Combination Therapy With Intrapleural Doxycycline and Talc in Reduced Doses Is Effective in Producing Pleurodesis in Rabbits*

Oner Dikensoy, MD; Zhiwen Zhu, MD; Edwin Donnelly, MD, PhD, FCCP; Georgios T. Stathopoulos, MD; Kirk B. Lane, PhD; and Richard W. Light, MD, FCCP

Background: It has been suggested that talc and doxycycline might be acting through different pathways in creating pleurodesis. We hypothesized that combining doxycycline and talc in half the usual doses would be synergistic in inducing pleurodesis.

Methods: Thirty-two rabbits were equally allocated into four groups: group 1, half-dose combination (5 mg/kg of doxycycline and 200 mg/kg of talc slurry); group 2, quarter-dose combination (2.5 mg/kg of doxycycline and 100 mg/kg of talc slurry); group 3, half-dose doxycycline (5 mg/kg of doxycycline); and group 4, half-dose talc (100 mg/kg of talc slurry). The pleurodesis scores from historical groups that received a full dose of talc (400 mg/kg) or doxycycline (10 mg/kg) were also compared to those obtained in the current study. Pleural fluid lactate dehydrogenase and protein levels were measured 24 h after the injection. Pleurodesis was graded from 1 (none) to 8 (> 50% symphysis) by two observers blinded to treatment groups. All rabbits underwent an ultrasonic examination on each side of their chest for the evaluation of pleurodesis.

Results: The mean pleurodesis score in the half-dose combination group was significantly higher than that in the half-dose talc group, half-dose doxycycline group, and the historical full-dose talc group (p = 0.009, p = 0.01, and p < 0.05, respectively). The quarter-dose combination group also had a significantly higher mean pleurodesis score compared to the half-dose talc group (p = 0.013). The difference between the historical full-dose doxycycline and the half-dose combination or quarter-dose combination groups was not significant (p > 0.05). A significantly positive correlation existed between the pleurodesis score and the ultrasound scores (r = 0.876, p = 0.000000005).

Conclusions: This study demonstrates that the combination of half doses of talc and doxycycline is more effective than the half dose of either drug alone or the full dose of talc in producing pleurodesis in rabbits. In addition, ultrasound is an accurate imaging modality for the evaluation of pleurodesis, in that the absence of pleural gliding on ultrasound correlates well with the presence of a pleurodesis in rabbits.

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Key words: combination; doxycycline; pleural effusion; talc; ultrasound

Abbreviations: IQR = intraquartile range; LDH = lactate dehydrogenase

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a pleurodesis. None of the agents are ideal, all with differing degrees of efficacy and adverse effects.3–6 The ideal agent for chemical pleurodesis should have the following features: low cost, mild or no side effects, worldwide availability, and ease of administration at the bedside. There is a large body of evidence that talc (either as poudrage or slurry) and tetracycline (or its derivatives, especially doxycycline) are two of the most effective sclerosing agents in treatment of malignant pleural effusions.1–13 Although some studies1–9 suggested slightly better effectiveness for talc (76 to 94.5%) than doxycycline10–13 in humans (79 to 88%), the differences were not statistically significant, and the route of administration of the agents was varied in most publications. In a recent, large, randomized controlled study by Dresler at al,14 the efficacy of talc either in poudrage form (78%) or slurry form (71%) was relatively low. In terms of cost-effectiveness and availability, talc is superior to doxycycline. However, the recent association of talc with serious, and even lethal, respiratory failure has stirred considerable concerns and debate. In the study by Dresler et al,14 comparing talc administered as an aerosol at thoracoscopy (insufflation) and talc administered as a slurry in 501 patients with documented malignant pleural effusion, a treatment-related mortality was reported in 9 of 242 patients in the talc insufflation group and in 7 of 240 patients in the talc slurry group. However, injection of the tetracycline derivatives is at times extremely painful,15 and parenteral doxycycline is not available worldwide.

It has been suggested previously that talc and doxycycline might act through different pathways.16 Also, it has been suggested that serious side effects attributed to talc slurry occur in a dose-dependent manner.17 Lower doses would be expected to cause less severe side effects but also to be less effective in producing pleurodesis. If this is so, one can speculate that the combination of a lower amount of each agent may generate a synergy and therefore produce a better pleurodesis with lower incidence of side effects. To the best of our knowledge, combining sclerosing agents to provide synergy in the production of pleurodesis has never been studied before. We hypothesized that the combination of half doses of doxycycline and talc slurry would result in a pleurodesis at least as good as each agent by itself.

**Materials and Methods**

The study protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee. The methods used were similar to those described in our previous studies.18–19

**Chest Tube Insertion**

New Zealand white rabbits weighing 1.5 to 2 kg were anesthetized with an IM injection of 35 mg/kg of ketamine hydrochloride (Fort Dodge Animal Health Laboratories; Fort Dodge, IA) and 5 mg/kg of xylazine hydrochloride (Fermenta; Kansas City, MO). The chest was shaved, and the skin was sterilized with 10% povidone iodine (Baxter; Deerfield, IL). The rabbit was placed in the left lateral decubitus position, and a skin incision < 2 cm was made midway between the tip of the right scapula and the sternum, approximately 2 cm above the costal margin. A chest tube (silicone tube, 0.062-inch internal diameter and 0.125-inch outer diameter; Braintree Scientific; Braintree, MA) was created with five extra openings near the distal end to enhance drainage. The chest tube was then inserted by blunt dissection into the right pleural cavity and was secured at the muscle layers with purse-string sutures. The proximal end of the chest tube was tunneled underneath the skin and drawn out through the skin posteriorly and superiorly between the two scapulae. A three-way stopcock (Medex; Dublin, OH) was attached to the skin end of the chest tube via an adapter through which pleural air was evacuated from the pleural space. The three-way stopcock was then removed from the chest tube, and the exterior end of the chest tube was sealed with a one-way valve with a cap (Medex) via the adapter and was sutured to the skin. Any pleural fluid or air that accumulated could be aspirated through the one-way valve. A local anesthetic was applied into the incision area before it was sutured since the rabbits were not administered full general anesthesia. The left pleural cavity was used for control.

**Intrapleural Injections**

Thirty-two rabbits were allocated into following groups: group 1, half-dose combination (n = 8; 5 mg/kg of parenteral doxycycline in 2 mL of saline solution, and 200 mg/kg of talc in 3 mL of saline solution); (2) quarter-dose combination (n = 8; 2.5 mg/kg of parenteral doxycycline in 2 mL of saline solution, and 100 mg/kg of talc in 3 mL of saline solution); group 3, half-dose doxycycline (n = 8; 5 mg/kg of parenteral doxycycline in 2 mL of saline solution); and (4) half-dose talc (n = 8; 100 mg/kg of talc in 3 mL of saline solution). Parenteral doxycycline (Human Label; Burns Veterinary Supply; Norcross, GA) was dissolved in 2 mL of 0.9% sodium chloride (Baxter). Asbestos-free talc powder (Sigma; St. Louis, MO) was gas sterilized using ethylene oxide and was then mixed with the 0.9% sodium chloride solution. In combination groups, each drug was injected separately through the chest tubes, with doxycycline being the first agent followed by talc slurry. The injection of the agents was followed by injection of at least 2 mL of 0.9% sodium chloride to clear the dead space of the chest tube. The total volume that each animal received was completed to 7 mL with the additional 0.9% sodium chloride injection. We also used our historical data for full doses of talc19 and doxycycline20 for the statistical comparisons.

**Measurements for Pleural Fluid and Follow-up**

After the intrapleural injection, the chest tube was aspirated at 24-h intervals for any pleural fluid that had accumulated. The volumes of the aspirated fluids were recorded. The levels of protein and lactate dehydrogenase (LDH) were measured in the fluid collected 24 h after the injection. The protein and LDH levels were determined with an automated analyzer (Johnson & Johnson; Rochester, NY). The lower and upper limits of normal serum LDH are 120 IU/L and 200 IU/L, respectively, by this method. Gentamicin, 2 mg/kg, was administered IM q24h until the removal of the chest tubes. The chest tube was removed under light sedation when the pleural fluid drainage was < 2 mL over the preceding 24 h.
Thoracic Ultrasound

Prior to surgery, all rabbits underwent an ultrasonic examination at three marked sites (anteriorly in the midclavicular line, laterally in the mid axillary line, and posteriorly in a line below the scapula) on each side of chest in the sitting position. At each site, the absence of a gliding sign was evaluated and graded as follows: 0, gliding sign present; 1, gliding sign questionable; and 2, gliding sign absent. The total score was calculated as the sum of the three grades at each site. Other ultrasonic findings such as existence of pleural fluid or pleural thickening were also noted. Each rabbit was again examined using ultrason 14 days after the intrapleural injection. Thoracic ultrasound was performed by an experienced radiologist (E.D.) [ATL Ultramark 9; Philips; Bothell, WA] with a compact linear 10–5 MHz probe. The ultrasonographer was blinded as to which pleurodesis agent was used. Prior to ultrasonic examination, the fur over the entire thoracic area of each rabbit was removed.

Grading for Pleurodesis

The rabbits were killed on day 14 by carbon dioxide euthanasia following sedation. The thorax was removed en bloc. The lungs were expanded by the injection of 50 mL of 10% neutral-buffered formalin into the exposed trachea. The trachea was then ligated, and the entire thorax submerged into 10% neutral-buffered formalin solution for at least 48 h. To assess the pleurodesis, the pleural cavity was carefully exposed as previously described. A consensus grading was reached by two investigators (K.B.L. and R.W.L.) blinded to the treatment group on the degree of macroscopic pleurodesis using a semiquantitative scheme. The degree of pleurodesis was graded using the following scale as described previously: 1 = no adhesions between the visceral and parietal pleura; 2 = rare adhesions between the visceral and parietal pleura with no synphysis; 3 = a few scattered adhesions between the visceral and parietal pleura with no synphysis; 4 = many adhesions between the visceral and parietal pleura with no synphysis; 5 = many adhesions between the visceral and parietal pleura with synphysis involving <5% of the hemithorax; 6 = many adhesions between the visceral and parietal pleura with synphysis involving 5 to 25% of the hemithorax; 7 = many adhesions between the visceral and parietal pleura with synphysis involving 25 to 50% of the hemithorax; and 8 = many adhesions between the visceral and parietal pleura with synphysis involving >50% of the hemithorax. Adhesions were defined as fibrous connections between the visceral and parietal pleura. Synphysis was present if the visceral and parietal pleura were difficult to separate as a result of adhesions.

Statistical Analysis

The data were expressed as mean ± SD when they were normally distributed, and as median (intraquartile range [IQR]) when they were not normally distributed. One-way analysis of variance was used to compare the means, and the Kruskal-Wallis test was used to compare the medians between the groups. The Dunn method and the Holm-Sidak method were used for the comparison of medians and means, respectively, when the F values reached statistical significance. The correlations were assessed by Pearson product moment correlation. All data were analyzed with statistical software (Sigma Stat V3.0; Jandel Scientific; San Rafael, CA); p < 0.05 was considered significant.

Table 1—The Grading of Pleurodesis for Comparison With Ultrasound*

<table>
<thead>
<tr>
<th>Grade</th>
<th>Pleurodesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No synphysis between the visceral and parietal pleura</td>
</tr>
<tr>
<td>2</td>
<td>Minimal amount of synphysis (&lt;5%) of total area of hemithorax</td>
</tr>
<tr>
<td>3</td>
<td>Synphysis of 5 to 25% of the hemithorax</td>
</tr>
<tr>
<td>4</td>
<td>Synphysis of 25 to 50% of the hemithorax</td>
</tr>
<tr>
<td>5</td>
<td>Synphysis of &lt;50% of the hemithorax</td>
</tr>
</tbody>
</table>

*Synphysis by definition indicated that the visceral and parietal pleura were difficult to separate due to adhesions.

RESULTS

All 32 rabbits survived without any major distress until death. A right hemothorax occupying 90% of the hemithorax was observed at autopsy in one rabbit in the half-dose combination group, and this rabbit was excluded from the data analysis. This rabbit also had massive ascites at the time of death.

The injection of the half-dose combination was effective in producing a pleurodesis. The mean pleurodesis score in the half-dose combination group (7.1 ± 1.4) was significantly higher than that in the half-dose talc (3.2 ± 1.6) and half-dose doxycycline groups (4.1 ± 1.6) [p = 0.009 and p = 0.01, respectively; Fig 1]. The quarter-dose combination group also had a significantly higher pleurodesis score (5.7 ± 1.2) compared to the half-dose talc group (p = 0.013). When pleurodesis scores from historical groups of full dose (400 mg/kg) of talc (4.1 ± 2) and full dose (10mg/kg) of doxycycline (7 ± 0.9) [n = 13 and n = 8, respectively] were compared to the pleurodesis scores in the present study, the half-dose combination group had a higher mean pleurodesis score than the historical group of full-dose talc (p < 0.05). There were no significant differences in the mean pleurodesis scores between the historical group of full-dose doxycycline, the half-dose combination, and the quarter-dose combination groups (p > 0.05). The left pleural score in each rabbit in all four groups was 1.

The intrapleural injections resulted in exudative pleural effusion in each group. The pairwise multiple comparison by the Dunn method showed that pleural fluid volume at 24 h was higher both in the historical full-dose doxycycline group and in the half-dose doxycycline group than that in the historical full-dose talc and the half-dose talc groups (p ≤ 0.001). Additionally, pleural fluid volume at
48 h was significantly higher in the historical doxycycline and the quarter-dose combination groups than that in the historical talc group (p ≤ 0.001). There was no significant difference between pleural fluid volume in the half-dose combination group and the other groups at 24-h and 48-h time points. Pleural fluid volume at 72 h was significantly higher in the half-dose combination and the quarter-dose combination groups than those in the historical full-dose talc group (p ≤ 0.001). Similarly, total pleural fluid volumes in the half-dose combination, half-dose doxycycline, quarter-dose combination, and historical doxycycline groups were significantly higher than that in the historical talc group (p ≤ 0.001) [Table 2].

The pleural fluid protein and LDH levels in the four different groups are shown in Table 3. The mean pleural fluid protein levels were higher in the half-dose combination group compared to the half-dose talc group (p = 0.006). The pleural fluid LDH levels were significantly higher in the half-dose combination group compared to the half-dose talc group (p < 0.05).

There was a significantly positive correlation between the right pleurodesis score and the pleural fluid volumes, LDH, and protein levels (Table 4). Thus, the effectiveness of pleurodesis tended to be better in the presence of greater inflammation as indicated by a higher pleural fluid volume, LDH, or protein levels. Visualization of the lung gliding sign with ultrasound was very useful in predicting whether a pleurodesis was present. Prior to the injection of the sclerosing agents, the ultrasound gliding score was 0 in all animals. The correlation between the ultrasound gliding scores obtained immediately before death and the pleurodesis scores was very good (r = 0.876, p = 0.00000000005) [Fig 2].

**Discussion**

This study demonstrates that the combination of half doses of talc and doxycycline is more effective than the half dose of either drug alone or the full dose of talc in producing pleurodesis in rabbits. Moreover, the mean pleurodesis scores were not significantly different between rabbits receiving the historical full dose of doxycycline, the half-dose combination group, or the quarter-dose combination group. These results support our hypothesis that combining two different agents that act through different pathways may generate additive effects in

**Table 2—Volume of Aspirated Pleural Fluid in Each Group**

<table>
<thead>
<tr>
<th>Groups</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-dose combination, n = 8</td>
<td>3.75 (2-6)</td>
<td>3.7 (2.5-5.2)</td>
<td>7 (5.2-9.5)</td>
<td>25 (18.2-30.5)</td>
</tr>
<tr>
<td>Quarter-dose combination, n = 8</td>
<td>2 (1-4.2)</td>
<td>4.5 (2.2-6)</td>
<td>6.5 (2-9)</td>
<td>22.5 (8.2-30)</td>
</tr>
<tr>
<td>Half-dose doxycycline, n = 8</td>
<td>3.7 (3-7)†</td>
<td>3.7 (2.2-6)</td>
<td>4.5 (0.5-7.5)</td>
<td>15.7 (9.7-28)</td>
</tr>
<tr>
<td>Half-dose talc, n = 8</td>
<td>0.7 (0.5-1)</td>
<td>1.5 (1.7)</td>
<td>1.5 (0.6-5)</td>
<td>3.2 (2.2-11.7)</td>
</tr>
<tr>
<td>Historical doxycycline, n = 13</td>
<td>5.2 (3.5-6.7)†</td>
<td>5.5 (3.2-8.2)</td>
<td>2.2 (0-8.5)</td>
<td>13.2 (7.2-29.2)</td>
</tr>
<tr>
<td>Historical talc, n = 8</td>
<td>0.5 (0.3-1.1)</td>
<td>0.5 (0-1)‡</td>
<td>0 (0-0)¶</td>
<td>1 (0.6-2.2)¶</td>
</tr>
<tr>
<td>p Value</td>
<td>≤ 0.001</td>
<td>≤ 0.001</td>
<td>≤ 0.001</td>
<td>≤ 0.001</td>
</tr>
</tbody>
</table>

*Data are presented as median (IQR).
†Volume in the half-dose doxycycline group was significantly higher than that in the half-dose talc and historical talc groups.
‡Volume in the historical doxycycline group was significantly higher than that in the half-dose talc and historical talc groups.
¶Volume in the historical talc group was significantly lower than that in the historical doxycycline and quarter-dose combination groups.
| Volume in the historical talc group was significantly lower than that in the half-dose combination and quarter-dose combination groups.
††Volume in the historical talc group was significantly lower than that in the half-dose combination, half-dose doxycycline, quarter-dose combination, and historical doxycycline groups.
creating pleurodesis. The mechanism of pleurodesis resulting from the intrapleural administration of sclerosing agents is incompletely understood. It has been hypothesized that intrapleural injection of a sclerosing substance causes injury to the mesothelial cells ranging from cuboidal transition to total cell desquamation.21 This injury likely results in the elaboration of cytokines, particularly transforming growth factor-β, tumor necrosis factor-α, interleukin-1, and interleukin-8 into the pleural fluid.22,23 It is likely that the injury produced by different sclerosing agents varies from agent to agent. The injection of the tetracycline derivatives results in severe acute chest pain, while the injection of talc can result in ARDS.

It has been suggested previously that talc and doxycycline might be acting through different pathways in creating pleurodesis.16 Cheng et al16 showed that the pleurodesis observed at 28 days was significantly inhibited in the talc group, but not in the doxycycline group, when anti-tumor necrosis factor-α Fab fragments were administered concomitantly. However, corticosteroid administration can significantly inhibit the pleurodesis in rabbits induced by talc24 and doxycycline25 but not by transforming growth factor-β.26

There are only few previous studies that investigated whether the combination of two different agents administered intrapleurally was more effective than either agent alone in controlling malignant pleural effusion. Rusch et al27 evaluated intrapleural cisplatin and cytarabine in 46 patients with malignant pleural effusions from a variety of solid tumors. A single dose of cisplatin, 100 mg/m², plus cytarabine, 1,200 mg, was instilled into the pleural space via a chest tube and was immediately removed. No recurrence of the effusion was considered a complete response. Partial response was defined as a ≥ 75% decrease in the amount of the effusion on serial chest radiographs. The overall response rate at 3 weeks was 49% (18 of 37 patients). The theory behind combining two antineoplastic agents in this study was obtaining a synergy in the antitumor effect (intrapleural chemotherapy) rather than the pleurodesing effect. Tanaka and Sato28 instilled OK-432 (an antitumor immunomodulator prepared from Streptococcus pyogenes) and minocycline (a tetracycline derivative) through a chest tube for pleurodesis in 11 patients with malignant pleural effusion. Pleural effusion was diminished in all patients without recurrence, allowing the drainage tubes to be successfully removed. High fever was observed in all patients and acute renal failure in one. In a recent study by Kishi et al,29 the efficacy of pleurodesis using both OK-432 and doxorubicin (an antineoplastic agent) for 20 patients with malignant pleural effusion was evaluated. After complete removal of the pleural effusion, OK-432 and 30 mg of doxorubicin were injected via an inserted chest tube. Responses were defined as follows: (1) complete

### Table 3—Pleural Fluid Protein and LDH Levels in the Study Groups*

<table>
<thead>
<tr>
<th>Pleurodesis Groups</th>
<th>Pleural Fluid Protein, g/dL</th>
<th>LDH, IU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half-dose combination, n = 8</td>
<td>3.9 ± 0.6</td>
<td>5,393 (1,857–3,405)</td>
</tr>
<tr>
<td>Quarter-dose combination, n = 8</td>
<td>3.3 ± 0.6</td>
<td>1,911 (1,469–1,765)</td>
</tr>
<tr>
<td>Half-dose doxycycline, n = 8</td>
<td>3.6 ± 0.7</td>
<td>2,940 (792–1,342)</td>
</tr>
<tr>
<td>Half-dose talc, n = 8†</td>
<td>2.6 ± 0.9</td>
<td>856 (694–1,131)</td>
</tr>
<tr>
<td>p value</td>
<td>0.035</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD or as median (IQR).†Pleural protein and LDH are significantly higher in the half-dose combination group compared to that in the half-dose talc group.

### Table 4—Correlation by Pearson Product Moment Correlation of Pleural Fluid Features With Right Pleurodesis Scores in All 53 Rabbits Including Historical Groups

<table>
<thead>
<tr>
<th>Pleural Fluid Features</th>
<th>No. of Rabbits</th>
<th>Correlation Coefficient</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume</td>
<td>53</td>
<td>0.550</td>
<td>0.001</td>
</tr>
<tr>
<td>24-h volume</td>
<td>53</td>
<td>0.441</td>
<td>0.0009</td>
</tr>
<tr>
<td>48-h volume</td>
<td>53</td>
<td>0.483</td>
<td>0.0002</td>
</tr>
<tr>
<td>72-h volume</td>
<td>50</td>
<td>0.313</td>
<td>0.002</td>
</tr>
<tr>
<td>LDH level</td>
<td>45</td>
<td>0.381</td>
<td>0.03</td>
</tr>
<tr>
<td>Protein level</td>
<td>45</td>
<td>0.464</td>
<td>0.01</td>
</tr>
<tr>
<td>Gliding sign</td>
<td>32*</td>
<td>0.876</td>
<td>0.0000000005</td>
</tr>
</tbody>
</table>

*Ultrasound was not performed in historical groups.

![Figure 2. The linear regression analysis between the pleurodesis score and the absence of gliding sign (r = 0.876, p < 0.00000000005; n = 32).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/22033/)
response, no fluid accumulation for > 4 weeks; (2) partial response, a marked decrease or no increase of pleural fluid for > 4 weeks; and (3) no response, reaccumulation of pleural fluid within 4 weeks. In total, 16 patients (80%) had a complete response, 2 patients (10%) had a partial response, and 2 patients had no response. The main side effects were fever and pain that were easily treated with nonsteroidal anti-inflammatory drugs. None of these studies used reduced doses rather than regular doses for pleurodesis. Therefore, to our knowledge, the present study is the first to assess synergy resulting from combining two different sclerosing agents with reduced doses to create pleurodesis in rabbits.

The pleural inflammation following instillation of a sclerosing agent is indicated by the accumulation of exudative pleural fluid with high WBC counts, protein, and LDH levels. In all of the 32 rabbits in the current study, the aspirated pleural fluid was an exudate with total volumes of 2 to 44 mL, and high LDH (> 1,900 U/L) and protein levels (> 2.90 g/dL). It also was bloody appearing in 81% of the rabbits. The positive correlation between the pleural score and fluid volumes, LDH, and protein levels in the current study supports the importance of inflammation in pleurodesis. We did not measure the pleural WBC count in this present study because our group has previously demonstrated that some of the talc particles remain in the pleural space for a variable length of time and can be counted as WBCs by automated cell counters.

In the present study, pleural fluid LDH levels were significantly higher in the half-dose combination group compared to the half-dose talc group. Additionally, the pleural fluid protein level was significantly higher in the half-dose combination group compared to the half-dose talc group. These findings suggest that combining talc and doxycycline in half doses might induce a greater inflammatory response in the pleura compared to a half dose of each drug alone.

To-and-fro movement of the visceral and parietal pleural surfaces can be seen with ultrasonic imaging during respiratory movements, and this motion is termed the pleural gliding sign. We have previously shown that the absence of a gliding sign is correlated with the presence of pleural symphysis at necropsy. The present study confirms the usefulness of the absence of a gliding sign for predicting pleurodesis in rabbits. In reality, the correlation coefficient for the relationship between the gliding sign and pleural symphysis was higher in the present study ($r = 0.876$) than in the previous study ($r = 0.506$). It remains to be demonstrated whether the absence of a gliding sign is indicative of pleurodesis in humans.

It should be noted that the gliding sign will also be absent if there is a pneumothorax.

A large right hemothorax occurred in one rabbit in the half-dose combination group. Although a high incidence of hemothorax and substantial mortality has been reported in rabbits administered intrapleural tetracycline derivatives without chest tube drainage, the insertion of a chest tube and daily aspirations of the accumulating fluid has prevented hemothorax and led to a better pleurodesis in rabbits. However, neither hemothorax nor death have been reported in human subjects receiving intrapleural tetracycline or its derivatives in whom the pleural fluid is drained with a chest tube.

The hemothorax is hypoechoic with a relatively homogeneous echotexture and several visible septations by ultrasonic examination. The visceral layer of the pleura could not be identified because of the size of the hemothorax. Therefore, gliding sign was absent in the rabbit with hemothorax.

Intense pleural pain caused by the intrapleural injection of tetracycline or its derivatives is a well-known complication of pleurodesis. Usually, conscious sedation with benzodiazepines or analgesia with a narcotic drug is recommended before starting pleurodesis with tetracycline or its derivatives. In our study, we did not observe signs of pain in any rabbit, but this is probably due to the fact that the doxycycline was injected while the rabbit was still under anesthesia.

Certain limitations to the present study should be noted. First, the animals were killed at 14 days, while animals in many other studies evaluating pleurodesis were killed at 28 to 30 days. However, more recent studies have shown that the mean degrees of macroscopic pleurodesis after an intrapleural tetracycline derivative or talc at 14 days and 28 days are virtually identical. Therefore, the timing of death should not affect the conclusions of the study. Second, historical controls for full-dose doxycycline and talc were used in the present study rather than concomitant controls. Since our results with doxycycline pleurodesis over the years have been consistent, we believed that the use of the additional rabbits was unnecessary. Third, since rabbits have a thin pleura while humans have a thick pleura, one must be careful in extrapolating results in rabbits to humans. Nevertheless, we believe that results in rabbits can provide guidance for pleurodesis in humans, as both talc and doxycycline have been shown to be effective pleurodesing agents in both species.

We conclude that combining half doses of talc and doxycycline is more effective than half doses of either drug alone or a full dose of talc, and is as effective as a full dose of doxycycline alone in
producing pleurodesis in rabbits. In addition, ultrasound is an accurate imaging modality for the evaluation of pleurodesis, in that the absence of pleural gliding on ultrasound correlates well with the presence of a pleurodesis in rabbits.

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