Lung Damage in Experimental Pleurodesis Induced by Silver Nitrate or Talc*
1-Year Follow-up

Francisco S. Vargas, MD, FCCP; Leila Antonangelo, MD; Vera Capelozzi, MD; Marcelo A.C. Vaz, MD; Eduardo H. Genofre, MD; Evaldo Marchi, MD, FCCP; and Lisete R. Teixeira, MD

Study objective: To compare the lung damage caused by intrapleural silver nitrate (SN) with that caused by talc over a 12-month period.

Design: One hundred forty rabbits received an intrapleural injection of 0.5% SN or 400 mg/kg talc slurry in 2 mL saline solution. Groups of 10 rabbits were killed after 1, 2, 4, 6, 8, 10, or 12 months. The macroscopic pleurodesis, microscopic lung changes (ie, collapse, hemorrhage, and edema), and cellular infiltrates (number and proportion of cells) were graded on a scale of 0 to 4.

Results: The mean (± SEM) adhesion score after SN injection (3.3 ± 0.1) was higher (p < 0.001) than that after talc injection (2.3 ± 0.1). The mean alveolar collapse score was greater (p < 0.001) 1 month after SN injection (2.2 ± 0.3) than after talc injection (0.2 ± 0.1) and was similar from the second month on. The degree of parenchymal hemorrhage, by alveolar collapse score, (SN injection, 0.2 ± 0.1; talc injection, 0.2 ± 0.0) and edema (SN injection, 0.4 ± 0.1; talc injection, 0.3 ± 0.1) was minimal in both groups (p > 0.05). One month after the injection, the total number of inflammatory cells was greater (p < 0.001) in rabbits that had received SN injections (2.7 ± 0.3) than in those that had received talc injections (1.2 ± 0.1). From the second month on, cellularity decreased and became similar in both groups. The cellular profile was different, with a predominantly neutrophilic reaction after talc injection and a predominantly eosinophilic reaction after SN injection.

Conclusions: Rabbits injected with intrapleural 0.5% SN had significantly higher scores for adhesions than did those that had received talc injections, with mild and reversible alveolar collapse and an eosinophilic responses, conditions showing a clear tendency to normalize with time.

Key words: lung damage; pleural effusion; pleurodesis; silver nitrate; talc

Abbreviation: SN = silver nitrate

The ideal sclerosing agent for inducing effective pleurodesis has not yet been identified. Ideally, it should be easily administered, safe, inexpensive, and widely available. None of the agents presently used meet all of these criteria. The agent most commonly used is talc, which has been accepted as the sclerosant of choice for the local treatment of recurrent pleural diseases. Talc is effective in humans (success rate, approximately 90%) and in experimental studies. However, serious concerns exist about its safety, and evidence is available that the intrapleural administration of talc induces ARDS in up to 8% of patients, with an overall mortality rate of 1.4.

Silver nitrate (SN) has been suggested in experimental studies to be an effective agent for inducing pleurodesis. It had been used with success in pa-
tients with pneumothorax, but was abandoned in the 1980s for no clear reason. The morbidity observed after SN injections apparently was related to the high concentrations used (ie, 1 to 10%). However, in our animal model, the effectiveness at a lower concentration (0.5%) was comparable to that produced by tetracycline and was superior to that induced by talc. For these reasons, SN can be considered for use in humans. However, it is a substance (pH, 5.5) with irritative and caustic properties, so we must recognize and study the possibility of lung damage before its use.

The purpose of this study was to evaluate lung damage during a 12-month follow-up of intrapleural injections of SN or talc.

**Materials and Methods**

This study was approved by the Ethics Committee of the Heart Institute (InCor) at the University of São Paulo Medical School, and the methods are similar to those previously described. New Zealand white rabbits (weight range, 2.0 to 2.5 kg) were anesthetized, and, under aseptic conditions, a 2-cm skin incision was made in the right hemithorax. The intercostal muscles were dissected, and, under direct vision, a 25-gauge needle was inserted into the pleural space and the sclerosant was injected. In sequence, the muscles and the skin were closed with sutures. After surgery, the rabbits were monitored, and, when necessary, they received buprenorphine subcutaneously.

The rabbits were divided into two groups of 70 rabbits each. One group received 2 mL 0.5% SN (pH, 5.3), and the other received 400 mg/kg sterilized talc slurry (pH, 8.4) in a total volume of 2 mL. Brazilian talc is asbestos-free and has a mean particle diameter of 25.4 μm (10th percentile, 6.4 μm; 90th percentile, 50.5 μm). Ten rabbits from each group were killed after 1, 2, 4, 6, 8, 10, or 12 months by an IV lethal injection of pentobarbital. The thorax was removed en bloc, and the expanded lungs were submerged in a 10% formalin solution for at least 48 h. The liver and spleen were removed for further examination.

Necropsy was performed by one of the investigators (LRT), who was blinded to the treatment received by the animals. The right pleural cavity was exposed, and the sternum and ribs were removed so that the lung could be evaluated. The gross pleural adhesions were classified into four grades: 1, fewer than three adhesions; 2, more than three adhesions, but fewer than five; 3, more than five adhesions; and 4, severe) according to the extension and severity of the histopathologic lesions present in the lung tissue. The differential cell count was performed by examining 400 cells and differentiating between polymorphonuclear (ie, neutrophils plus eosinophils) and mononuclear cells (ie, macrophages plus lymphocytes plus plasma cells). Use of a neutrophilic infiltrate was considered when >50% of nucleated cells were neutrophils, and use of an eosinophilic infiltrate was considered when >10% were eosinophils. The left hemithorax was used as a control.

**Statistical Analysis**

All data are expressed as the mean ± SEM. The mean scores in the two different treatment groups were compared with unpaired t test analysis. When the data failed the normality test, the nonparametric Mann-Whitney rank sum test was used to compare the values. The temporal evolution was compared with the one-way analysis of variance. Differences were considered to be significant at p < 0.05.

**Results**

The intrapleural administration either of SN or talc slurry did not cause distress in any of the animals. They rapidly regained normal feeding and returned to normal activities. None of the rabbits required any additional treatment. Deposits of talc in the pleural cavity were seen in rabbits from all groups.

The intrapleural instillation of SN resulted in a significantly greater degree of pleurodesis than did that with the talc slurry. The mean adhesion score after the administration of intrapleural SN (3.3 ± 0.1) was significantly higher than that observed after talc administration (2.3 ± 0.1; p < 0.001). It was significantly higher (p < 0.05) at each time point in the SN group except at 2 months (Table 1, Fig 1).

All lungs showed changes in the airspace configuration (ie, alveolar collapse), alveolar capillary permeability (ie, edema and hemorrhage), cellular components of the inflammatory reaction (ie, neutrophils, eosinophils, and mononucleated cells), and reparative process (ie, fibroblastic cells). The extension and distribution of the parenchymal changes were not homogeneous throughout the pulmonary tissue. Table 1 and Figure 1 show the mean values (± SE) and the temporal evolution of the subjective and semiquantitative evaluation of the histologic parameters in the lung tissue after talc or SN administration.

The mean alveolar collapse score was higher after SN administration but had statistical differences only 1 month after injection (p < 0.05). Parenchymal hemorrhage and edema were detected in small and similar amounts in all groups during the 12 months of the study.

The total number of inflammatory cells increased significantly (p < 0.05) in the SN group 1 month after injection, but after this time the cellularity decreased progressively, and achieved similar amounts and distribution in both groups.

www.chestjournal.org
Differential quantification of inflammatory cells showed statistically significant differences in both groups. In this context, a temporal decline curve was observed, with a predominance of neutrophils after talc and eosinophils after SN administration. All livers and spleens collected after intrapleural talc or SN administration were considered to be structurally normal with no inflammatory changes.

**Discussion**

The present study demonstrates that in this rabbit model, 0.5% SN produced more intrapleural adhesions than did talc (400 mg/kg). It also shows that 1 month after the intrapleural injection of SN, the lung architecture was mildly and reversibly affected with a discrete alveolar collapse and minimal signs of hemorrhage and edema. Two months after SN injection, the parenchymal changes were minimal and similar to those observed after talc injection. The local inflammatory responses were different after talc and SN administration. The cellular infiltrate was neutrophilic after talc administration, and eosinophilic after SN administration. The eosinophilic response observed after SN administration tended to be more pronounced in regions near the injection site of the agent, probably reflecting a late phase of a locally induced type I hypersensitivity reaction. No systemic inflammatory changes were observed either after talc or SN administration. Livers and spleens were considered to be histologically normal.

The mechanisms responsible for pleurodesis are not completely understood. It is accepted that the first events are the injury manifested by the denudation of the mesothelial cells and the development of an exudative pleural effusion. The reparative phase is complex and involves the inflammatory reaction to injury, regeneration of the damaged cells, migration of connective tissue cells, synthesis of extracellular matrix proteins, and collagenization with the acquisition of wound strength.

Several factors should be considered regarding the process occurring from the instillation of the sclerosing agent to the tissue repair or the development of a pleural symphysis. The degree of pleurodesis caused by the sclerosant depends on a series of events including the capacity to secrete or degrade collagen (i.e., mesothelial cells, enzymes as metalloproteinases and enzymatic inhibitors). The duration, extension, and intensity of the inflammatory response could influence the result. Talc pleurodesis is inhibited by corticosteroids and by tumor necrosis factor-α-blocking antibodies in experimental models.

The ideal sclerosing agent has not been identified. All of the available agents have significant disadvantages. Talc is effective, but concerns exist about its safety. SN is inexpensive, widely available, and...

---

**Table 1—Macroscopic Pleural Adhesions and Microscopic Lung Changes After the Intrapleural Injection of Talc or SN**

<table>
<thead>
<tr>
<th>Changes</th>
<th>1 mo</th>
<th>2 mo</th>
<th>4 mo</th>
<th>6 mo</th>
<th>8 mo</th>
<th>10 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macroscopic pleural changes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adhesions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>1.7 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>2.3 ± 0.3</td>
<td>2.1 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>SN</td>
<td>3.1 ± 0.2</td>
<td>3.1 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.1 ± 0.1</td>
<td>3.0 ± 0.2</td>
<td>3.4 ± 0.2</td>
<td>3.5 ± 0.1</td>
</tr>
<tr>
<td><strong>Microscopic parenchymal changes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar collapse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>0.2 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>SN</td>
<td>2.2 ± 0.3</td>
<td>1.2 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.2 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>SN</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>0.2 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>SN</td>
<td>0.9 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Total cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.3</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>SN</td>
<td>2.7 ± 0.3</td>
<td>1.7 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Neutrophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>2.5 ± 0.1</td>
<td>2.4 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>2.6 ± 0.1</td>
<td>2.2 ± 0.4</td>
<td>0.9 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>SN</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.0</td>
<td>0.9 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talc</td>
<td>1.1 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>1.0 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>SN</td>
<td>3.0 ± 0.0</td>
<td>3.0 ± 0.0</td>
<td>2.7 ± 0.3</td>
<td>2.9 ± 0.1</td>
<td>2.3 ± 0.4</td>
<td>1.3 ± 0.5</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

*Data given as mean ± SE.

†p < 0.05, talc × SN.
effective in producing pleural adhesions. In this animal model, SN had a significantly higher score for pleural adhesions than did talc throughout the follow-up. However, SN might produce lung damage, thus reducing pulmonary function. The question is, how much more severe is the lung lesion when SN is injected into the pleural cavity?

The present study shows the changes of the lung architecture 1 month after the intrapleural injection of talc or SN and compares the damage over 1 year. The results clearly demonstrate that both agents caused only discrete parenchymal lung changes during the 12 months of the study.

The morphologic changes found on the parenchymal configuration after intrapleural SN injection were characterized predominantly by alveolar collapse. This was more evident after SN injection than after talc injection and was observed only 1 month after the intrapleural injection. From the second month onward, the alveolar collapse decreased and became minimal, which was similar to that observed after talc injection. We hypothesize that this finding could be associated with the viscoelastic and mechanical properties of the chest wall, especially the surface of the pleura. The more precocious and severe pleural fibrosis produced by SN could reduce pulmonary compliance, decreasing lung expansion and consequently alveolar spaces.

The second mechanism involved in the development of the parenchymal changes is the altered capillary permeability, probably caused by severe inflammation with passive diffusion of liquid through the pleura into the alveolar tissue. The minimal intra-alveolar hemorrhage and edema were histologically visualized at least during the first 8 months after both sclerosants had been administered, tending to be slightly more pronounced after SN injection than after talc injection.

The inflammation observed 1 month after intrapleural SN injection was greater than that produced by talc injection. From the second month of the study on, a tendency toward decreased inflammation was observed, becoming similar to that seen after talc administration. The greater inflammatory reaction seen after SN administration could be a consequence of the pleural damage.

The hallmark of the inflammatory process caused by the sclerosing agents was the specific cell recruitment in the pulmonary tissue. In this context, an exuberant neutrophilic reaction was produced by talc with maximal intensity 1 month after the intrapleural injection. On the other hand, an inflammatory reaction with eosinophilic predominance was found in the SN group, probably reflecting a hypersensitivity reaction induced by this sclerosing agent.

In conclusion, the present study demonstrates that the intrapleural injection of 0.5% SN produces more pleural adhesions than does the intrapleural injection of talc.
of 400 mg/kg talc slurry. The pleurodesis induced by SN persisted throughout the 1-year follow-up period. However, because SN is a caustic substance, the intrapleural injection could produce lung damage. Despite this characteristic of SN, 1 month after its injection the lung lesion was moderate, and during the other 11 months of the study it was mild, which was similar to the conditions produced by talc injection. We hypothesize that the alveolar collapse observed after SN injection could be due to changes in the mechanical properties of the lung, especially the reduction of pulmonary compliance, a consequence of the pleural adhesions. Finally, the changes observed in the lung architecture are reversible and not severe, with a clear tendency to normalize over time. These findings need to be confirmed before considering the use of SN as a sclerosing agent in patients with recurrent malignant pleural effusions.

REFERENCES
7 Vargas FS, Teixeira LR, Vaz MAC, et al. Silver nitrate is superior to talc slurry in producing pleurodesis in rabbits. Chest 2000; 118:808–813