Silver Nitrate Is Superior to Talc Slurry in Producing Pleurodesis in Rabbits*  

Francisco S. Vargas, MD, FCCP; Lisete R. Teixeira, MD; Marcelo A.C. Vaz, MD; Alipio O. Carmo, PharmD; Evaldo Marchi, MD; Patricia M. Cury, MD; and Richard W. Light, MD, FCCP

Study objective: The ideal agent for producing pleurodesis has not been identified. Although talc is the agent most commonly used at the present time, there are concerns about its safety. Silver nitrate is a possible alternative agent. The purpose of the present study was to compare the effectiveness of intrapleural silver nitrate and talc slurry in producing pleurodesis in rabbits. Additionally, the total amount of pleural collagen and the distribution of thick and thin collagen fibers were studied.

Design: Two groups of 10 rabbits received either 0.50% silver nitrate or 400 mg/kg talc in a total volume of 2 mL intrapleurally. The animals were killed 28 days after injection, and the pleural spaces were assessed grossly for evidence of pleurodesis and microscopically for evidence of inflammation and fibrosis. Collagen was assessed with the van Gieson’s and picrosirius stains.

Results: The macroscopic pleurodesis (scale, 0 to 4; mean ± SEM) resulting from the intrapleural injection of silver nitrate (3.4 ± 0.2) was significantly better (p < 0.001) than that resulting from talc (1.6 ± 0.1). The mean degree of microscopic pleural fibrosis induced by silver nitrate (3.3 ± 0.3) was significantly higher (p = 0.003) than that induced by talc (1.8 ± 0.1). The mean amount of microscopic pleural collagen (van Gieson’s) was significantly greater (p < 0.001) in the rabbits that received silver nitrate (3.0 ± 0.2) than in those that received talc (1.6 ± 0.2). The distribution of thick and thin collagen fibers did not differ between the groups.

Conclusions: We conclude that, in our rabbit model, intrapleural silver nitrate was more effective than talc in producing a pleurodesis. (CHEST 2000; 118:808–813)

Key words: collagen; pleural effusion; pleurodesis; picrosirius; silver nitrate; talc

Currently, talc is accepted by many as the agent of choice for creating a pleurodesis in patients with either a pneumothorax or a malignant pleural effusion. Talc became popular for several reasons: (1) in the experimental situation, it is effective in producing a pleurodesis; (2) in humans, it appears to be more effective than the tetracycline derivatives or chemotherapeutic agents; (3) in humans, its administration does not interfere with subsequent chemotherapy; (4) it is widely available; and finally, (5) it is less expensive than other sclerosing agents. However, there are serious concerns about its safety. ARDS, necessitating the use of mechanical ventilation, develops in 8% of patients who are given talc intrapleurally. The overall mortality related to the intrapleural administration of talc appears to be about 1%. Accordingly, the search for a better agent continues.

For editorial comment see page 577

Silver nitrate appears to be a possible candidate for a better agent. It is inexpensive and widely available throughout the world. Prior studies demonstrated that silver nitrate was very effective at...
creating a pleurodesis in patients with a pneumotho-
ran.7 However, its use was essentially abandoned in the mid-1980s because of severe side effects, mainly chest pain requiring large doses of opiates, and because of the formation of large amounts of pleural fluid requiring thoracentesis.8 We theorized that the concentrations of silver nitrate used previously (1 to 10%) were too high and led to severe injury of the pleura, as evidenced by the intense chest pain and large amounts of pleural fluid. We therefore studied the effectiveness of lower concentrations of silver nitrate in producing pleurodesis. We demonstrated that the intrapleural administration of 2 mL of 0.5% silver nitrate produced a pleurodesis in rabbits that was comparable to that produced by 35 mg/kg tetracycline.9

The purpose of the present study was to compare the gross and microscopic effects of the intrapleural injection of 0.5% silver nitrate and 400 mg/kg talc slurry, with particular attention to the distribution and characterization of collagen. We hypothesized that the silver nitrate would be at least as effective as talc slurry in producing a pleurodesis. We further hypothesized that the pleurodesis resulting from silver nitrate would be characterized by a higher percentage of mature collagen fibers. We reasoned that the pleurodesis resulting from talc would have more immature collagen fibers because talc remains in the pleural space for prolonged periods and presumably continues to act as an irritant, which could result in the continuing production of collagen.

Materials and Methods

The rabbits were divided into two groups of 10 rabbits. One group received silver nitrate 0.5% (Merck; Darmstadt, Germany), whereas the other received sterilized talc slurry, 400 mg/kg (Xilolite; Salvador, Brazil), each in a total volume of 2 mL. The talc was asbestos-free and had a mean particle diameter of 25.4 µm, with the 10th percentile being 6.4 µm and the 90th percentile being 50.5 µm. The talc was contaminated with small amounts of dolomite, kaolinite, chlorite, and forsterite. The talc slurry was gently shaken immediately before injection. Rabbits were killed 28 days after injection by a lethal injection of pentobarbital into the marginal ear vein. The thorax was removed en bloc. The lung was expanded by injection of 10% formalin intratracheally. After tracheal injection, the trachea was ligated, and the entire thorax was submerged in 10% neutral buffered formalin for at least 48 h.

The necropsy was performed by one of the investigators (L.R.T.), who was blinded to the treatment group. Each pleural cavity was carefully exposed by making bilateral incisions through the diaphragms and through all the ribs in approximately the midclavicular line. The sternum and the medial portions of the anterior ribs were removed to allow evaluation of the pleural cavities. The left hemithorax received no injection and served as a control.

The degree of gross pleurodesis was graded according to the following scheme: 0 = normal; 1 = less than three adhesions; 2 = more than three adhesions, but localized; 3 = generalized scattered adhesions; and 4 = complete obliteration of pleural space by the adhesions.

The presence of hemothorax was graded on a scale of 0 to 4, with 0 indicating no hemothorax, 1 indicating a hemothorax that involved < 15% of the hemithorax, 2 indicating involvement from 15 to 33%, 3 indicating involvement from 33 to 75%, and 4 indicating involvement of 75% of the hemithorax. At gross examination, the diagnosis of hemothorax was made if there were blood clots in the pleural space. Atelectasis was classified as none (0), partial (1), or complete (2). Mediastinal shift was classified as none (0), slight (1), moderate (2), or severe (3).

Samples of the visceral pleura and lung from each hemithorax were obtained and placed in 10% neutral buffered formalin. These tissue samples were processed routinely and stained with hematoxylin-eosin, picrosirius,10 and van Gieson’s11 stains. These tissue samples were processed routinely and stained with hematoxylin-eosin, picrosirius,10 and van Gieson’s11 stains. The degree of microscopic inflammation and fibrosis from the hematoxylin-eosin slide and the total collagen from the van Gieson’s slide were graded by one of us (L.R.T.), who was blinded to the treatment. The inflammation and fibrosis were graded as none (0), equivocal (1), mild (2), moderate (3), or severe (4). Collagen fibers were subdivided into thick fibers and thin fibers using picrosirius staining, as reported by Andrade et al.10 The tissue sections were stained for 1 h in a 0.2% solution of Sirius Red, Direct Red 80 (Aldrich; Milwaukee, WI). The enhancement of collagen birefringence elicited by picrosirius staining is specific for collagen. Thin (immature) fibers are weakly birefringent and green, whereas thick (mature) fibers are strongly birefringent and yellow.12 For this study, the percentage of immature thin fibers was graded as 1 (0 to 25%), 2 (26 to 50%), 3 (51 to 75%), or 4 (76 to 100%).

Statistical Analysis

All data are expressed as the mean ± SEM. The mean scores for pleurodesis, microscopic fibrosis, inflammation, collagen, and collagen fiber type in the two different treatment groups were compared using unpaired t test analysis. When the data failed tests of normality, the Mann–Whitney rank sum test was used to compare the median values in the two treatment groups. Differences were considered significant when p < 0.05.

Results

The intrapleural instillation of silver nitrate resulted in a significantly greater degree of pleurodesis than did talc slurry (Table 1). The mean pleurodesis
score after the intrapleural injection of 0.50% silver nitrate (3.4 ± 0.2) was significantly higher (p < 0.001) than that obtained after talc (1.6 ± 0.1; Fig 1). Eight of nine rabbits (89%) that received silver nitrate 0.50% had an effective pleurodesis (score 3 or 4), and no rabbit that received talc had a score.

The scores on the left hemithorax were 0 for all the animals in both groups, except one animal in the silver nitrate group that had a score of 2 (Table 2).

When the pleura was examined microscopically, the intrapleural administration of silver nitrate also resulted in more fibrosis (Table 1). The degree of pleural fibrosis after the administration of 0.50% silver nitrate (3.3 ± 0.3) was significantly greater (p = 0.003) than that after administration of talc (1.8 ± 0.1; Fig 2). The degree of pleural inflammation on the injected side was similar in both groups (Table 1). The mean degree of alveolar inflammation and fibrosis was also significantly higher in the silver nitrate group than in the talc group (Table 1). On the control side, rabbits in the silver nitrate group had significantly more pleural fibrosis and inflammation (Table 2), but in only one rabbit did the score exceed 1, and that was a fibrosis score of 3.

The intrapleural injection of silver nitrate resulted in the production of significantly more collagen than did the intrapleural injection of talc (Table 1). The mean total collagen score in the animals that received silver nitrate (3.0 ± 0.2) was significantly greater than that in the talc group (1.6 ± 0.2; p < 0.001; Fig 3). There was no difference in the collagen subtypes between the groups (Table 1). On the control side, there was no observable collagen in the rabbits that received talc and only a small amount (0.3 ± 0.5) in the rabbits that received silver nitrate (Table 2).

The intrapleural administration of either silver nitrate or talc slurry did not cause distress in any of the rabbits.

---

**Table 1—Results From Macroscopic and Microscopic Examination of the Right Side After Intrapleural Instillation of Sclerosant**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Silver Nitrate</th>
<th>Talc</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleurodesis score (macroscopic)</td>
<td>3.4 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pleural fibrosis</td>
<td>3.3 ± 0.3</td>
<td>1.8 ± 0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Pleural inflammation</td>
<td>2.1 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>0.152</td>
</tr>
<tr>
<td>Total collagen</td>
<td>3.0 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Thin collagen fibers</td>
<td>1.9 ± 0.2</td>
<td>1.7 ± 0.1</td>
<td>0.592</td>
</tr>
<tr>
<td>Thick collagen fibers</td>
<td>2.1 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>0.592</td>
</tr>
<tr>
<td>Right side mediastinal shift</td>
<td>1.6 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Hemothorax</td>
<td>0.6 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>0.430</td>
</tr>
<tr>
<td>Atelectasis</td>
<td>2.1 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>0.005</td>
</tr>
<tr>
<td>Alveolar inflammation</td>
<td>2.1 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Alveolar fibrosis</td>
<td>1.1 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Values are reported as mean ± SEM.

**Table 2—Results From Macroscopic and Microscopic Examination of the Left Side (Control) After Intrapleural Instillation of Sclerosant on Right Side**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Silver Nitrate</th>
<th>Talc</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleurodesis score (macroscopic)</td>
<td>0.2 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.71</td>
</tr>
<tr>
<td>Pleural fibrosis</td>
<td>0.9 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.015</td>
</tr>
<tr>
<td>Pleural inflammation</td>
<td>0.9 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Total collagen</td>
<td>0.3 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.23</td>
</tr>
<tr>
<td>Left side mediastinal shift</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.97</td>
</tr>
<tr>
<td>Hemothorax</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Atelectasis</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Alveolar inflammation</td>
<td>0.8 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Alveolar fibrosis</td>
<td>0.1 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*Values are reported as mean ± SEM.

---

**Figure 1.** Comparison of pleurodesis score on right side in talc and silver nitrate groups (mean ± SEM).

**Figure 2.** Comparison of pleural fibrosis score on right side in talc and silver nitrate groups (mean ± SEM).
the animals. One rabbit in the silver nitrate group died during the initial anesthesia. All the other rabbits rapidly regained a normal feeding pattern and resumed normal activities after the injection. None of the rabbits required any medication for vocalization, tachypnea, or restlessness. The mean weight gain in the rabbits that received silver nitrate (0.53 ± 0.13 kg) and those that received talc slurry (0.65 ± 0.03 kg) did not differ significantly.

The intrapleural injection of silver nitrate resulted in significantly more mediastinal shift than did the intrapleural injection of talc slurry (Table 1). The only two rabbits with a hemothorax (one with a grade 2 and one with a grade 3) were in the silver nitrate group. Six of the 10 rabbits that received silver nitrate had grade 2 or higher atelectasis of the ipsilateral lung, whereas none of the rabbits that received talc had any atelectasis. On the control side, no rabbit in either group had atelectasis or hemothorax.

**DISCUSSION**

The present study demonstrates that in this rabbit model, silver nitrate at a concentration of 0.5% is more effective than talc, 400 mg/kg, in inducing pleurodesis. Microscopically, silver nitrate stimulates the production of more fibrosis and more collagen, but the proportion of thin and thick collagen fibers in the two groups is similar.

Conventional pleurodesing agents induce pleurodesis by creating an injury to the pleura, which leads to pleural fibrosis and fusion of the visceral and parietal pleura. The events that transpire between the insult to the pleura and the progression to fibrosis are not completely understood. The evolution of tissue repair is complex and involves a number of interrelated processes, including acute inflammatory reaction to the injury (day 1), regeneration of the damaged cells, migration of connective tissue cells to the damaged area (days 3 to 5), synthesis of extracellular matrix proteins (days to several weeks), and collagenization with acquisition of wound strength (progressive from the first week to several weeks). Collagen is probably the most important protein in the production of a pleurodesis. Collagen synthesis occurs primarily in fibroblasts. The initial step in its production is synthesis of collagen propeptides (α-chains). While the propeptides are still intracellular, they undergo several modifications and aggregate to form procollagen molecules, which are characterized by their three-chain helices. In this phase, the procollagen is still soluble and remains intracellular. The procollagen molecules are subsequently secreted, and once extracellular, they aggregate to form fibrils called tropocollagen, which are insoluble. Accompanying fibril formation is the oxidation of specific residues resulting in cross-linkages between α-chains of adjacent tropocollagen molecules and the formation of thin immature collagen fibers. These thin collagen fibers mature by reacting with other collagen fibers and losing water to become thick mature collagen fibers. The thick collagen fibers are responsible for the resistance of the visceral pleura to stretching, which gives strength to the pleurodesis.

Whether a pleural injury results in pleurodesis or normal tissue repair depends on several interrelated factors. First, the degree of injury is important—the greater the injury, the more likely one is to develop pleurodesis. Second, mesothelial cells are thought to play a critical role in the repair process that follows serosal injury. Mesothelial cells can secrete collagen, enzymes such as metalloproteinases that degrade collagen, and inhibitors of the metalloproteinases. We theorize that whether pleurodesis or normal tissue repair occurs depends on the balance between procollagen and anticollagen factors; pleurodesis will occur when the procollagen factors are predominant. Third, the duration, extent, and intensity of the inflammation will influence the end result as shown by the facts that pleurodesis in rabbits from intrapleural talc is inhibited by systemic corticosteroids and that the intrapleural administration of tumor necrosis factor-α blocking antibodies can also reduce the degree of pleurodesis after intrapleural talc.

The search for a better sclerosing agent continues, because all of the available agents have significant drawbacks. Talc appears to be the most effective agent, but there are concerns about its safety.

![Figure 3. Comparison of total collagen score on right side in talc and silver nitrate groups (mean ± SEM).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21952/)

Figure 3. Comparison of total collagen score on right side in talc and silver nitrate groups (mean ± SEM).

* p < 0.001
Bleomycin is less effective than the other sclerosing agents, and the tetracycline derivatives at times induce a great amount of pain. The primary advantages of silver nitrate are that it is inexpensive and widely available. The present study confirms our previous observation that the intrapleural injection of 2 mL of 0.5% silver nitrate is effective at producing a pleurodesis.\(^9\) The mean degree of pleurodesis in the present study (3.4) was identical to that reported in the previous study.\(^9\)

In the present study, silver nitrate was significantly better than talc, 400 mg/kg, in producing a pleurodesis. The mean degree of pleurodesis after the talc in the present study was less than has been reported previously with the same dose and methodology for talc slurry.\(^19\) It is possible that the differences in the results are related to the talc itself, inasmuch as it is known that talc preparations vary widely from country to country.\(^18,20\)

We had hypothesized that the pleurodesis resulting from silver nitrate would be characterized by a higher percentage of mature collagen fibers, because the talc persists in the pleural space and would be expected to incite continued pleural inflammation and the production of additional immature collagen. However, we could find no support for this hypothesis from the present study. Although the total amount of collagen was significantly greater in the rabbits that received silver nitrate than in those that received talc, there was no difference in the proportion of thin (immature) and thick (mature) collagen. It should be noted that, when the level of inflammation after the two different agents was assessed 28 days after injection (Table 1), the degree of pleural inflammation was actually greater after the silver nitrate than it was after the talc. It is possible that, if we had studied our animals at a later time (eg, 60 days), there would have been a difference in the proportion of thin and thick collagen fibers.

The use of silver nitrate for pleurodesis was abandoned in the past because of the severe side effects associated with its use. The present study suggests that the intrapleural administration of this lower concentration of silver nitrate produces few side effects. None of the rabbits appeared distressed after injection of silver nitrate. All of the rabbits rapidly regained a normal feeding pattern and resumed normal activities after injection. Moreover, the weight gain in both groups was similar. The presence of the mediastinal shift and atelectasis and the occurrence of two hemothoraces in the silver nitrate group were worrisome at first glance. However, it should be noted that these changes were less than those observed after the intrapleural administration of the tetracycline derivatives.\(^8\) With the tetracycline derivatives, these complications can be prevented if the animals are treated with chest tubes. The rabbits in the present study did not receive chest tubes.

In conclusion, this study demonstrated that a low dose of silver nitrate (0.5%) is more effective than talc, 400 mg/kg, in producing a pleurodesis in rabbits. The administration of silver nitrate produces a greater degree of macroscopic and microscopic fibrosis and is associated with the presence of more collagen. Silver nitrate is inexpensive, widely available, and produces an effective pleurodesis without significant side effects in rabbits. The efficacy and safety of silver nitrate as a pleurodesis agent should be compared with talc in randomized controlled studies in humans.

ACKNOWLEDGMENT: The authors thank Drs. Y.C. (Gary) Lee and Paul Branca and Ms. Sheila Rupp for their careful review of the article.

REFERENCES


