Talc Slurry Pleurodesis*

Pleural Fluid and Histologic Analysis

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Although talc slurry pleurodesis is effective for control of malignant pleural effusions and recurrent pneumothorax, the mechanisms of pleurodesis remain incompletely defined. We instilled 70 mg/kg of sterile asbestos-free talc slurry into the pleural space of New Zealand white rabbits and studied the inflammatory response at 1, 2, 3, 7, 15, 30, 60, 90, and 120 days by observing pleural fluid and histologic characteristics. Talc slurry caused mesothelial denudement and an exudative neutrophilic pleural effusion that resolved after 48 h. A transient mononuclear vasculitis was seen within the lung at 1, 2, and 3 days after instillation. Pleural adhesions were minimal and did not increase in number over time. Talc was found outside of the pleural space in mediastinal lymph nodes (4 of 23 animals examined), kidney (1 of 6), and spleen (4 of 10). The predominant cause of pleurodesis with talc slurry instillation is an acute pleural injury similar to the tetracycline class agents.

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Key words: pleural effusion; pleural fluid; pleurodesis; talc

Pleurodesis by mechanical and chemical pleural irritation has been extensively studied during the past century. Tetracycline,1 minocycline,2 doxycycline,3-4 quinacrine,1 bleomycin,1 Corynebacterium parvum, various antineoplastic agents, and talc5-10 have been studied in animals and humans for the treatment of pleural effusion and pneumothorax. These agents have had an overall complete success rate that has varied from 0 to 93% in the treatment of malignant pleural effusions.11

Talc (Mg₃Si₄O₁₀(OH)₂) is a highly effective pleurodesis agent. A recent review of over 1,200 talc pleurodesis procedures12 confirmed excellent success rates when talc is administered via slurry (87%) or poudrage (93%) and in the treatment of both pneumothorax (91%) and pleural effusion (91%). Talc poudrage at the time of thoracoscopy currently is the most common method of administration; however, the use of talc slurry through a chest tube has gained popularity in patients who do not require a diagnostic thoracoscopy or who are unable to tolerate thoracoscopy.

Although talc has been used as a pleurodesis agent since 1935, a paucity of information is available concerning the type and time course of cellular injury and repair. Early descriptions of the talc pleural reaction by Bethune5 (1935), Hanrahan et al6 (1941), and Singer et al7 (1941) focused on gross and histologic findings at 2 to 6 weeks after talc insufflation or slurry injection. More recently, Mathlouthi and colleagues8 instilled talc slurry through the thoracoscope in dogs and described findings at days 1, 2, 7, 15, and 30 following pleurodesis. We used a rabbit model to examine the histologic and pleural fluid changes that occur following talc slurry pleurodesis.

METHODS

Twenty-eight New Zealand white rabbits weighing 2 to 3 kg were lightly anesthetized with 80 to 100 mg of ketamine hydrochloride (Ketalar, Parke-Davis, Morris Plains, NJ) and 0.25 mg/kg acepromazine (PromAce, Aveco, Fort Dodge, Iowa) via the lateral ear vein. The right chest wall was shaved and a 1.0-cm skin incision was made midway between the spine and scapular tip. With aseptic technique, an 18-gauge plastic catheter (Cathion IV Striped, Critikon, Tampa, Fla) was placed percutaneously into the right pleural space.

USP asbestos-free talc (Standard Chemicals, Chicago) was dry heat sterilized at 175°C for 8 h. Rabbits received an intrapleural injection of 70 mg/kg talc in 3 mL 0.8% NaCl that was extrapolated from the usual human dose of 5 g in a 70-kg individual. Immediately after instillation, the catheter was removed. Postoperative administration of a single dose of 0.04 mg/kg subcutaneous buprenorphine (Norwich Eaton, Norwich, NY) provided narcotic analgesia for an additional 12 h.

Animals were killed with intravenous pentobarbital at 1, 2, 3, 7, 15, 90, 60, 90, and 120 days. Using a ventral midline approach, the rabbits underwent autopsy and the thorax was resected en toto. All visualized pleural fluid was aspirated through a diaphragmatic incision. Total cell count was determined by hemocytometry, and differential cell counts were performed after cytopsin preparation and Leukostat staining (Fisher Diagnostics, Fisher Scientific, Orangeburg, NY). Pleural fluid lactate dehy-
Table 1—Pleural Fluid Analysis Following Intrapleural Instillation of Talc Slurry

<table>
<thead>
<tr>
<th></th>
<th>24 h</th>
<th>48 h*</th>
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<tbody>
<tr>
<td>Pleural fluid volume, mL</td>
<td>2.9±0.5 1</td>
<td>1.7±0.6</td>
</tr>
<tr>
<td>RBCX10^6/μL</td>
<td>38±17</td>
<td>24±13</td>
</tr>
<tr>
<td>WBCX10^9/μL</td>
<td>3.3±0.6</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>56±2</td>
<td>27±2</td>
</tr>
<tr>
<td>Absolute neutrophilsX10^9/μL</td>
<td>1.8±0.4</td>
<td>0.6±0.2</td>
</tr>
<tr>
<td>Macrophages/monocytes, %</td>
<td>42±2</td>
<td>66±6</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>2±0</td>
<td>6±1</td>
</tr>
<tr>
<td>pH</td>
<td>7.41±0.03</td>
<td>7.44±0.01</td>
</tr>
<tr>
<td>Lactate dehydrogenase, IU/L</td>
<td>484±102</td>
<td>528±197</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td>3.7±0.1</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>146±12</td>
<td>131±5</td>
</tr>
</tbody>
</table>

*Pleural fluid was not present after day 2.

1Mean±SEM.

dehydrogenase, glucose and total protein measurements were made on an analyzer (Beckman CX7 Analyzer, Brea, Calif); pleural fluid pH determinations were made on a blood gas analyzer (System 1304 pH/Blood Gas Analyzer, Instrumentation Laboratory, Lexington, Mass).

The right hemithorax was entered and pleural adhesions were noted prior to transection at the midline. Lungs, thoraces, livers, spleens, and kidneys were fixed in 10% formalin. Histologic sections were prepared from the parietal pleura at the fourth intercostal space. A transverse circumferential section from the right lower lobe was obtained 1 cm from the lung base. Specimens were embedded in paraffin and cut; all specimens were stained with hematoxylin-eosin and Masson's trichrome stains. Some specimens were stained with alcian blue and high iron diamine with and without hyaluronidase. Gross and histologic analysis was performed concurrently by three investigators (L.K., R.H., C.S.) on all rabbits.

**Results**

**Pleural Fluid**

Animals had a small volume of pleural fluid at 24 and 48 h following talc slurry administration (2.9 mL and 1.7 mL, respectively). There was less than 0.2 mL pleural fluid in all animals at other time points. The 24-h nucleated cell response was modest (3,300 cells per microliter) and declined on the second day. Neutrophils were the predominant cell at 24 h (56%) while monocytes and macrophages were predominant by 48 h. Pleural fluid pH ranged from 7.38 to 7.48. Pleural fluid was exudative with lactate dehydrogenase levels of 484 and 528 IU/L at 24 and 48 h, respectively. Findings are summarized in Table 1.

**Gross Pathology**

All specimens had visible collections of talc that ranged in size from 0.5 to 10 mm. The collections were noted most frequently in the ventral (dependent) areas but were also found on the entire visceral and parietal pleural surface. Fibrin strands connecting visceral and parietal pleural surfaces were noted at each time point. On occasion, these strands were associated with visible collections of talc. Before day 15, these adhesions were thin and filmy. At no time was complete pleural symphysis observed.

**Histology: Visceral Pleura**

As expected from gross examination, talc particles and the associated tissue reaction were focal. Specimens obtained 24 h following pleurodesis demonstrated denudement of mesothelial cells with mononuclear cell infiltration into the subpleural connective tissue matrix and peripheral airspaces of the lung. Edema of the submesothelial connective tissue was noted on day 2 specimens and appeared independent of whether talc was within the microscope field (Fig 1).

The lung developed a patchy histiocytic inflammatory reaction on day 1, most marked around subpleural venules and arterioles (Fig 2) that extended

**Figure 1.** The inflamed and edematous visceral pleura 2 days after talc slurry is denuded of mesothelial cells. The normal rabbit visceral pleura is very thin (approximately two cells thick). No talc is visible in this field (hematoxylin-eosin, original X250).

**Figure 2.** The lung at day 2 demonstrates a patchy perivascular mononuclear infiltrate without talc demonstrated (Masson's trichome, original X100).
centrally along bronchovascular bundles. The mononuclear inflammation extended from capillaries to adjacent alveoli that were filled with large alveolar macrophages. No particles of talc could be detected in the lung with polarization. These lesions regressed completely by day 7.

Patchy pleural thickening was evident in every animal and peaked in thickness between 3 and 7 days following talc slurry instillation. At these times, reactive cuboidal mesothelial cells covered a thickened loose connective tissue matrix that extended above and below the discontinuous fibroelastic lamina. Fibrin formed the central core of the thin adhesions rising from the pleural connective tissue matrix. Specimens at days 30, 60, 90, and 120 days all demonstrated flattened mesothelial cells overlying thickened pleural connective tissue. Fewer inflammatory cells were noted with the passage of time (Fig 3).

Small and large collections of talc were discovered in all specimens examined (Figs 3 and 4). Small particles were incorporated into the thickened pleural connective tissue while large collections were found within the interlobar fissures and on both pleural surfaces. Within 7 days, these large collections were infiltrated by mononuclear inflammatory cells, fibroblasts, and collagen. Occasional multinucleate giant cells were evident.

Glycosaminoglycans were noted within all areas of pleural thickening on alcian blue stains. High iron diamine-alcian blue stains suggested the presence of hyaluronic acid in the pleural inflammatory reaction.

**Parietal Pleura**

Similar inflammatory reactions were seen on the parietal pleura. At times after 7 days a mononuclear infiltrate was demonstrated between the intercostal muscle and the pleura. As on the visceral pleural surface, small and large talc collections were noted adjacent to and incorporated within the parietal pleural inflammatory reaction.

**Other Tissues**

Microscopic examination of mediastinal lymph nodes, kidney, liver, and spleen sections revealed birefringent talc in 4 of 23 (17%), 1 of 6 (17%), 0 of 12 (0%), and 4 of 10 (40%), respectively. Tissue reaction to talc particles was noted in all of these sections (Figs 5 and 6).

**Discussion**

Talc as a slurry, as opposed to talc insufflation, has been criticized as a method of administration for pleurodesis because of nonuniform distribution in the pleural space. We considered that a better understanding of the mechanisms of talc pleurodesis would be helpful in resolving the issue of route of administration. Therefore, we analyzed pleural fluid and gross and histologic pathologic findings in an animal model to better characterize the mechanisms of pleural symphysis.

Early work by Bethune reported the gross and histologic anatomy in six cats and six dogs that received talc insufflation. No pleural fluid was noted on daily radiographs; pleural inflammation was not noted at the first autopsy at 1 week although filmy adhesions had formed. Hanrahan and colleagues performed talc poudrage in six dogs, noting a giant cell foreign body reaction in the pleura infiltrating the lung for 2 to 3 mm at 6 weeks; mediastinal thickening had occurred. Singer et al administered talc slurry to rabbits, noting pleural thickening at 2
weeks with macrophage and giant cell prominence; talc was noted in mediastinal lymph nodes.

More recently, Mathlouthi et al instilled talc slurry through the thoracoscope in dogs and described findings at days 1, 2, 7, 15, and 30 after administration. An inflammatory exudate was noted on the pleural surface at early time points. Extrapleural fibrin deposition was described at 7 and 15 days.

Our most impressive findings included denudement of the mesothelium by 24 h, neutrophil predominant pleural fluid formation, progressive thickening of the pleura through 7 days with adherent fibrin in areas of mesothelial denudement and basement membrane injury, and slow resolution of mononuclear predominant pleural inflammation as fibroblasts proliferate and collagen is deposited. These histologic findings are identical to those seen with tetracycline, doxycycline, and minocycline.1,2,13

It appears that the mechanism of talc pleurodesis may be different in some aspects from other chemical pleurodesis agents. The volume of pleural fluid following talc slurry instillation is small and transient compared with tetracycline, minocycline, bleomycin, and quinacrine. Furthermore, despite the fact that interleukin 8, a chemoattractant for neutrophils, is produced by mesothelial cells after talc phagocytosis,14,15 the absolute number of polymorphonuclear leukocytes in pleural fluid is less than with the tetracycline class agents.

The difference between high clinical efficacy and the relatively small number of adhesions that we and others have observed may be due to a number of factors. One possibility is that the persistence of pleural talc is responsible for chronic inflammatory
stimulation that slowly increases adhesion number and strength. However, this was not supported by the pathologic findings observed in the specimens from our animals at 60, 90, and 120 days that had small numbers of thin adhesions. While we cannot exclude the possibility that talc causes pleurodesis by altering pleural fluid and solute exchange in some unknown way, the more likely reason for the difference in adhesion numbers between our study and others is related to particle size,16 dose, or concomitant abrasion of the pleural surface performed in some animal models.9

The pulmonary abnormalities seen in the animals have not been described previously after talc pleurisy. Because the perivascular location is so prominent, the pathogenesis is likely related to some injurious agent within the circulation. One possibility is that the pulmonary vascular lesions are a response to talc particles too small to observe at light microscopy. In much the same way that the pleural surface was found injured without talc particles observed, the vasculitis might also represent more proximal talc injury with downstream inflammatory mediators responsible for the pathologic condition. Of interest is the possibility that these lesions might be the cause of the clinical syndrome causing respiratory failure after high-dose talc pleural instillation.12,17,18 Respiratory failure in these cases was of rapid onset and resembled the adult respiratory distress syndrome.

Extensive literature describes the presence of talc in many organs following intravenous infusion19-22 and abdominal surgery (with presumed contamination from gloves).23,24 There is a single report of talc in a liver specimen of a patient with inhalation exposure to talc.22 Singer and colleagues7 noted talc in rabbit mediastinal lymph nodes following talc slurry. Lobar intrapulmonary talc fibrosis has been seen in a patient who received talc and quinacrine in the presence of a bronchopleural fistula.25 To our knowledge, this is the first report of talc in other organs following intrapleural administration.

We postulate that intrapleural talc moves into the parietal pleural lymphatics and is transported to the mediastinal lymph nodes and thoracic duct where it enters the systemic circulation.26 In the absence of overwhelming exposure, it is unlikely that the entry of small particles of talc into the systemic circulation has clinical significance. Asbestos-free talc has never been associated with malignancy27,28 and extrapleural organ failure has not been described.

In summary, talc slurry causes mesothelial denudement that is followed by a low-grade mononuclear cell inflammatory process. Pleural fluid production is transient and of small volume. The neutrophilic influx is less intense than following most other forms of pleural injury. Pleural adhesions form early after talc administration and do not increase over time. Despite the fact that talc enters the systemic circulation, the tissue reaction in extrapleural sites is minimal.

REFERENCES
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