Influence of Antiinflammatory Drugs (Methylprednisolone and Diclofenac Sodium) on Experimental Pleurodesis Induced by Silver Nitrate or Talc*

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Study objective: To determine whether the administration of antiinflammatory drugs interferes with experimental pleurodesis induced by silver nitrate or talc.

Study design: Two groups of 30 white New Zealand rabbits were scheduled to receive an intrapleural injection of 0.5% silver nitrate or 400 mg/kg of talc. Each group was further classified into three subgroups (10 animals each), which received the following: (subgroup 1) the sclerosing agent only, (subgroup 2) the sclerosing agent plus 1 mg/kg of methylprednisolone, and (subgroup 3) the sclerosing agent plus 1.1 mg/kg of diclofenac sodium. The antiinflammatory agents were administered IM 24 h before the sclerosing agent and daily during the first week, followed by once-weekly injections until death at 28 days. At this time, the pleural cavity was macroscopically evaluated, and samples of pleura and lungs were collected for further microscopic examination.

Measurements and results: The degree of pleural adhesions was higher after silver nitrate administration ($p = 0.019$). No reduction in the adhesions was observed after administering antiinflammatory drugs to this group ($p > 0.05$). Conversely, the adhesion score was significantly reduced after administration of both prednisolone ($p = 0.028$) and diclofenac ($p = 0.032$) to the animals that received talc. Administration of the antiinflammatory agents did not influence microscopic pleural or lung changes induced by silver nitrate or talc.

Conclusion: These results show that the sustained systemic administration of antiinflammatory agents (steroidal or nonsteroidal) reduces the degree of pleural adhesions in animals with talc-induced pleurodesis but does not affect silver nitrate-induced pleurodesis. Extrapolation of these results to humans suggests that the use of antiinflammatory drugs should be avoided in patients with talc-induced pleurodesis and that appropriate clinical studies with silver nitrate should be conducted in patients chronically treated with these antiinflammatory agents.

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Key words: antiinflammatory drugs; pleurodesis; silver nitrate; talc

Chemical pleurodesis is used in the treatment of recurrent pleural diseases, especially in patients with malignant effusions when pleural symphysis is required. Chemical pleurodesis is used in the treatment of recurrent pleural diseases, especially in patients with malignant effusions when pleural symphysis is required. Intrapleural instillation of a sclerosing agent causes damage to the mesothelial layer, initiating an inflammatory process that culminates in the development of fibrosis that obliterates the pleural space.

The inflammation produced during the initial stage of pleurodesis is potentially susceptible to modulation with antiinflammatory agents, which, in theory, can reduce the amount of fibrosis expected. Experimental studies have shown that corticosteroids reduced the degree of pleurodesis induced by talc or doxycycline but not transforming growth factor-$
\beta$. In a recent study, Lardinois et al demonstrated in pigs that the administration of nonsteroidal antiinflammatory drugs during the perioperative period also reduces pleural adhesions caused by abrasion. Based on this evidence, we asked if the pleu-
rodesis induced by a caustic sclerosing agent (silver nitrate) could be inhibited by interfering in the inflammatory and fibrotic process. These findings would be of clinical importance, as a significant number of patients who are candidates for pleurodesis are affected by other diseases with treatments based on the long-term use of antiinflammatory agents. The objective of the present study was to investigate the effect of systemically administered methylprednisolone or diclofenac sodium on experimental pleurodesis induced by silver nitrate or talc, both effective sclerosing agents in humans.6

**Materials and Methods**

The study protocol was approved by the Ethics Committee of the Heart Institute (InCor), University of São Paulo Medical School, and animal use conformed to the National Council guidelines. Sixty white New Zealand rabbits weighing 2 to 3 kg were classified into two groups of 30 animals each and were scheduled to receive an intrapleural injection of either 0.5% silver nitrate or 400 mg/kg of talc (in a total volume of 2 mL). Each group was further divided into three subgroups (10 animals each) and managed as follows: one subgroup received only the sclerosing agent (nitrate or talc), the second subgroup received the sclerosing agent plus 1 mg/kg of the corticosteroid methylprednisolone (prednisolone), and the third group received the sclerosing agent plus 1.1 mg/kg of the nonsteroidal antiinflammatory agent diclofenac sodium (diclofenac).

We utilized the talc currently used to induce pleurodesis in our clinical practice and also used in our previous experimental studies, a magnesium silicate asbestos-free particle (Magnetisa; Brumado, Bahia, Brazil), with median diameter of 25.4 μm (range, 6.4 to 50.5 μm). The antiinflammatory agents were administered IM 24 h before intrapleural injection of the sclerosing agent. After the procedure, the animals received one daily injection of antiinflammatory agent during the first week, followed by one weekly injection until death at 28 days.

The method used was similar to that reported in previous studies by our group. After the induction of anesthesia by the IM injection of 35 mg/kg of ketamine hydrochloride and 5 mg/kg of xylazine hydrochloride, the animals were prepared for aseptic surgery. A 0.5-cm incision was made in the right hemithorax, and a catheter (6F chest tube) was tunneled through the subcutaneous tissue to prevent possible attempts at removal by the animal. Next, the parietal pleura was exposed, and the catheter was inserted into the pleural space, followed by suture, fixation, and injection of the sclerosing agent. The drain was maintained for a minimum period of 72 h and removed when the amount of aspirated pleural fluid was < 1 mL/d.3,10 In all animals, the surgical procedures were performed on the right hemithorax, while the left hemithorax was used as a control.

After 28 days, the animals were killed by an IV injection of 40 mg/kg of pentobarbital into the marginal ear vein. The thorax was removed en bloc, and the lungs were expanded with 60 mL of 10% formalin and submerged in this solution for at least 48 h. After this period, each pleural cavity was exposed using the method previously described by our group.2,3,5,8 Then, with the thorax opened, we evaluated the presence of macroscopic pleural adhesions and collected fragments from the pleura and pulmonary parenchyma for later microscopic analysis.2,3,5,8

The degree of pleural adhesions was established as described previously2,3,5,8 using the following scoring system: 0 = normal pleural space, 1 = one to three small adhesions, 2 = more than three adhesions (lung easily separated from the thorax), 3 = generalized adhesions (showing areas where the lung could only be separated from the thoracic wall with difficulty), and 4 = complete obliteration of the pleural space by adhesions. Samples of the visceral pleura and lung were obtained from the anterior lower lobes and placed into a 10% neutral buffered-formalin solution. After processing these tissue samples for histologic examination and staining them with hematoxylin-eosin, the microscopic slides were evaluated for the presence of inflammation and fibrosis. Inflammation and fibrosis were graded on a scale of 0 to 4 according to the intensity of the process, where 0 = normal pleura or parenchyma, 1 = equivocal, 2 = mild changes, 3 = moderate changes, and 4 = marked changes.2,3,5,8

The microscopic analysis was done by an experienced examiner (L. A.) blinded to the treatment agent. We collected samples from only one site because no significant variations are observed when samples are taken from different pulmonary lobes.9 When adhesions or symphysis were present at the site of dissection, the samples were obtained by careful dissection in order to keep the integrity of the visceral pleura.

The results are reported as the mean and SD. Statistical significance was determined via the Mann-Whitney rank-sum test and t test using statistical software (SigmaStat; Jandel Scientific; San Rafael, CA). Differences were considered to be significant at p < 0.05.

**Results**

At death, pleurodesis, characterized by the presence of macroscopic pleural adhesions, was more evident after the intrapleural injection of 0.5% silver nitrate (3.2 ± 1.1) than talc (2.2 ± 0.8) [p = 0.019]. Administration of the antiinflammatory drugs prednisolone (3.5 ± 0.5; p = 0.578) or diclofenac (3.3 ± 1.0; p = 0.870) did not reduce the number of pleural adhesions in the animals injected with silver nitrate (3.2 ± 1.1). However, the adhesion score was significantly lower in the groups receiving prednisolone (1.3 ± 0.7; p = 0.028) or diclofenac (1.2 ± 0.7; p = 0.032) when talc was used as the sclerosing agent (2.2 ± 0.8) [Fig 1].

With respect to microscopic changes, the pleural inflammation produced after the instillation of the sclerosing agent was mild after silver nitrate (2.0 ± 0.9) and minimal after talc (1.2 ± 0.2), with no significant difference between the two agents (p = 0.053; Table 1). There was no significant difference in the degree of pleural inflammation induced by silver nitrate observed in animals receiving prednisolone (1.3 ± 0.5; p = 0.136) or diclofenac (2.2 ± 1.6; p = 0.795). Similarly, there was no significant difference in the degree of pleural inflammation induced by talc in animals receiving prednisolone (0.9 ± 0.3; p = 0.515) or diclofenac (0.7 ± 0.5; p = 0.170).

Pleur al fibrosis was significantly more evident in the animals that received silver nitrate than in those that received talc (3.2 ± 0.6 vs 1.8 ± 0.8, p < 0.001; Table 1). Similar to the macroscopic pleural adhe-
sion scores, the degree of microscopic fibrosis did not differ between the group receiving silver nitrate plus corticosteroid (3.4 ± 0.5; p = 0.548) or diclofenac (3.2 ± 1.0; p = 0.935). In contrast to the observation made for macroscopic pleural adhesions, neither prednisolone (1.5 ± 1.0; p = 0.426) nor diclofenac (1.4 ± 0.8; p = 0.366) significantly influenced the degree of microscopic pleural fibrosis in the group receiving talc (Table 1).

The mean alveolar inflammation and fibrosis scores also reflected the minimal changes caused by the sclerosing agents; these scores were significantly lower in the animals receiving talc (p < 0.05; Table 1). The antiinflammatory treatment did not influence the changes observed when only the sclerosing agent was introduced into the pleural space. No changes were observed in the left hemithorax, which was used as a control for the right hemithorax (ie, a score of zero on the macroscopic and microscopic evaluations was obtained in all animals studied).

**Discussion**

This study demonstrates that the sustained systemic administration of antiinflammatory agents (steroidal or nonsteroidal) reduces the degree of pleurodesis induced by the intrapleural injection of talc but not silver nitrate, and confirms the previously demonstrated superiority of silver nitrate over talc as a pleural sclerosing agent in the experimental animal model.7,8 The mechanisms responsible for the production of pleurodesis are not completely established. It is believed that the injection of a sclerosing agent into the pleural space damages the mesothelial cells, triggering an inflammatory process characterized by a neutrophilic exudate.1 This initial change culminates in the development of adhesions between the visceral and parietal pleura, obliterating the virtual pleural space. Factors that modulate these reactions include the degree of injury, the ability of mesothelial cells and fibroblasts to secrete collagen, and the balance between metalloproteinases and plasminogen activators, in addition to the relationship among inflammation, cytokines, and fibrinolysis.1

As the duration, extent, and intensity of inflammation established in the pleural space influence the final outcome of pleurodesis,1,3 the use of antiinflammatory agents can inhibit this process, consequently reducing pleural adhesions. This finding has been

**Table 1—Scores Corresponding to the Degree of Microscopic Pleuropulmonary Changes**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pleural Inflammation</th>
<th>Pleural Fibrosis</th>
<th>Alveolar Inflammation</th>
<th>Alveolar Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver nitrate</td>
<td>2.0 ± 0.9</td>
<td>3.2 ± 0.6†</td>
<td>1.5 ± 0.7†</td>
<td>1.3 ± 0.9†</td>
</tr>
<tr>
<td>Plus prednisolone</td>
<td>1.3 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>1.7 ± 1.5</td>
<td>0.7 ± 0.8</td>
</tr>
<tr>
<td>Plus diclofenac</td>
<td>2.2 ± 1.6</td>
<td>3.2 ± 1.0</td>
<td>1.9 ± 1.0</td>
<td>1.3 ± 1.7</td>
</tr>
<tr>
<td>Talc</td>
<td>1.2 ± 0.2</td>
<td>1.8 ± 0.8</td>
<td>0.7 ± 0.5</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Plus prednisolone</td>
<td>0.9 ± 0.3</td>
<td>1.5 ± 1.0</td>
<td>0.5 ± 0.7</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Plus diclofenac</td>
<td>0.7 ± 0.5</td>
<td>1.4 ± 0.8</td>
<td>0.6 ± 0.5</td>
<td>0.0 ± 0.0</td>
</tr>
</tbody>
</table>

*Values are presented as the mean ± SD.
†p < 0.05, silver nitrate vs talc.
previously documented in experimental studies demonstrating that the administration of steroidal antiinflammatory drugs (corticosteroids) reduced the effectiveness of pleurodesis induced by doxycycline and talc, while the administration of a nonsteroidal antiinflammatory drug (diclofenac) decreased the degree of pleurodesis produced by mechanical abrasion. Conversely, another study showed that the systemic administration of corticosteroids does not interfere with pleurodesis induced by the intrapleural injection of transforming growth factor-β. In extrapolating these results to humans, we may infer that, depending on the sclerosing agent instilled, previous or concomitant antiinflammatory treatment can interfere with the effectiveness of the pleurodesis achieved, thus impairing the appropriate management of pneumothorax or pleural effusions.

In experimental studies, silver nitrate has been shown to be a more effective sclerosant than talc, a finding again confirmed in the present study. A previous clinical study has shown that the effectiveness of silver nitrate is similar to that of talc in inducing pleurodesis for the control of malignant pleural effusions, with no evidence of respiratory distress syndrome. In the present study, we also found that, in contrast to talc-induced fibrosis, the degree of silver nitrate-induced pleurodesis remained unchanged after the systemic administration of antiinflammatory drugs. The group of animals that received talc and antiinflammatories (steroidal or nonsteroidal) showed a significant reduction in pleural adhesions, suggesting a clear decrease in the effectiveness of pleurodesis; interestingly, no changes in pleural fibrosis were observed. Of note, although the pleural inflammation showed no difference (p < 0.053) between silver nitrate and talc, a greater number of animals would probably lead to a statistically significant difference.

Although silver nitrate induced more alveolar inflammation and fibrosis than did talc, the changes were minimal and similar to those described in our previous studies, the systemic use of antiinflammatory drugs did not change these parameters. The reduction in the degree of talc-induced pleurodesis to the administration of prednisolone was expected, since corticosteroids inhibit early and late components of the inflammatory process. During the initial phase, these drugs reduce the tissue edema, fibrin deposition, capillary dilatation, leukocyte migration, and phagocytosis; their action during the late phase of the inflammatory process includes a reduction in fibroblast proliferation, collagen deposition, and healing. Similarly, diclofenac also reduced the degree of talc-induced pleurodesis. The exact mechanism by which nonsteroidal antiinflammatory drugs influence collagen deposition and the occurrence of adhesions is not completely understood. However, similar to corticosteroids, nonsteroidal antiinflammatory drugs reduce leukocyte migration, edema formation, capillary dilatation, and fibrosis production by suppressing prostaglandin synthesis. In addition, these drugs act as immunomodulatory agents, blocking the endogenous production of prostaglandin E; this prostaglandin is involved in the regulation of collagenase, an enzyme with an important function in the derangement and remodeling of collagen.

In contrast to the findings with talc, we observed no reduction in the degree of pleurodesis with the use of the antiinflammatory agents (steroidal or nonsteroidal) in animals that received intrapleural silver nitrate. This observation supports the theory that these sclerosing agents produce pleurodesis by different mechanisms. Silver nitrate has an intense caustic action, which appears to induce generalized necrosis of mesothelial cells. Thus, compared with talc, silver nitrate likely causes much more intense inflammation, which is less responsive to systemically administered antiinflammatory agents; in contrast, talc seems to have a mild and gradual action, mainly provoking mesothelial cell apoptosis. Due to its biopersistence, cells are believed to remain viable causing a more prolonged and progressive release of inflammatory mediators. Thus, regular administration of antiinflammatory agent should act in a more effective way, since it interferes with both the early and late phases of inflammation pleural.

Another viewpoint is based on the demonstration that in vitro, cell toxicity and lysis occur in a dose- and time-dependent fashion. Silver nitrate seems to induce a more intense injury than talc, suggesting that silver nitrate would be more toxic to the mesothelial cell.

Based on these findings, we speculate that talc stimulates mesothelial cells to actively coordinate the inflammatory response and that silver nitrate probably modulates this response by other mechanisms. Regarding pleural effusion, our findings indicate that the number of leukocytes in the group that received silver nitrate plus antiinflammatory drugs is higher when compared to the group receiving with silver nitrate alone (data not shown). We also can speculate that the antiinflammatory agents when used in correspondent clinical doses in silver nitrate pleurodesis are not able to inhibit the inflammatory process.

One limitation of our study is the low score of pleurodesis obtained with talc when compared to silver nitrate. Although, ideally, the doses used of both agents should be of “equivalent potency,” these doses are very difficult to define due to variation of the in vivo response. New studies should be done.
using different doses of these sclerosing agents to test the influence of antiinflammatory drugs in similar score pleurodesis.

The present study has important clinical implications. If the results obtained in rabbits are extrapolated to humans, the concomitant use of antiinflammatory agents may compromise the effectiveness of talc-induced pleurodesis. This finding is important since many patients who undergo pleurodesis (e.g., patients with chronic lung diseases and pneumothorax or those undergoing chemotherapy) use antiinflammatory drugs either as analgesics or as a supplement to other treatments. Thus, we should consider the recommendation of not using antiinflammatory treatment during the induction of pleurodesis, especially if talc is chosen as the sclerosing agent.

As silver nitrate is a potent pleurodesis-inducing agent and is insensitive to the action of antiinflammatory agents, human studies should be undertaken to define its usefulness in patients who are receiving long-term treatment with antiinflammatory drugs. A randomized study in patients submitted to talc or silver nitrate pleurodesis, using antiinflammatory or analgesic drugs, could be done to confirm our experimental results.

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