Talc Slurry Is an Effective Pleural Sclerosant in Rabbits*

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Insufflated talc is probably the most effective agent for creating a pleurodesis both in the clinical situation and in animals. However, the insufflation of talc requires an invasive procedure such as thoracoscopy or thoracotomy. Recently, there have been reports that talc in a slurry was effective in the clinical situation. The objective of this project was to determine whether talc in a slurry at varying doses is an effective sclerosant in an experimental model in rabbits. Talc, 50, 100, 200, and 400 mg/kg, in a 2-mL slurry was injected intrapleurally through a small catheter in male rabbits. Eleven rabbits received each dose. Twenty-eight days after the instillation, the animals were killed. The pleural spaces were assessed grossly for evidence of pleurodesis and microscopically for evidence of fibrosis and inflammation.

The degree of pleurodesis (on a scale of 0 to 4) after the injection of 50, 100, 200, and 400 mg/kg of talc was 1.1 ± 0.9, 1.5 ± 1.1, 2.7 ± 0.6, and 3.4 ± 0.5, respectively. The degree of microscopic fibrosis similarly increased with increasing doses of talc. These scores were similar to those we have reported with the tetracycline derivatives. In contrast to the results with tetracycline derivatives, none of the rabbits developed fibrosis and pleurodesis. From this study, we conclude that talc in a slurry is not effective in rabbits but does not produce fibrosis. The tetracycline derivatives.

Key words: pleural effusion; pleurodesis; pneumothorax; talc

The search for the ideal agent for creating a pleurodesis in patients with chronic pleural effusions or pneumothorax continues. The first agents used for this purpose were the antineoplastic agents such as nitrogen mustard.1 Subsequently it became apparent that these agents produced a pleurodesis not due to their antineoplastic actions but rather because they produced pleural fibrosis. Following this observation, agents such as quinacrine2 and tetracycline,3 which produced pleural fibrosis, gained acceptance. In the 1980s, tetracycline was the agent most commonly used due to its effectiveness in an animal model,4 its effectiveness in humans,5 its low cost, its ease of administration, and its wide availability.

However, in the early 1990s, the company that had been manufacturing parenteral tetracycline ceased to produce it because of more stringent manufacturing requirements for parenteral antibiotics. This led to a scramble to find a substitute. The leading candidates were the tetracycline derivatives minocycline,6,7 and doxycycline,8,9 bleomycin,10,11 aerosolized talc,12,13 and Corynebacterium parvum.14,15

However, there are problems with all the above agents. In the experimental situation, the intrapleural administration of minocycline or doxycycline, particularly at higher doses, leads to the development of marked atelectasis, pleural effusion, and a high mortality.6 Bleomycin is very expensive and since it does not produce a pleurodesis in rabbits with a normal pleura,17 it certainly should not be used for pneumothorax or benign pleural effusions. Insufflated talc is probably the best agent for producing pleurodesis in patients with malignant pleural effusions18 or pneumothorax18 and it is very effective in the animal, but it must be administered in conjunction with an invasive procedure (thoracotomy).

Corynebacterium parvum is not available in the United States.

Another possible alternative is talc in a slurry. Four series have demonstrated that it is highly effective in treating malignant pleural effusions.19-22 The objective of the present study is to determine whether talc in a slurry is an effective sclerosant in an experimental model in rabbits and if so to define the appropri-
ate dose.

**METHODS**

The methods we used were similar to those we have described previously. New Zealand white male rabbits weighing 2.5 to 4.0 kg were lightly anesthetized with ketamine hydrochloride, 35 mg/kg, plus xylazine hydrochloride, 5 mg/kg intramuscularly. The thorax was prepared for aseptic surgery by shaving the right chest wall and then cleaning it with povidone-iodine and alcohol. A 0.3-cm skin incision was made midway between the spine and the sternum. Then a 16-gauge intravenous catheter placement unit was inserted into the pleural space. The end of the catheter was removed so that the right lung could collapse, thereby preventing damage to the lung via the catheter’s needle. The needle was removed and the plastic catheter was left in place. A stopcock was attached to the end of the catheter. Through this, all air was aspirated from the pleural space. Verification of the catheter’s position in the pleural space was obtained by documenting inspiratory pressure drops with a pressure transducer. The talc slurry was then injected through the catheter. Following the injection of the slurry, a small amount of fluid was aspirated to again verify proper placement of the catheter and the lack of a pneumothorax. After the catheter was removed, the wound site was cleaned with povidone-iodine. Since the wound was small, suturing of the site was rarely necessary.

After the surgery, the rabbits were closely monitored for clinical evidence of pain (vocalization, tachypnea, and restlessness). All rabbits received 0.3 mL of buprenorphine immediately after recovery from surgery and every 12 h thereafter when they appeared to have any distress. The left hemithorax received no injection and served as a control.

We studied the effects of intrapleural talc in four groups of rabbits. One group of animals received each of the following four doses of talc: 50, 100, 200, and 400 mg/kg. We attempted to have at least ten rabbits in each group. The talc was obtained (Sigma Chemical) and certified to be asbestos free. The talc was sterilized in an autoclave. The talc was suspended in a total volume of 2 mL of bacteriostatic saline solution and the suspension was shaken vigorously just prior to the injection.

Rabbits were killed 28 days after the injection by the injection of an euthanasia solution into an ear vein. The thorax was removed from the remainder of the rabbit in block. Small incisions were made in the diaphragm to allow better access of the fixative (10% formalin) to the pleural cavities. Attempts were then made to expand the lungs by the injection of the fixative into a plastic catheter (6 mm diameter) that had been inserted into the exposed trachea. After the trachea was ligated with 2-0 silk, the entire thorax was submerged in 10% formalin solution for at least 48 h.

The necropsy was performed by one of us (R.W.L.) who was blinded as to which dose of talc the animal had received. At least one of the other coinvestigators was present at each necropsy. Each pleural cavity was carefully exposed by making bilateral incisions through the diaphragms and through all the ribs in approximately the midclavicular line. In this manner, the sternum and the medial portions of the anterior ribs were removed so that the lung and pleural cavities could be evaluated. The presence or absence of hemothorax (clotted blood in the pleural space) and the position of the mediastinum in each animal was recorded.

The degree of pleurodesis observed grossly was graded according to the following scheme: 0, normal pleural space; 1, no adhesions but pleural space inflated as evidenced by roughness and fibrin deposition; 2, few scattered adhesions; 3, generalized scattered adhesions; and 4, complete obliteration of the pleural space by adhesions.

At the time that the thorax was assessed grossly, samples of the parietal pleura, visceral pleura, and lung from each hemithorax were obtained and placed in neutral buffered 10% formalin. Samples were obtained of the lower lobes with the contiguous parietal pleura. Attempts were made to sample the most representative region. These tissue samples for histologic examination were processed routinely and stained with hematoxylin-eosin. The microscopic slides were evaluated blindly by one of us (N.S.W.) for the presence of inflammation and fibrosis. The degree of inflammation and fibrosis was graded from 0 to 4 for absent, equivocal, mild, moderate, or marked, respectively.

**Statistical Analysis**

All data are expressed as the mean ± SD. To compare the effects of the different doses of talc, the test results with the different medications were compared using one-way analysis of variance. If the F value achieved statistical significance (p<0.05), then the means were compared using Tukey’s test. Differences were considered significant when p<0.05. If the p value was less than 0.01, this was also noted.

**RESULTS**

Talc in a slurry was effective in a dose-dependent fashion in producing a pleurodesis (Table 1). The mean degree of pleurodesis for the highest dose (400 mg/kg) was 3.36 ± 0.51, which was significantly higher than the degree of pleurodesis with the two lower doses of talc. The scores for the amount of pleurodesis on the left were 0 in all rabbits.

None of the rabbits died during the 28 days after the intrapleural injection of talc. No rabbit had a hemothorax and none of the rabbits had a mediastinal shift or severe atelectasis of the underlying lung like we had observed previously in animals injected with the tetracycline derivatives. When the rabbits were examined grossly, talc was visible through the right diaphragm. Then when the thorax was opened.

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**Table 1—Mean Values for Gross Pleurodesis Score and Microscopic Pleural Fibrosis and Inflammation With the Four Different Doses of Talc and on the Control Side**

<table>
<thead>
<tr>
<th>Dose of Talc, mg/kg</th>
<th>n</th>
<th>Gross Pleurodesis</th>
<th>Microscopic Fibrosis</th>
<th>Microscopic Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (left)</td>
<td>44</td>
<td>0.0 ± 0.0*</td>
<td>0.39 ± 0.61*</td>
<td>0.75 ± 0.68*</td>
</tr>
<tr>
<td>50</td>
<td>11</td>
<td>1.09 ± 0.90†</td>
<td>1.82 ± 0.57*</td>
<td>1.64 ± 0.77†</td>
</tr>
<tr>
<td>100</td>
<td>11</td>
<td>1.55 ± 1.08†</td>
<td>2.09 ± 0.67*</td>
<td>1.82 ± 0.39</td>
</tr>
<tr>
<td>200</td>
<td>11</td>
<td>2.73 ± 0.62</td>
<td>2.55 ± 0.78</td>
<td>1.64 ± 0.48†</td>
</tr>
<tr>
<td>400</td>
<td>11</td>
<td>3.36 ± 0.48</td>
<td>3.27 ± 0.86</td>
<td>2.36 ± 0.64</td>
</tr>
</tbody>
</table>

*p<0.01 when compared with 400 mg/kg
|f*p<0.05 when compared with 400 mg/kg
talc was visible throughout the pleural space, particularly in the anterior part of the thoracic cavity. In the rabbits with incomplete pleurodesis, most of the adhesions were anteriorly (inferiorly) where the talc had collected.

When the pleura was examined microscopically, the results for the pleural fibrosis (Table 1) paralleled those for gross pleurodesis. The mean degree of fibrosis increased with increasing doses of talc. The score with 400 mg/kg of talc was 3.27 ± 0.9, which was significantly greater (p<0.01) than the score with the 50 or 100 mg/kg dose of talc. The mean degree of pleural inflammation was greater in the group that received the highest dose of talc than it was in any other group. There was a significant (p<0.05) difference in the mean degree of inflammation between the 400-mg/kg group and the 50-mg/kg group and the 200-mg/kg group.

The intrapleural injection of talc derivatives appeared to have very little effect on the underlying lung (Table 2). The degree of fibrosis and the degree of inflammation tended to be slightly greater in the lung on the side of the injection (right lung) than on the other side, but the changes were minimal and there were no statistically significant (p>0.05) differences in the means.

**DISCUSSION**

The present study demonstrates that talc in a slurry is an effective agent for producing pleurodesis in the rabbit model. Talc at a dose of 400 mg/kg produced gross and microscopic pleural fibrosis to a degree that was at least as high as that produced by the intrapleural injection of tetracycline, 35 mg/kg, or minocycline 7, 10, 20, or 40 mg/kg. Moreover, the intrapleural administration of talc did not lead to fibrothorax or hemothorax and none of the rabbits died after the injection as did a significant percentage or the rabbits after tetracycline or minocycline intrapleurally. Therefore, at least in the rabbit model, talc in a slurry appears to be the agent of choice.

To our knowledge, there has only been one previous study comparing tetracycline, 500 mg, insufflated talc, 1 g, mechanical dry gauze abrasion, Nd:YAG laser photocoagulation, and argon beam electrocoagulation of the parietal pleura for their ability to produce a pleurodesis in dogs weighing 25 to 35 kg. They found that talc insufflation produced results that were basically identical to those produced by mechanical abrasion and that were better than those produced by tetracycline, argon-beam electrocoagulation, or Nd:YAG laser photocoagulation.

In a previous study, we assessed the capability of tetracycline, 35 mg/kg, and minocycline, 4, 7, 10, 20, and 40 mg/kg, to produce pleurodesis in rabbits. We found that the degree of gross pleurodesis produced by tetracycline, 35 mg/kg, and the higher doses of minocycline (20 or 40 mg/kg) was comparable to that produced by 400 mg/kg of talc in the present study. Interestingly, the intrapleural administration of tetracycline and the higher doses of minocycline in the previous study were associated with the development of a marked fibrothorax and large hemothoraces in a substantial proportion of the animals. There was also significant late mortality in these animals and almost all the animals who died had a hemothorax. Fibrothorax and bloody pleural effusions were also observed in another study in which the animals were killed at 14 days. In contrast, none of the rabbits who received talc intrapleurally in the present study developed either a fibrothorax or a hemothorax.

The fact that the rabbits who received the tetracycline derivatives developed fibrothorax, while those who received the talc did not, suggests that these two types of agents produce a pleurodesis by different mechanisms. However, the mechanism by which either of these agents produces a pleurodesis is not completely known. Antony and associates have shown that tetracycline stimulates mesothelial cells in culture to release a growth-factor-like activity for fibroblasts.

Previous clinical studies have suggested that talc is probably the most efficacious sclerosant for malignant pleural effusions. In a recent review of the literature, talc completely controlled the pleural effusion in 95% of 165 patients. This success rate was higher than that with the tetracycline derivatives, C parvum, or any of the antineoplastic agents. Although most of the reports used insufflated talc, four reports using talc in a slurry have been reported with an overall success rate of 90% in 131 patients. Insufflated talc administered at the time of thoracoscopy has also been shown to be an effective therapy for benign pleural effusions.

Previous studies have also documented that insufflated talc is an effective agent for creating a pleurodesis and preventing recurrent pneumothorax. If

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<td>0.77 ± 0.60</td>
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<td>50</td>
<td>11</td>
<td>0.09 ± 0.50</td>
<td>0.82 ± 0.40</td>
</tr>
<tr>
<td>100</td>
<td>11</td>
<td>0.64 ± 1.03</td>
<td>1.10 ± 0.54</td>
</tr>
<tr>
<td>200</td>
<td>11</td>
<td>0.18 ± 0.40</td>
<td>1.18 ± 0.60</td>
</tr>
<tr>
<td>400</td>
<td>11</td>
<td>0.45 ± 0.52</td>
<td>1.00 ± 0.63</td>
</tr>
</tbody>
</table>
four previous series\textsuperscript{27-30} are combined, only 4 of 104 (4\%) of the patients had a recurrence. It appears that insufflated talc is more effective in preventing recurrent pneumothorax than the tetracycline derivatives. In the Veterans Affairs cooperative study,\textsuperscript{31} the recurrence rate during the observation period in the 104 patients who received tetracycline was 25\%, while in the study of Almind and associates,\textsuperscript{29} the recurrence rate after tetracycline administration was 13\%. To our knowledge, there are no reports evaluating the efficacy of talc slurry in preventing recurrent pneumothorax.

It appears that the intrapleural administration of talc is associated with relatively few side effects. One possible significant side effect is the development of respiratory failure. The intrapleural administration of talc has been reported to cause acute pneumonitis\textsuperscript{32} and adult respiratory distress syndrome.\textsuperscript{33} Both of these reports involved only patients who had received talc in a slurry, and in retrospect, we speculate that the patients possibly had reexpansion pulmonary edema rather than an adverse reaction to talc. In the recent series reported by Kennedy and coworkers,\textsuperscript{22} 5 of 58 patients (9\%) had respiratory failure associated with the talc slurry pleurodesis. In two of the patients, the respiratory failure was not thought to be due to the talc. A third patient received simultaneous bilateral talc instillation that was followed by the development of fever, bilateral infiltrates, and hypoxic respiratory failure requiring intubation and mechanical ventilation. The other two patients developed increasing hypoxia, but recovered without requiring mechanical ventilation. There were no episodes of respiratory failure reported in the other three series\textsuperscript{19-21} with a total of 88 patients who used talc in a slurry. Further studies are necessary to document the incidence of respiratory failure following talc in a slurry and to document whether the incidence is different than with other compounds. Similar problems have been reported after the intrapleural administration of bleomycin.\textsuperscript{34}

In the past, there was concern about the possibility that the asbestos in talc could lead to the development of malignant mesothelioma and other asbestos-related diseases. However, Lange and coworkers\textsuperscript{35} studied 114 patients 22 to 35 years after talc pleurodesis for spontaneous pneumothorax and found no patients with mesothelioma. Additionally, asbestos-free talc is now available. The intrapleural administration of talc can lead to severe chest pain,\textsuperscript{30} but it is our impression that the pain associated with insufflated talc is less than that associated with tetracycline derivatives. Most patients treated with talc in a slurry will develop a temperature elevation.\textsuperscript{22}

In conclusion, the results of the present study demonstrate that talc in a slurry is a very effective pleural sclerosant in normal rabbits. The intrapleural administration of talc to rabbits does not lead to fibrothorax and hemothorax as does the administration of the tetracycline derivatives. If a patient with a pleural effusion undergoes thoracoscopy or thoracotomy, then insufflated talc is probably the method of choice for producing a pleurodesis. If such an invasive procedure is not performed, then talc in a slurry appears to be a good choice for producing a pleurodesis. Talc slurry should be evaluated for its safety and efficacy in preventing recurrence of pneumothorax.

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