea 4 weeks post-Teflon injection showing fibroblast proliferation and collagen deposition; hematoxylin and eosin stain; magnified ×20 (A) and ×400 (B). a+

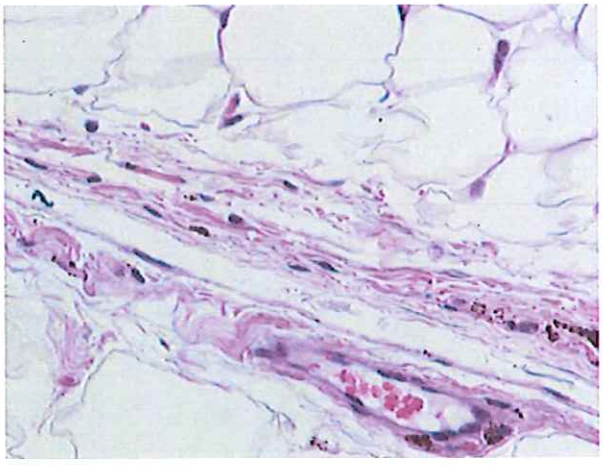

FIGURE 2. Posterior membrane of pig trachea 8 weeks post glycerin injection showing lack of fibroblastic response; hematoxylin and eosin stain; magnified ×20 (A) and ×400 (B). a+

TABLE 1. Average Number of Fibroblasts Counted in a Calibrated Grid With an Area 0.01 mm^2 at ×1000 With ± SD

Weeks After Teflon Injection	No. Fibroblast Counted on Each Slide	Mean ± SD
1	29, 31, 22, 23, 30, 20, 28, 22, 25, 31	26.1 ± 4.2*
2	31, 28, 27, 44, 33, 36, 42, 43, 40, 53	37.7 ± 8.2*
3	—	—
4	47, 61, 57, 49, 55, 52, 52, 53, 60, 47	53.3 ± 5.0*
8	75, 80, 60, 85, 62, 67, 79, 61, 68, 57	69.4 ± 9.7*
12	24, 32, 28, 31, 30, 34, 32, 32, 19, 33	29.5 ± 4.7*
Control	0	0

Week 1 compared with weeks 2, 4, and 8.
Week 2 compared with weeks 4 and 8.
Week 4 compared with weeks 8 and 12.
Week 8 compared with weeks 12.
*$P < 0.01$.

still need to be investigated and addressed before implementation in humans. Bronchoscopy before euthanizing the animals was not performed, thus we were unable to evaluate the luminal cross sectional area after Teflon injection. Another potential limitation to this method is that it would be technically difficult for patients with diffuse disease, as this would likely require many injection sites and multiple procedures.

The described reaction of relatively short-lived fibroblast proliferation followed by long-term collagen deposition is a potentially desirable effect in the treatment of ECAC. Collagen deposition could increase the rigidity of the trachea and give structural support to diseased areas, of similar concept as TBP. However, Teflon injection has the large advantage of being able to be performed bronchoscopically and therefore, would be much less invasive. As such, our results could potentially shed light into an alternative method of treatment of ECAC. Further studies are necessary to determine the longevity of collagen deposition, and if the fibroblast proliferation and collagen deposition seen after Teflon injection could serve to strengthen and stabilize the structurally feeble site of airway collapse.

REFERENCES

1. Baxter JD, Dunbar JS. Tracheomalacia. *Ann Otol Rhinol Laryngol.* 1963;72:1013–1023.
2. Nuutinen J. Acquired tracheobronchomalacia. A clinical study with bronchological correlations. *Ann Clin Res.* 1977;9:350–355.
3. Zhang J, Hasegawa I, Feller-Kopman D, et al. AUR Memorial Award Dynamic expiratory volumetric CT imaging of the central airways: comparison of standard-dose and low-dose techniques. *Acad Radiol.* 2003;10:719–724.
4. Gilkeson RC, Ciancibello LM, Hejal RB, et al. Tracheobronchomalacia: dynamic airway evaluation with multidetector CT. *AJR Am J Roentgenol.* 2001;176:205–210.
5. Boiselle PM, Feller-Kopman D, Ashiku S, et al. Tracheobronchomalacia: evolving role of dynamic multislice helical CT. *Radiol Clin North Am.* 2003;41:627–636.
6. Ikeda S, Hanawa T, Konishi T, et al. Diagnosis, incidence, clinicopathology and surgical treatment of acquired tracheobronchomalacia. *Nihon Kyobu Shikkan Gakkai Zasshi.* 1992;30:1028–1035.
7. Palombini BC, Villanova CA, Araujo E, et al. A pathogenic triad in chronic cough: asthma, postnasal dripsyndrome, and gastroesophageal reflux disease. *Chest.* 1999;116:279–284.
8. Jokinen K, Palva T, Sutinen S, et al. Acquired tracheobronchomalacia. *Ann Clin Res.* 1977;9:52–57.
9. Jokinen K, Palva T, Nuutinen J. Chronic bronchitis. A bronchologic evaluation. *ORL J Otorhinolaryngol Ralat Spec.* 1976;38:178–186.
10. Feist JH, Johnson TH, Wilson RJ, et al. Acquired tracheobronchomalacia: etiology and differential diagnosis. *Chest.* 1975;68:340–345.
11. Johnson TH, Mikita JJ, Wilson RJ, et al. Acquired tracheomalacia. *Radiology.* 1973;109:576–580.
12. Iwamoto Y, Miyazawa T, Kurimoto N, et al. Interventional bronchoscopy in the management of airway stenosis due to tracheobronchial tuberculosis. *Chest.* 2004;126:1344–1352.
13. Ferguson GT, Benoist J. Nasal continuous positive airway pressure in the treatment of tracheobronchomalacia. *Am Rev Respir Dis.* 1993;147:457–461.
14. Bolot G, Poupart M, Pignat JC, et al. Self-expanding metal stents for the management of bronchial stenosis and bronchomalacia after lung transplantation. *Laryngoscope.* 1998;108:1230.
15. Susanto I, Peters JI, Levine SM, et al. Use of balloon-expandable metallic stents in the management of bronchial stenosis and bronchomalacia after lung transplantation. *Chest.* 1998;114:1330–1335.
16. Eisner MD, Gordon RL, Webb WR, et al. Pulmonary function improves after expandable metal stent placement for benign airway obstruction. *Chest.* 1999;115:1006–1011.
17. Ernst A, Feller-Kopman D, Becker HD, et al. Central airway obstruction. *Am J Respir Crit Care Med.* 2004;169:1278–1297.
18. Lehman JD, Gordon RL, Kerlan RK Jr, et al. Expandable metallic stents in benign tracheobronchial obstruction. *J Thorac Imaging.* 1998;13:105–115.
19. Bolliger CT. Airway stents. *Semin Respir Crit Care Med.* 1997;18:563–570.
20. Noppen M, Piérard D, Meysman M, et al. Bacterial colonization of central airways after stenting. *Am J Respir Crit Care Med.* 1999;160:672–677.
21. Wright CD, Grillo HC, Hammoud ZT, et al. Tracheoplasty for expiratory collapse of central airways. *Ann Thorac Surg.* 2005;80:259–266.

22. Majid A, Guerrero J, Gangadharan S, et al. Tracheobronchoplasty for severe tracheobronchomalacia: a prospective outcome analysis. *Chest*. 2008;134:801–807.
23. Herzog H, Heitz M, Keller R, et al. Surgical therapy for expiratory collapse of the trachea and large bronchi. In: Grillo HC, Eschapasse H, eds. *International Trends in General Thoracic Surgery (Vol 2)*. Philadelphia, PA: WB Saunders; 1987:74–90.
24. Ernst A, Odell D, Michaud G, et al. Central airway stabilization for tracheobronchomalacia improves quality of life in patients with COPD. *Chest*. 2011;140:1162–1168.
25. Kirchner FR, Toledo PS, Svoboda DJ, et al. Studies of the larynx after teflon injection. *Arch Otolaryng*. 1966;88:74–78.
26. Dedo HH, Urrea RD, Lawson L. Intracordal injection of Teflon in the treatment of 135 patients with dysphonia. *Ann Otol Rhinol Laryngol*. 1973;82:661–667.
27. Chertin E, Colhoun E, Velayudham M, et al. Endoscopic treatment of vesicoureteral reflux. An eleven to seventeen years follow-up. *J Urol*. 2003;170:1541–1547.
28. Chertin E, Puri P. Endoscopic treatment of vesicoureteral reflux: does it stand the test of time? *Eur Urol*. 2002;42:598–606.
29. Fujiwara M, Patino R, Kukes G, et al. Study of the trachea after teflon injection (abstract). *Am J Respir Crit Care Med*. 2012;A68:A2129.
30. Sigma Plot user's guide, SPSS Inc, Chicago, IL; 2001:401–420.
31. Murgu SD, Cherrison LJ, Colt HG. Respiratory failure due to expiratory central airway collapse. *Respir Care*. 2007;52:752–754.