Acute inflammatory response secondary to intrapleural administration of two types of talc

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All authors reviewed and approved the manuscript.

Short Title:
Inflammatory response secondary to talc
**Abstract**

Intrapleural instillation of talc has been used in the treatment of recurrent pleural effusions but can in rare instances, result in respiratory failure. Side effects seem to be related to composition, size and inflammatory power of talc particles. The aim of this study was to evaluate the inflammatory response to intrapleural injection of small (ST) or mixed (MT) talc. One hundred rabbits received intrapleural talc, 50 with ST (median 6.41 \( \mu \)m) and 50, with MT (median 21.15 \( \mu \)m); thirty five composed the control group. Cells, LDH, C reactive protein (CRP), IL-8 and VEGF were evaluated in serum and BAL at 6, 24, 48, 72 and 96 hours. Lung histology and presence of talc were also analyzed. Statistics: Anova and t-test. Most of the parameters showed greater levels in the animals injected with talc than in the control, suggesting a systemic and pulmonary response. Higher serum levels of CRP and IL-8 were observed in the animals injected with ST. Talc particles were observed in both lungs with no differences between groups. Lung cells infiltrate was more evident in the ST group. We conclude that talc of larger particles should be preferred in clinical practice in order to induce a safer pleurodesis.

**Key Words:** Bronchoalveolar lavage, Cytokines production, Inflammatory mediators, Pleurodesis, Rabbits
Introduction

Patients with malignant pleural disease frequently present recurrent pleural effusion, justifying the recommendation of pleurodesis. Among the proposed sclerosing agents, talc is the most used, as it is widely available, at low cost and high efficacy [1-7]. However, despite the common use, its administration may be questionable due to the development of undesirable side effects [3, 7, 8-12].

It is important to bear in mind that talc particles have been found in samples from bronchoalveolar lavage (BAL) and pulmonary tissues of patients undergoing pleurodesis [7, 11]. It is believed that starting from the pleural cavity, talc particles migrate to the pulmonary parenchyma through the stomas of the parietal pleura, reach systemic circulation, and later on return to the lungs [13]. Another pathway of dissemination would be through the mesotelial layer by rupture or invagination of the intercellular spaces of the visceral pleura, and reaching the adjacent pulmonary parenchyma [14, 15]. In this scenario, the smaller the size of the particle, the higher the chance of migration. In fact, countries where smaller talc particles are used, in general present a greater incidence of respiratory failure following pleurodesis [16]. However, contradictory to these facts, there is in the literature a necroscopic reference to a patient who died as a result of respiratory failure after undergoing pleurodesis, with no evidence of systemic migration of talc [12]. This report allows us to consider the existence of other mechanisms, one being the composition of the talc itself. In this case, the local inflammatory reaction could have contributed to an unfavorable outcome, as a result of the production of inflammatory markers which could reach the pulmonary parenchyma by the visceral pleura or to produce a systemic response and later on, an acute pulmonary response [12].

In this case, even though the dissemination of talc is recognized, there are few clinical or experimental studies evaluating the response of inflammatory markers in the bronchoalveolar lavage [17]. Thus, the aim of this study is to analyze, in an experimental model, the pulmonary and systemic changes secondary to the intrapleural administration of different sizes of talc particles.
Materials and Methods

The project was approved by the local ethics committee and was conducted in the laboratory of pleural diseases (University of Sao Paulo Medical School, Brazil).

In this study (figure 1) 135 New Zealand rabbits (2-3 Kgs) were divided in two groups: control (n=35) and experimental (n=100). In the control group, five animals underwent only bronchoalveolar lavage (simple control); five were used for lung histological analysis (control without undergoing BAL) and twenty five, underwent an intrapleural injection of saline with BAL (sham control). The experimental group (n=100) received in the right hemithorax, an intrapleural injection of talc (400mg/kg) diluted in saline. Of these, 50 animals received calibrated talc and 50, mixed talc as used in clinical practice. A sampling of bronchoalveolar lavage was collected from 25 animals in each subgroup. Of the remainder (n=25), no bronchoalveolar lavage was carried out to avoid interference in the histological analysis of the lungs. The calibrated talc is predominantly constituted of particles of small size (ST), being supplied by the Sigma Aldrich Company (Steinheim, Germany); the mixed talc (MT) containing particles of varying sizes was supplied by the Magnesita Company (São Paulo, Brazil). The composition and the size of the particles are shown in table 1.

Prior to carrying out these procedures, the animals were sedated by an intramuscular injection of Ketamine Chlorohydrate (35mg/Kg) and Xylazine Chlorohydrate (5mg/Kg). For the euthanasia, pentobarbital (60mg/Kg) was injected in the marginal vein of the ear, after 6, 24, 48, 72 or 96 hours of the intrapleural injection of talc. Immediately after this procedure, the abdominal cavity was opened and a blood sample was taken from the inferior vein cava.

In order to obtain samples of the bronchoalveolar lavage, the trachea was dissected and opened. A catheter (#8) was placed into the main bronchus to infuse 20ml of physiological solution, first to the left and then to the right side, avoiding mixture of BAL samples. For the histological analysis of the lungs, the thorax was removed in block and 10% formalin was injected through the trachea to avoid the collapse of the lungs.

The cytological study included a hemogram and the quantitative analysis of the bronchoalveolar lavage. Measurements of the lactate dehydrogenase (LDH – semi-automated UV kinetic method), urea
(colorimetric assay) and C reactive protein (CRP - immunoaglutination) were carried out in blood and in lavage supernatant.

Samples were stored at -80°C for future measurement of the cytokines Interleukin-8 (IL-8 – Opt EIA, Pharmingen, San Diego, CA) and vascular endothelial growth factor (VEGF –R&D Systems, Minneapolis, MN, USA) - ELISA method. In order to correct the concentration of the biochemical parameters analyzed in the bronchoalveolar lavage, the relation between serum and BAL urea was used [18, 19]. The histological analysis of the pulmonary parenchyma included the semi-quantitative evaluation of cellular infiltrate, edema, capillaritis, hemorrhage and thrombosis which are parameters associated with ARDS. The evaluation was done by two independent pathologists (LA and VLC), who were blinded to allocation. Ten randomized fields (x400) were analyzed and the results were expressed as score (0 to 3) according to the degree of pulmonary impairment [20].

Statistical analysis
The data were expressed as a median and quartiles (25 and 75%) or mean and standard deviation, depending on the distribution of the samples. To compare the results obtained with both types of talc, the t-Student or Mann-Whitney test was used. In order to compare the findings from the right and left lungs, we use the t-paired test (normal distribution) or Wilcoxon (non-normal distribution). The temporal analysis of the variables was carried out by variance analysis (ANOVA). Tukey or Dunn multiple comparison tests were used when significant differences were found (p<0.05). The SigmaStat program (SPSS Inc, San Raphael, USA) was used for the statistical analysis.

Results
No significant differences were observed between Sham and simple control (figures 2 to 4).

The leukocytes count and the LDH (blood and BAL) measurement did not reveal significant differences between both types of talc and the control group and not even between the left and right lungs (BAL).

Compared to the control group, after 6 hours the animals injected with talc showed a marked increase in the serum levels of CRP, which remained high throughout the whole study period (96 hours), reaching their highest value after 24 hours of the procedure. The small talc particles induced a
greater systemic inflammatory response, with statistical significance after 24 hours of the administration (figure 2). The levels of CRP were untraceable in the bronchoalveolar lavage of both study groups.

Among the groups that received talc, the serum levels of VEGF increased after 24 hours, and remained high throughout the whole evaluated period. This increase was independent of the size particles. In relation to BAL, both lungs presented high VEGF levels after 6 hours of the intrapleural injection and remained high during the 96 hours, without significant differences between the two types of talc. It should be noticed that in the first 72 hours, the BAL from the right lung (injected side) presented higher VEGF levels than the not injected side (figure 3).

In relation to interleukin 8, in both types of talc, the serum levels remained high throughout all the study period. Analyzing the BAL, we observe on the right, higher levels than the control at all times. Comparing with the mixed talc, the animals injected with talc of small particles presented higher levels at 72 and 96 h. The injection of talc did not cause significant differences in the levels of IL-8 on both LBA sides. On the left, the animals injected with ST presented higher levels of IL-8 at all times, a fact observed from the 24 h on in the animals injected with mixed talc (figure 4).

Based on the histological evaluation, we did not observe significant differences between the two experimental groups for most of the parameters. Only the cellular infiltrate was more pronounced from 48 h on in the animals injected with small talc (figure 5).

Discussion

We observed that both types of talc when injected in the pleural cavity produce an acute pulmonary and systemic inflammatory response which tends to be more pronounced in the animals injected with small talc.

Previous studies have shown that talc particles could be found in the bronchoalveolar lavage and lung tissue of patients undergoing pleurodesis [7, 11]. Among the factors related to the inflammatory response some, such as the size, composition of the talc particles, and the injected dose, should be taken into consideration [7,11,16,17,20]. Our study supports the observation that the early clinical manifestation, in particular the acute respiratory failure, appears in the first 96 hours following the procedure. In the present study, both types of talc produced an acute inflammatory
response; blood levels of CRP, VEGF, and IL-8 rose in the first 48 hours of the procedure, with a fall at the subsequent time intervals. The bronchoalveolar lavage tended to present similar response for VEGF and IL-8.

The maximum levels of IL-8 (BAL) were obtained after 24 hours of the intrapleural injection. This behavior was noted later on in the serum, suggesting that the pulmonary inflammatory response precedes the systemic one. Similar results were observed by Genofre et al [21] who cited this behavior after analyzing blood and pleural fluid in rabbits submitted to talc pleurodesis.

It is of interest to point out that the small talc particles induced a more pronounced inflammatory response as demonstrated by the increasing of CRP and IL-8 levels. Maskell et al [22] evaluating the gradient of alveolar-arterial pressure of oxygen and the C reactive protein, also showed the presence of a more evident systemic and pulmonary inflammation after the use of small talc particles.

The evaluation of the pulmonary response showed an increase in the bronchoalveolar lavage levels of VEGF and IL-8. Despite not observing a significant difference between the IL-8 levels in the two lungs, this did not occur with VEGF, which showed higher levels in the right lung of animals injected with small talc. These results suggest that the overflow of inflammatory mediators from the pleura, the first injured site, can stimulate their bilateral pulmonary production and then, reach the blood stream. This hypothesis could explain the case reported by Gill et al [12] in which a patient submitted to pleurodesis with talc developed lung failure and died with no talc particles having been found in the necropsy examination.

In order to compare the IL-8 and VEGF levels (blood and BAL) we used a factor to correct the BAL dilution. We obtained higher levels of IL-8 and VEGF in the fluid covering the alveoli than in blood suggesting, once more, that the lung is the target organ in the inflammatory response induced by the intrapleural talc injection. The correlations between IL-8 and VEGF levels in both talc groups (ST and MT) and in both hemi-thoraces reflect an acute shared response of the lungs to the pleural injury. Despite the higher levels of IL-8 and VEGF on the right side, the positive correlations suggest that after the injection, the lungs become unique in reacting to injury.
In blood, the leukocytes count and the IL-8 and VEGF levels were similar to those observed in previous studies, reflecting a more pronounced response to the injection of small talc particles [23,24]. When we analyze these results in the light of the acute respiratory distress syndrome (ARDS), some observations can be made and questions raised. According to Meduri et al [25], the majority of patients with ARDS, irrespective of their etiology, presented higher serum levels of IL-8, IL-1β and IL-6 over the first 24 hours after the beginning of clinical manifestation. Individuals who maintain high levels of these markers generally die, whereas those who present a reduction of these levels after 48 hours, almost always have a favorable outcome. In the present study, the rise and fall of these markers were similar to those described by Meduri et al. Despite the IL-8 levels (blood and BAL) having remained high over all experimental period, none of the animals showed signs compatible with ARDS. However, increased levels of IL-8 (blood and BAL) in the animals of the ST group suggest that the particles size influence the inflammatory response. The mechanisms by which this response occurs have not been fully clarified and need more investigation.

The presence of talc particles in intra and extra thoracic organs has also been a topic of study. Ferrer et al. [16] suggest that the small particles may cross over the pleural lymphatic stomas (6.2 µm in humans) reaching the lymphatic blood vessels and afterwards the systemic circulation, inducing a systemic and pulmonary inflammatory response. In our study, both talc particles were observed in the lungs, although with no significant difference between the groups (figure 6). These findings permit us to speculate that the migration of talc to the lungs may occur through systemic circulation. Nevertheless, we must reiterate the limitations of the method used to quantify talc particles. The digital image analysis system used does not enable us to detect particles smaller than 2 µm. As these particles are more common in the small talc particles, this quantity may be underestimated, leading to an erroneous conclusion that the dispersion of talc was similar in both groups.

The histological changes were discrete and similar in both study groups (ST and MT). Only the lymphomononuclear infiltrate showed a tendency to be more pronounced in the animals injected with small talc. As none of the animals developed acute respiratory distress syndrome, we
interpreted this finding as part of the acute inflammatory response triggered by the lungs.

Finally, it should be reiterated that although the talc may be mainly composed of silica and magnesium, other chemical elements may be present. In this study, despite the similar composition of both types of talc, the small one had a higher percentage of iron. Experimental studies using asbestos fibers with iron (crocidolite) have shown an increase in the inflammatory response and a greater cellular damage [26,27]. However, we do not know of studies linking the chemical composition of talc to the inflammatory response observed in pleurodesis, as well as, with the triggering of ARDS.

In conclusion, this study reinforces the use of calibrated talc with larger particles in the clinical practice for pleurodesis induction. The intent is to make the procedure safer and freer of adverse effects including the possibility of death.
References
23. Marchi E, Vargas FS, Acencio MMP, Antonangelo L, Teixeira LR, Genofre EH, Light RW. Talc and silver nitrate induce systemic inflammatory effects


Table 1.  
Talc: elemental composition and size of particles

<table>
<thead>
<tr>
<th>Talc</th>
<th>ST Small particles</th>
<th>MT Mixed particles</th>
</tr>
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<tbody>
<tr>
<td>Elemental composition (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silicon</td>
<td>64.754</td>
<td>69.746</td>
</tr>
<tr>
<td>Magnesium</td>
<td>26.799</td>
<td>26.801</td>
</tr>
<tr>
<td>Iron</td>
<td>5.823</td>
<td>0.687</td>
</tr>
<tr>
<td>Aluminium</td>
<td>2.137</td>
<td>2.229</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.487</td>
<td>- - - - -</td>
</tr>
<tr>
<td>Chlorine</td>
<td>- - - - -</td>
<td>0.536</td>
</tr>
<tr>
<td>Size ((\mu)m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D10</td>
<td>2.19</td>
<td>6.66</td>
</tr>
<tr>
<td>D50</td>
<td>6.41</td>
<td>21.15</td>
</tr>
<tr>
<td>D90</td>
<td>17.82</td>
<td>52.56</td>
</tr>
</tbody>
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D10, D50 or D90: 10, 50 or 90% of the particles are of a lesser diameter.

**Figure 1:** Distribution of rabbits for the experiment.
Figure 2: C reactive protein (CRP) levels in the blood of rabbits undergoing intrapleural injection of small (ST) or mixed (MT) talc.
**Figure 3:** VEGF levels in blood and bronchoalveolar lavage (BAL) of rabbits undergoing intrapleural injection of small (ST) or mixed (MT) talc. Comparison with the control group and between the right and left sides.

**Figure 4:** Interleukin-8 (IL-8) levels in blood and bronchoalveolar lavage (BAL) of rabbits undergoing intrapleural injection of small (ST) or mixed (MT) talc.
Figure 5: Photomicrography of lung parenchyma of rabbits undergoing intrapleural injection of small talc. Presence of lymphomononuclear infiltrate. HE.

Figure 6: Photomicrography of lung parenchyma of rabbits undergoing intrapleural injection of small (A) or mixed (B) talc. Presence of particles talc. HE stain, polarization light.