The short-term administration of Ketoprofen does not decrease the effect of Pleurodesis induced by talc or Doxycycline in rabbits

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Summary
Objective: To determine whether the concomitant administration of ketoprofen, a non-steroidal anti-inflammatory drug (NSAID) has any effect on the pleurodesis induced by talc or doxycycline in rabbits.

Methods: Four groups of seven New Zealand rabbits were assigned to receive the following treatments: 400 mg/kg of talc intrapleurally only (group 1), 400 mg/kg of talc plus 1 mg/kg of ketoprofen intramuscularly (group 2), 10 mg/kg of doxycycline intrapleurally only (group 3) and 10 mg/kg of doxycycline plus 1 mg/kg of ketoprofen intramuscularly (group 4). Intramuscular administration of ketoprofen began 4 h before the intrapleural administration of the sclerosing agents, followed by twice daily administrations for 1 week. Pleural fluid was collected 24, 48 and 72 h after intrapleural injections. Pleurodesis was evaluated macroscopically and microscopically after 14 days.

Results: The concomitant use of ketoprofen at 1 mg/kg does not decrease the WBC, LDH, and protein in pleural fluid at 24 h following intrapleural injection of talc or doxycycline. There were no significant differences in the macroscopic pleurodesis scores, the degree of microscopic pleural fibrosis, the thickness of the pleura or the percent of the pleura occupied with angiogenesis.

Conclusions: The study shows that the short-term systemic administration of NSAIDs does not affect the efficacy of pleurodesis induced by talc or doxycycline in rabbits.

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Introduction

Pleurodesis is a common treatment for patients with symptomatic recurrent pleural effusions or spontaneous pneumothorax. Many different agents have been administered into pleural cavity to induce a pleurodesis. It is thought that most of these agents induce the pleurodesis by injuring the pleura which leads to inflammation in the pleural space and subsequently pleurodesis if the degree of inflammation is sufficient. We have shown previously that when pleurodesis is induced in rabbits with talc or doxycycline, the concomitant use of systemic corticosteroids decreases the inflammatory process and diminishes the efficacy of the pleurodesis.

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as post-surgery analgesics in the management of pain associated with inflammatory conditions. Since inflammation is crucial for the production of pleurodesis, a reasonable question is whether NSAIDs adversely affect the production of a pleurodesis. Ketoprofen, an NSAID, is a reversible, short-acting, non-selective cyclooxygenase inhibitor, which particularly blocks the synthesis of thromboxane. Under in vitro conditions, it has been shown that ketoprofen stabilizes lysosomal membranes, restrains secretion of lysozomal enzymes from granulocytes, and prevents the aggregation of platelets as well as the immigration of leukocytes. However, there is no study examining the effects of ketoprofen on pleurodesis.

The aim of this study was to evaluate the effects of ketoprofen on the pleurodesis in rabbits resulting from talc or doxycycline. We hypothesized that the administration of ketoprofen would attenuate the pleural inflammation induced by talc or doxycycline and thus decrease the efficacy of pleurodesis in rabbits.

Materials and methods

Surgery procedure

The protocol was approved by Vanderbilt University Institutional Animal Care and Use Committee. The methods used were similar to those we have described previously. New Zealand white rabbits weighing 1.5–2.0 kg were anesthetized with 35 mg/kg ketamine hydrochloride (Fort Dodge Animal Health Laboratories; Fort Dodge, IA.) plus 5 mg/kg xylazine hydrochloride (Fermenta; Kansas City, MO) intramuscularly. The chest was shaved and cleansed with 10% povidone iodine (Baxter, Deerfield, IL). A small skin incision (<2 cm) was made between the tip of the scapula and the sternum approximately 2 cm above the costal margin. With the rabbit in the supine position, a chest tube, which was made from intravenous solution set tubing (Braintree Scientific, Braintree, MA) with five extra openings near the distal end, was inserted into the right pleural cavity by blunt dissection. The pleural cavity was closed and the chest tube was fixed with a purse-string suture. The exterior end of the chest tube was tunneled under the skin, drawn out of the skin posteriorly between two scapulae and sealed with a two-way valve with cap (Medexinc, Hilliard, OH) via an adapter and sutured to the skin. Air was aspirated from the right pleural cavity with a 20 cc syringe.

After chest tube placement seven rabbits were assigned to each of the following treatment groups: 400 mg/kg talc in 2 ml saline intrapleurally (talc control group, n = 7) 400 mg/kg talc in 2 ml saline plus ketoprofen 1 mg/kg twice a day intramuscularly (talc pre-treatment group, n = 7) 10 mg/kg doxycycline in 2 ml saline intrapleurally plus ketoprofen 1 mg/kg twice a day intramuscularly (doxycycline pre-treatment group, n = 7). Ketoprofen (1 mg/kg) was started 4 h before the intrapleurally administration of the agents and was administered twice a day intramuscularly thereafter for 1 week. Gentamicin 2 mg/kg was administered intramuscularly during the surgery and at 24 h intervals as long as the chest tubes were in place. The chest tube was removed when the pleural fluid drainage was less than 2 ml over the preceding 24 h.

The pleural space was aspirated every 24 h after chest tube placement and the volume of the fluid was recorded. The protein and lactate dehydrogenase (LDH) levels at 24 h were determined with an automated analyzer (Johnson & Johnson, Rochester, NY). The upper limit of normal for serum LDH was 220 IU/L. Total leukocyte count was measured using an automated counter (Coulter Electronics, Luton, UK).

The rabbits were sacrificed 14 days following chest tube placement. At sacrifice, the rabbit was anesthetized with pentobarbital (150 mg/kg ip) and then asphyxiated with CO2. 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 via a plastic catheter (6 mm diameter). Then the trachea was ligated and the entire thorax submerged into 10% neutral-buffered formalin solution for at least 48 h before the pleurodesis was graded.

Grading of pleurodesis score

The grading system for the pleurodesis score was on a scale of 1–8 as described in our previous studies. The grading procedure was performed by two investigators (R.W.L. and K.B.L.) who were blinded to the treatment group of the rabbit.

The degree of pleurodesis was evaluated as follows:

1. No adhesions between the visceral and parietal pleura.
2. Rare adhesions between the visceral and parietal pleura but not symphysis.
3. A few scattered adhesions between the visceral and parietal pleura but not symphysis.
4. Many adhesions between the visceral and parietal pleura, but no symphysis.
5. Many adhesions between the visceral and parietal pleura and a minimal amount of symphysis (<5%) of total area of hemithorax.
6. Many adhesions between the visceral and parietal pleura—symphysis in 5–25% of the hemithorax.
7. Many adhesions between the visceral and parietal pleura—symphysis in 25–50% of the hemithorax.
8. Many adhesions between the visceral and parietal pleura—symphysis in >50% of the hemithorax.

Adhesions were defined as fibrous connections between the visceral and parietal pleura whereas the symphysis was...
defined as fusion of the visceral and parietal pleura such that they were difficult to separate due to adhesions.

**Microscopic examination of pleura**

**Pleural fibrosis**

Samples of the visceral pleura and lung from the right hemithorax were collected and placed in 10% neutral buffered formalin. The tissue samples were stained with hematoxylin-eosin (H&E) for histologic examination. The pleural inflammation and fibrosis were graded as none (0), equivocal (1), mild (2), moderate (3), or severe (4), as previously described. The thickness of visceral pleura was measured using the Leica Q500IW Imaging Workstation, Image Processing and Analysis System (Leica Imaging Systems Ltd., Cambridge, UK). With this system, the image obtained was transformed from pixels to micrometers. Measurements were obtained at 10 different points on each sample, and the mean of these 10 measurements was reported.

**Pleural angiogenesis**

The degree of pleural angiogenesis (vascular density) was measured by immunostaining for factor VIII antigen (von Willebrand factor) as we have described previously. The measured by immunostaining for factor VIII antigen (von Willebrand factor) as we have described previously. The degree of pleural angiogenesis (vascular density) was measured by immunostaining for factor VIII antigen (von Willebrand factor) as we have described previously.5 The measured by immunostaining for factor VIII antigen (von Willebrand factor) as we have described previously. The thickness of visceral pleura was measured using the Leica Q500IW Imaging Workstation, Image Processing and Analysis System (Leica Imaging Systems Ltd., Cambridge, UK). With this system, the image obtained was transformed from pixels to micrometers. Measurements were obtained at 10 different points on each sample, and the mean of these 10 measurements was reported.

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**Microscopic pleural adhesions**

The administration of ketoprofen did not significantly influence the pleurodesis scores (Fig. 1). The mean pleurodesis score of talc control group (4.43±0.90) did not differ significantly (P>0.05) from the group that also received ketoprofen (6.00±0.29). The mean pleurodesis scores of doxycycline control group (7.43±0.35) did not differ significantly from the group that also received ketoprofen (7.00±0.38).

### Table 1 Pleural fluid characteristics at 24 h after intrapleural injection of doxycycline or talc.

<table>
<thead>
<tr>
<th>Pleural fluid</th>
<th>Groups</th>
<th>Ketoprofen+doxycycline</th>
<th>Doxycycline</th>
<th>Ketoprofen+talc</th>
<th>Talc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>24 h</td>
<td>6.3±1.3</td>
<td>3.8±1.5</td>
<td>1.3±0.3</td>
<td>1.8±0.8</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>5.6±1.9</td>
<td>4.2±1.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>2.9±1.5</td>
<td>2.8±1.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WBC (cells/ul)</td>
<td>24 h</td>
<td>2601±719</td>
<td>3286±1030</td>
<td>2785±568</td>
<td>3532±1335</td>
</tr>
<tr>
<td></td>
<td>48 h</td>
<td>3147±771</td>
<td>1725±341</td>
<td>4442±1014</td>
<td>3576±910</td>
</tr>
<tr>
<td></td>
<td>72 h</td>
<td>3.2±0.2</td>
<td>2.7±0.3</td>
<td>2.9±0.4</td>
<td>3.4±0.5</td>
</tr>
</tbody>
</table>
Microscopic analysis

Pleural fibrosis and thickness
There were no significant differences (P > 0.05) in the microscopic grading score for pleural fibrosis between the talc control group (2.25 ± 0.39) and the pre-treatment talc group (3.02 ± 0.28), or between the doxycycline control group (2.47 ± 0.30) and the pre-treatment doxycycline group (2.42 ± 0.24) (Fig. 2). The rabbits that were pretreated with ketoprofen showed no significant difference from those given only doxycycline or talc in the thickness of the visceral pleura (Fig. 3).

Pleural angiogenesis
The administration of ketoprofen did not appear to decrease the degree of visceral pleural angiogenesis (Fig. 4). The vascular densities (percentage of the area) in rabbits that received talc with and without ketoprofen were 3.36 ± 0.28% and 4.09 ± 1.36%, respectively, and in rabbits that received doxycycline with and without ketoprofen were 3.09 ± 0.74% and 4.42 ± 0.77%, respectively. There were no significant differences in the degree of visceral pleural angiogenesis measured by immunostaining for factor VIII between ketoprofen pretreated rabbits and control rabbits (P > 0.05).

Discussion
The present study demonstrates that the pre-treatment of ketoprofen at 1 mg/kg does not decrease the WBC, LDH, and protein in pleural fluid at 24 h following intrapleural injection of talc or doxycycline. Furthermore, the short-term administration of ketoprofen at 1 mg/kg twice daily for one week does not reduce the efficacy of pleurodesis induced by talc or doxycycline in rabbits at 14 days. There were no significant differences in the macroscopic pleurodesis scores, the degree of microscopic pleural fibrosis, the thickness of the pleura or the percent of the pleura occupied with angiogenesis.

The mechanism of pleurodesis induced by most of the agents such as talc and doxycycline is not fully understood. It is commonly accepted that pleural inflammation plays a
key role in the production of a pleurodesis. The pleural inflammation is characterized by denudation of mesothelial cells, infiltration of neutrophils, development of exudative pleura fluid, increased vascular permeability and angiogenesis of pleura. Many kinds of cells, cytokines and fibrogenesis factors are involved in this process. Corticosteroids which have potent effects on inhibiting both the early and the latter manifestations of inflammation reduce the efficacy of pleurodesis. The results of our previous study showed that the pre-treatment and then weekly administration of systemic corticosteroids (triamcinolone, 0.8 mg/kg) essentially prevented an effective pleurodesis at 28 d.2 However, there is still controversy whether inflammation plays a pivotal role in the production of a pleurodesis, since the intrapleural administration of transforming growth factor (TGF)-beta can induce an effective pleurodesis without inducing a significant inflammatory response and the production of the pleurodesis is not inhibited by the systemic administration of corticosteroids.9

NSAIDs are currently used as anti-inflammatory drugs and analgesics due to their property of inhibiting cyclooxygenases (COX) that are involved in the formation of prostanooids. Two isoforms COX-1 and COX-2 exist, and COX-2 is thought to be the main source of prostanooids during inflammation.10–13 In the model of carrageenan-induced pleurisy in rats, Vinegar et al.14 showed that the early (<48h) inflammatory response in subpleural tissue is depressed by NSAIDs (aspirin and indomethacin) while corticosteroids decrease both the early and the late inflammatory response. Since systemic corticosteroids decrease the effectiveness of pleurodesis,2 the question is whether the use of NSAIDs can negatively affect the pleurodesis induced by sclerosing agents.

There are several previous studies15,16 that have been published on the effect of NSAIDs on pleurodesis. Lardinois et al.15 demonstrated that daily use of 2 mg/kg diclofenac for three weeks decreased the quality of pleurodesis in pigs after mechanical pleural abrasion. In a second study Teixeira et al.16 administrated 1.1 mg/kg diclofenac intramuscularly to rabbits that were given silver nitrate or talc before, then daily for seven days and thereafter weekly until the animals were sacrificed on day 28. They found that silver nitrate induced more pleural inflammation and more effective pleurodesis than did talc. In addition, the sustained systemic administration of NSAIDs reduced the degree of pleural adhesions in animals that received talc but not in those that received silver nitrate. They speculated that the anti-inflammatory agents did not reduce the pleurodesis score in rabbits receiving silver nitrate because the silver nitrate induced a greater inflammatory response which was still sufficient after the NSAIDs to result in a pleurodesis.

Why do the results in the present study differ from those of previous studies? There are several possible explanations. First, the NSAIDs used in our study are different from those used in previous studies. Ketoprofen, which used in our study, is relatively COX-1 selective, whereas diclofenac, which was used in previous studies, is relatively COX-2 selective. In general, the COX-2 enzyme is believed to locate at sites of inflammation and COX-1 enzyme is thought to be mainly within stomach and duodenum.10,11 However, it should be noted that until to date, there is no evidence that the more selective COX-2 NSAIDs are more effective anti-inflammatory agents than are the in the less selective COX-2 NSAIDs. Second, the length of time that the NSAIDs administered is different. In present study, ketoprofen was administered for 1 week, whereas in the other studies diclofenac was used for 3–4 weeks until the animals were sacrificed. Third, the conflicting results could be due to different doses of the NSAIDs. This is unlikely because the ketoprofen dose used in the present study and diclofenac doses used in the previous studies are the recommended dose in humans.11

In addition to the pleurodesis scores, other data from the present study supports our conclusions that ketoprofen does not affect the inflammatory response or the resulting pleurodesis. Ketoprofen had no effect on the levels of the WBC and LDH. These inflammatory markers had not been measured in the two previous studies on NSAIDs and pleurodesis.15,16 In our previous study,2 we showed that the degree of angiogenesis was closely correlated with the degree of pleurodesis. However, in the present study the administration of ketoprofen had no effect on the pleural angiogenesis.

The present study has important clinical implications. It demonstrates that the use of NSAIDs for the first 7 days after a pleurodesing agent is injected does not significantly reduce the effectiveness of pleurodesis. In clinical practice anti-inflammatory agents are frequently used for a few days in patients with chest tubes who undergo pleurodesis. However, we cannot eliminate the possibility that the administration of NSAIDs for a longer period may have a negative effect on pleurodesis.

Some limitations of our study should be noted. First, we notice that some inflammatory parameters seem higher in the NSAID group while some are lower and that there is no consistent pattern. This indicates that number of rabbits in each group, according to the power analysis, may be not enough to detect the difference with a P value lower than 0.5. Second, the study was done in rabbits and one must always be careful in extrapolating results in animals to human adults. This may be particularly important since rabbits have a thin visceral pleura while humans have a thick visceral pleura.

In conclusion, the present study demonstrates that the short-term systemic administration of NSAIDs does not affect the efficacy of pleurodesis induced by talc or doxycycline in rabbits. The effect of NSAIDs on pleurodesis in human requires confirmation in a clinical trial.

References


