

## Research Article

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# Efficacy of mistletoe for chemical pleurodesis in rats without malignancy

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**Abstract:** Chemical pleurodesis is an effective treatment modality to reduce recurrence of malignant effusion. Several agents have been used in chemical pleurodesis but, it is not yet clear which is better. Eighteen Sprague-Dawley rats were used and classified into three groups: a group intrapleurally injected normal saline (group A, n=6), 400mg/kg talc (group B, n=6), and 9mg/kg mistletoe extraction (ME) (group C, n=6). Autopsy was performed to evaluate the pleural adhesion, pathologic examination of pleura and lung and bronchoalveolar lavage fluid analysis 4 weeks after pleurodesis. Both group B and C showed an obvious pleural adhesion and there was no significant difference in grade of pleural adhesion between two groups ( $p=0.58$ ). The parietal pleural thickness in talc group than ME group was significantly thicker ( $p=0.002$ ) and the visceral pleura of talc group showed marked foreign body reaction with fibrosis and many multinucleated giant cells associated with talc crystal. This study suggests that pleurodesis using ME in condition without malignancy has comparable effect to pleurodesis using talc. However, additional experimental study in large animal or clinical

trials would be required to prove a safety and an efficacy of pleurodesis using ME.

**Keywords:** chemical pleurodesis, mistletoe extraction, animals

## 1 Introduction

Pleurodesis is a treatment modality to make an adhesion between visceral and parietal pleura and this procedure has the effect to reduce recurrence of malignant effusion, pneumothorax, chylothorax, etc [1-7]. Of procedures for pleurodesis, chemical pleurodesis is known as simply applicable method and several agents have been used in chemical pleurodesis [8-11]. There are tetracycline, doxycycline, bleomycin, talc etc as agents for chemical pleurodesis. Bleomycin is less efficacy in pleurodesis and expansive. In the past, tetracycline was commonly used, but is not currently produced. Of these, talc is known as a very effective agent, however small-particle talc could rarely develop acute respiratory distress syndrome and large-particle talc, known as relatively safe agent for systemic dissemination and potential secondary acute respiratory failures, is not yet available in some countries including Korea. Mistletoe extraction (ME) is an agent to be used in some patients with malignant pleural effusion and stimulation of antitumor immunity has been known as main mechanism of pleurodesis [12-14]. ME consists of mistletoe's lectins which are cytotoxic glycoprotein, visco-toxin which is amino acid peptide, avarious polysaccharides and alkaloids [15].

However, a few reports presented cases for the effect of ME for chemical pleurodesis in patients with non-malignant condition [1,3,4] in addition, the mechanism of ME for chemical pleurodesis is still not clear. We performed an experimental study about the effect of ME for chemical pleurodesis in rats with non-malignant condition and

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compared with effect of pleurodesis using talc which has been known as an effective agent.

## 2 Materials and methods

### 2.1 Animals

Sprague-Dawley female rats of mean weight 252.0 g (range: 220-302 g) were used. All animals received humane care in compliance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institutes of Health (NIH Publication no. 85-23, revised 1985). Twelve hours before surgery, rats were isolated and fasted with free access to water. This study was approved by the Institutional Review Board of the Pusan National University Hospital.

### 2.2 Experimental design

Sprague-Dawley rats were classified into three groups such as below.

Group A was treated with pleurodesis using normal saline

Group B was treated with pleurodesis using talc slurry (400mg/kg)

Group C was treated with pleurodesis using ME (9mg/kg, ABNOVaviscum® Injection)

### 2.3 Experimental procedures

#### 2.3.1 Anesthesia

Anesthesia was induced by intraperitoneal injection of 30 mg/kg of ketamine hydrochloride. Rats were then weighed and placed supine on an operating table and under sterile conditions; a 5mm skin incision was made after local injection of 1% lidocaine over the seventh intercostal space.

#### 2.3.2 Methods of intrapleural injection of agents

ME (9mg/kg) and talc slurry (400mg/kg) were administered via a 16-gauge PTFE catheter into the right pleural space. The presence of air in the pleural space was checked, and if present, was evacuated by using a

three-way stopcock, and then the catheter was removed. The animals were rotated to assure distribution of agent to the entire pleural surface. The control group received intrapleural saline by the same method. The muscles and the skin were closed sequentially with 2-0 silk sutures. The animals were maintained in adequate cages and fed according to the protocol of the animal.

Before we conducted this study, we had performed pilot studies to determine the optimal quantity of ME. We intrapleurally administered 2mg/kg, 6mg/kg, 9mg/kg, or 12mg/kg of ME, but there were no remarkable changes in the pleural cavity of rats that received 2mg/kg or 6mg/kg. We selected the lowest quantity of ME that produced recognizable changes in the pleural cavity. We determined the optimum quantity of talc for pleurodesis according to Muta's report [8].

#### 2.3.3 Measured parameter

Autopsies were performed by one investigator 4 weeks after the administration of each agent. Microscopic and macroscopic examination of lung parenchyma, pleura and pleural symphysis were investigated. After lung retrieval, bronchoalveolar lavage fluid analysis was performed.

##### 2.3.3.1 Gross finding of pleural adhesion

The degree of adhesion observed grossly was graded according to the following scheme: grade 0 - absent adhesion, grade 1 - minimal pleural adhesion, grade 2 - lesser than half pleural adhesion, grade 3 - more than half pleural adhesion, grade 4 - whole pleural adhesion.

##### 2.3.3.2 Pathologic finding

All samples were fixed in 10% buffered formalin, embedded in paraffin, cut with 4µm thickness and stained with hematoxylin and eosin (H&E) stain. The thickness of the visceral pleura and parietal pleura was measured with ocular micrometer. And the degree of inflammation of the lung parenchyma was evaluated. The intensity of inflammation was graded as 0 (no inflammation), 1 (mild), 2 (moderate) and 3 (marked).

##### 2.3.3.3 Bronchoalveolar lavage fluid (BALF) analysis

Collection of BALF was performed through a tracheal cannula by washing twice with 2ml saline. The total cell was determined using standard hematological procedures in 500 µl each sample. The total cell number was counted

by using hemocytometer. The remainder fluid was centrifuged at 900 xg for 5 min at 4 °C. The cell pellets were resuspended in PBS and slides were centrifuged by using a cytospin. Neutrophil was examined by counting at least 400 cells on smear prepared by using Wright-Geimas staining (Sigma-Aldrich, St. Louis, MO, USA). Total protein concentration in the BALF was measured with the WelProt™ Protein Assay kit (Welgene, Daegu, Korea) according to the manufacturer's instructions.

**Ethical approval:** The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

## 3 Results

### 3.1 Gross finding and degree of pleural adhesion

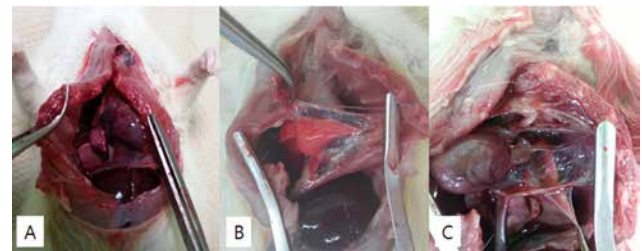
All of group A (n=6, saline group) showed grade 0 of pleural adhesion in gross finding. Of group B (n=6, talc group), five rats showed grade 3 and one showed grade 2. Of group C (n=6, ME group), four rats showed grade 3 and two showed grade 2 (Table 1) (Figure 1). Both group B and C showed a significant pleural adhesion and there was no significant difference in grade of pleural adhesion between two groups ( $p=0.58$ ).

### 3.2 Change of pleural and lung parenchyma

Visceral and parietal pleural thickness was observed on microscopic examination in group B and C and parietal pleura was thicker than visceral pleura in group B and C. The visceral and parietal pleural thickness in talc group

was significantly thicker than ME group ( $p=0.008$  and  $p=0.002$ ) and obvious pleural mesothelial reaction of pleura was observed in group B (Table 1). Especially, the visceral pleura of talc group showed marked foreign body reaction with fibrosis and many multinucleated giant cells associated with talc crystal (Figure 2). These injuries were not seen in the ME group and the pleura of ME group just showed chronic inflammation with fibrosis and mesothelial reaction.

To identify damage of lung parenchyma, analysis of BALF and microscopic evaluation were performed. In analysis of BALF, neutrophil count and protein were

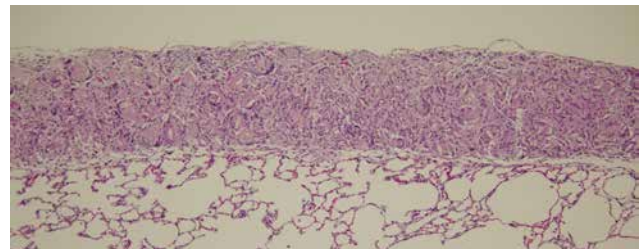


**Figure 1:** The pleural adhesion in gross findings

A. Group A (n=6, saline group) showed grade 0 of pleural adhesion.

B. Group B (n=6, talc group), five rats showed grade 3 of pleural adhesion.

C. Group C (n=6, Mistletoe extraction group), four rats showed grade 3 of pleural adhesion.

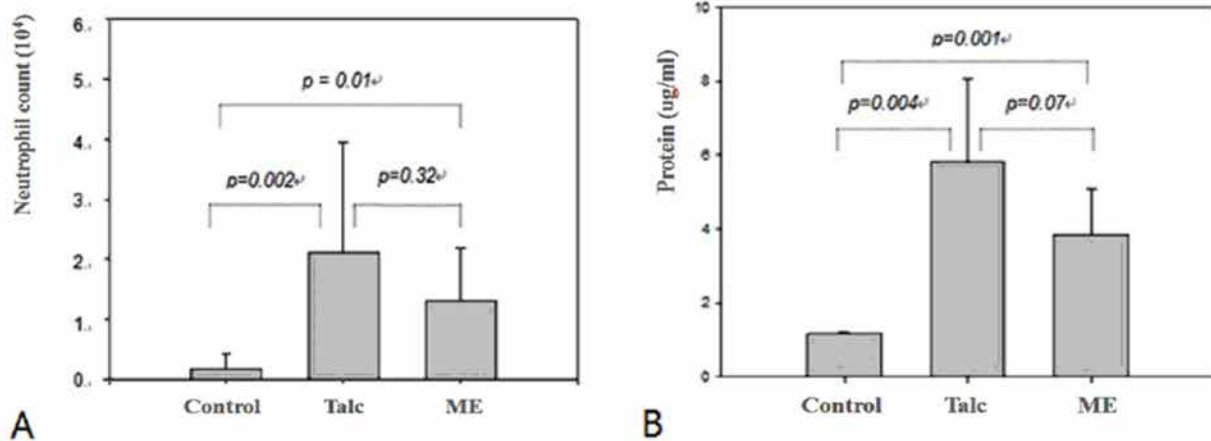


**Figure 2:** Pathologic findings of visceral pleura in talc group X100, This section shows no inflammatory reaction at the lung parenchyma. The visceral pleura is thickened due to foreign body giant cell reaction to talc crystal and fibrosis.

**Table 1:** Macroscopic and microscopic findings of pleural adhesion and thickness after pleurodesis

	Control (n=6)	Talc (n=6)	Mistletoe extraction (n=6)	<i>p</i> value*
Grade of adhesion	0	2.83	2.67	$P=0.58$
degree of visceral pleural thickening( $\mu$ m)	10	68.33 $\pm$ 69.11	60.0 $\pm$ 21.91	$P=0.008$
degree of parietal pleural thickening( $\mu$ m)	10	541.67 $\pm$ 294.98	210.0 $\pm$ 73.77	$P=0.002$

\* *p* value to compare between Talc group and Mistletoe extraction group

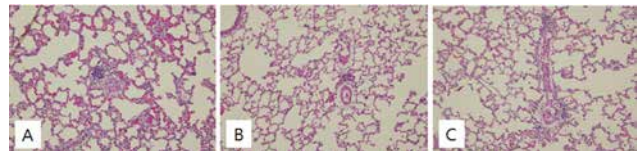


**Figure 3:** Bronchoalveolar lavage fluid analysis

A. The mean neutrophil count in the control group, talc group, and Mistletoe extraction (ME) group were  $0.17 \pm 0.25 \times 10^4$ ,  $2.11 \pm 1.84 \times 10^4$ , and  $1.31 \pm 0.86 \times 10^4$ , respectively. The neutrophil count in the talc group and ME group was significantly higher than that in the control group ( $p=0.002$  and  $0.01$ ) and there is no significant difference between talc and ME group ( $p=0.32$ ).

B. The mean protein concentration in the control group, talc group, and Mistletoe extraction (ME) group were  $1.21 \pm 0.2 \mu\text{g/ml}$ ,  $5.81 \pm 2.26 \mu\text{g/ml}$ , and  $3.85 \pm 1.26 \mu\text{g/ml}$ , respectively. The protein concentration in the talc group and ME group was significantly higher than that in the control group ( $p=0.004$  and  $p=0.001$ ) and there is no significant difference between talc

significantly increased in group B and C comparing with group A and there is no significant difference between group B and C (Figure 3). However, pathologically, inflammation of lung parenchyma in all group was not serious and mean degree of lung inflammation in control, talc, and ME group was 1.25, 1.00, and 1.17, respectively and there was no significant difference of intensity of inflammation in all group ( $p=0.82$ ) (Figure 4).



**Figure 4:** Pathologic findings of lung parenchyma

A. Control group, Mild inflammation, x200

B. Talc group, Mild inflammation, x200

C. Mistletoe extraction group, Mild inflammation, x200

### 3.3 Miscellaneous findings

Reactive pleural effusion was observed on radiologic image in some rats of ME group and some of them needed additional pleural drainage on third day after pleurodesis due to tachypnea and mean amount of drainage was 13.6ml (Figure 5).



**Figure 5:** The images by computed tomography in Mistletoe extraction group

A,B. The coronal plane of computed tomography shows pleural effusion in fissures (arrow) which could make lung atelectasis (triangle).

C. The horizontal plane of computed tomography also shows pleural effusion in fissures (asterisk).

## 4 Discussion

Of chemical agents for pleurodesis, authors compared mistletoe extraction with talc to investigate the efficacy of mistletoe extraction (ME, *Viscum album* L.) for pleurodesis in this experimental study. First, of agents for chemical pleurodesis, talc has been known as a clinically effective agent of pleurodesis [6,7], although it is debatable for its

safety. There are many experimental and clinical studies about safety of talc pleurodesis. Werebe et al. reported that talc crystals were found in every organ of all of the animals, which suggests a rapid absorption of talc through the pleural surface. They suggested that talc should therefore be avoided in young patients with benign diseases, and even in the majority with malignant diseases with a



very good prognosis [16]. But, Fraticelli *et al.* reported that calibrated talc would be required in case of intrapleural administration for pleurodesis to avoid systemic dissemination and potential secondary acute respiratory failures [17] and Janssen *et al.* reported that the use of large-particle talc for pleurodesis in malignant pleural effusion would be safe and not associated with the development of ARDS [18]. However, unfortunately, large-particle talc was not yet available in some countries including Korea.

Small-particle talc was used in this study. On macroscopic finding, most cases of pleurodesis using talc slurry showed high grade of pleural adhesion. Pathologically, the visceral pleura of talc group showed marked foreign body reaction with fibrosis and many multinucleated giant cells associated with talc crystal and many talc crystals were observed in thickened pleura of talc group rats. Although the talc crystal was not found in the lung parenchyma in this study, it could be systemically distributed by a rapid absorption through the pleural surface [16], and this is one of reasons that authors don't perform the talc pleurodesis in patients with benign disease or malignancy expecting good prognosis.

In ME group, grade of pleural adhesion similar to talc group was observed on macroscopic evaluation and chronic inflammation with fibrosis of visceral pleural was observed on microscopic evaluation, but marked foreign body reaction with fibrosis and many multinucleated giant cells associated with foreign body was not detected. In addition, ME (*Viscum album L.*) has been also known as an immune modulator and other positive effects, and there was no clinically significant toxic effect in patients with malignancy [9-11,19,20].

In conclusion, this study suggests that pleurodesis using ME in condition without malignancy has comparable effect to pleurodesis using talc and is not accompanied by serious problem such as accumulation of talc crystal showed in talc pleurodesis. Additional experimental study in large animal or clinical trials would be required to prove a safety and an efficacy of pleurodesis using ME in patients with benign disease or malignancy expecting good prognosis.

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**Conflict of Interest:** The authors declare that they have no conflict of interests.

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