Comparison of the Omentum, Pleura and Diaphragm for Tracheal Autograft Neovascularization in Rats

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Abstract

Study objectives: Pleura, diaphragm, pericardial fat pad, intercostal muscles and omentum can be used to protect and revascularize the bronchial suture line. To compare the efficiency of pleura, diaphragm and omentum, an experimental study was designed.

Design: Heterotopic tracheal autotransplantation was performed in 15 rats.

Interventions: The animals were divided into 3 groups. The heterotopic tracheal segment was transplanted into omentum, diaphragm and pleura cavity.

Measurements and Results: The tracheal Segment Necrosis Scoring System was used for pathologic examinations of the tracheal segment.

The pleural and diaphragmatic groups showed the least necrosis and there was significant statistical difference among these groups and the omental group. ($p<0.05$)

Conclusions: Our study showed that the pleura and diaphragm could be used as safely as omentum for protection and neovascularization of bronchial suture lines.

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Key words: *Neovascularization, Trachea, Experimental*

Introduction

Routine use of additional tissue to patch the bronchial suture line is controversial (1). Coverage of the suture line can revascularize and reduce bronchial complications in complex tracheobronchial surgery, combined therapy for stage IIIA lung cancer (2), closure of the postpneumonectomy bronchopleural fistula (3,4), and lung transplantations (5). Most commonly used structures are neighboring tissues such as pleura, pericardium, pericardial fat, diaphragm, omentum and intercostal muscles (1). Omentum is considered as the best covering material because a large amount is available and it has a good blood supply and plasticity (6). Pleural flaps have found limited use in the thoracic cavity. Pericardial flaps are highly desirable, as being thicker than pleura, and also carrying an inherent blood supply. Diaphragmatic flaps serve as a viable alternative for serratus anterior and latissimus dorsi muscle flaps in complex tracheobronchial reinforcement (2).

The purpose of the present study is to compare the capacity of the pleura, diaphragm and omentum to

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revascularize isolated tracheal segments in the experimental animals.

Material and Methods

Wistar Laboratories rats weighing 250-350mg were used. The animals were in good condition and humane care was given in all cases, in compliance with the guidelines formulated by the National Society for Medical Research, the "Guide for the Care and use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institute of Health. The experimental protocol was approved by the Animal Care Committee of the Marmara University Medical Faculty.

Rats were anesthetized in an induction chamber with halothene (3%) followed by continuous anesthesia with 2%-3% halothene in oxygen with spontaneous ventilation. In a sterile fashion the trachea was exposed and a three-ring section of cervical trachea was excised. Primary tracheal anastomosis was performed. The animals were divided into three groups;

Omental group (n=5): Tracheal autograft implanted into the greater omentum. After a midline laparotomy, the greater omentum was brought out of the peritoneal cavity. The trachea autografts were wrapped in the omentum and fixed by a 4/0 Prolene suture.

Diaphragmatic group (n=5): Tracheal autograft implanted into the diaphragm. After a left subcostal incision tracheal segments were wrapped in the diaphragm by folding the diaphragm over the tracheal segments using 4/0 Prolene sutures.

Pleural group (n=5): Tracheal autograft implanted into the pleura. After a right thoracotomy incision the tracheal segments were placed in the right hemithorax between the parietal and visceral layers of the pleura and the air in the hemithorax was evacuated by an angiocath while closing the incision.

After 14 days rats were sacrificed by injecting propofol intraperitoneally. The tracheal segments were removed with their surrounding tissues and fixed by immersing in the 10% neutral buffered formalin solution. The tissues were embedded in paraffin and 5m-thick cross-sections were cut and stained with hematoxylin and eosin. Histopathological viability was assessed by Nikon Optiphot 2 light microscopy, according to the scale shown in table 1.

Table 1: Tracheal Segment Necrosis Scoring System[a]

	Characteristics
Epithelium	
0	Intact, no ulceration
1	1% - 33% ulcerated
2	34% - 67% ulcerated
3	68% - 100% ulcerated
Submucosa	
0	Normal histology
1	1% - 33% necrosis/necrosis + inflammation
2	34% - 67% necrosis/necrosis + inflammation
3	68% - 100% necrosis/necrosis + inflammation
Cartilage	
0	Normal histology
1	1% - 33% necrosis
2	34% - 67% necrosis
3	68% - 100% necrosis

[a]These individual grades are summed together to give a final score. A score of 0 represents a segment with normal histology. Increasing scores, up to a maximum of 9, indicate increasing degrees of tissue necrosis.

A grade of zero represented normal histology and higher scores indicated increasing degrees of ulceration and necrosis. The scores for each variable were added together to give a tracheal segment necrosis score, with a possible maximum value of 9.

The results were presented as medians and range (maximum-minimum). Statistical analysis was performed using the Instat program on a personal computer. Kruskal-Wallis nonparametric ANOVA test and Dunn's Multiple Comparison test was used to compare the groups. P values less than 0.05 were considered significant.

Table 2: Median and range(maximum-minimum) necrosis scores in each group

Tracheal layers	Omentum	Pleura	Diaphragm
Epithelium	3 (2-3)	0 (0-1)	0 (0-2)
Submucusa	2 (1-2)	1 (0-1)	1 (1-2)
Cartilage	1(0-3)	0(0-0)	0(0-1)
Total	6(3-8)	1(0-2)	1(1-5)

Results

A total of 15 animals underwent heterotopic tracheal autotransplantation. Tracheal segment necrosis scores are summarized in table 2.

One way analysis of variance indicated that there was a significant difference among the groups (p:0.0033). Dunn's Multiple Comparison test showed significant difference between omental and pleural groups but there was no significant difference between pleural and diaphragmatic groups.

We performed the same statistical analysis to compare the ulceration and necrosis in each layer of the trachea between the three groups. The results were statistically worse, that is more necrosis and ulceration, in the omental group, and the best results were achieved in the pleural group.

Discussion:

Wrapping the bronchial suture line or bronchial stumps to protect and revascularize these structures is gaining wide acceptance. Since Morrison (7) reported in 1906 that the greater omentum was capable of revascularizing ischemic tissues and maintaining their viability without an apparent arterial blood supply, it has often been used in thoracic surgery for healing difficult wounds such as bronchial fistulas after lobectomy (8). In lung transplantation, it has been reported that omentopexy improves the healing of bronchial anastomosis markedly (9).

The pleura, azygos vein, pericardium, muscle flaps (2) and diaphragm(3) are the other neighboring structures that are more commonly used than the omentum in tracheobronchial surgery. Our study was planned to compare the effectiveness of these flaps. Although the pleura is thin, and has a delicate nature, due to its close relation and abundance can be used easily in most pulmonary operations. In our study the least necrosis and ulceration were seen in tracheal autografts left in the thoracic cavity.

Diaphragmatic flaps have been used for intrathoracic problems for more than 30 years. They have been successfully employed to reinforce spontaneous esophageal perforations and to close chest wall defects (10). Mineo and Amborgi (3) suggested that in closing postoperative bronchopleural fistulas diaphragmatic flaps should be considered. In our study, the diaphragmatic group had statistically better results than the omental group.

Whether omental wrapping can maintain the viability of tracheal graft is still controversial. Canine studies performed by Balderman and Weinblatt (11) showed negative data. So, additional attempts, like mechanical abrasion or addition of basic fibroblast growth factor were performed to increase tracheal graft viability after wrapping with omentum (5,12).

This is an experimental study in which the omentum, diaphragm or pleura were not dislodged from their original position. In clinical settings the omentum is transported into the thorax, the diaphragmatic flap is created and wrapped around the suture line, or pleural flaps are created and used accordingly. Clinical studies are needed to show the influence of pleura or diaphragm on protection and neovascularization of the suture lines.

In the experimental setup, our study showed that the pleura could be used for protection and neovascularization of the tracheobronchial suture lines as safely as diaphragm and omentum.

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