# Influence of Talc Dose on Extrapleural Talc Dissemination after Talc Pleurodesis

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**ABSTRACT**

This study was designed to ascertain, in a rabbit model, extrapleural talc deposition and the related inflammatory response following talc slurry pleurodesis with two clinical doses, 200 and 50 mg/kg. Histopathologic evaluations revealed that while numerous rabbits receiving a high dose had talc in the ipsilateral (70%) and contralateral (55%) lung, mediastinum (90%), pericardium (30%) and liver (25%), a small number of animals treated with a low dose showed talc in the ipsilateral lung (10%) and mediastinum (20%), and none in the contralateral lung, pericardium or liver. Hematologic and immunocytochemical analyses showed that a systemic inflammatory response develops shortly after pleurodesis with a high talc dose involving massive accumulation of neutrophils and macrophages in lung tissue. Zymography also revealed that the pulmonary expression of matrix metalloproteinases 2 and 9 was upregulated in both lungs in a dose-dependent manner soon after talc instillation. Furthermore, microscopic examination of lung specimens revealed that the higher the dose of talc, the greater the development of both fibrotic visceral pleural thickening and foreign-body granulomas. These findings show pleurodesis with a high talc dose to be associated with an increased risk of extrapleural talc deposition, which may originate undesirable acute and chronic inflammatory responses.

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## INTRODUCTION

Chemical pleurodesis consists of the introduction of a sclerosing agent into the pleural cavity to achieve symphysis of the two pleural layers and, thus, obliteration of the pleural space. Pleurodesis is indicated in the treatment of recurrent spontaneous pneumothoraces and relapsing symptomatic pleural effusions of either malignant or benign etiologies (1, 2). Among the different agents proposed for pleurodesis, talc is at present the most commonly used, either in a slurry or via thoracoscopic poudrage, with a high success rate of around 90% irrespective of the dose administered (3, 4). However, despite its popularity, its use remains controversial (5-8) owing to complications following talc pleurodesis.

In addition to minor side effects such as fever and chest pain, common to other sclerosing agents, the intrapleural administration of talc has been associated with acute severe adverse effects such as cardiovascular complications and acute respiratory failure. Whereas cardiovascular complications, including arrhythmias (9, 10), cardiac arrest (9, 11, 12) and myocardial infarction (13), appear to result from either the surgical procedure (3) or comorbid conditions (5, 7), it has been suggested that cases of acute respiratory failure, including acute respiratory distress syndrome (ARDS), are due to extrapleural talc dissemination (6, 8). Post-talc ARDS is a severe form of acute, sometimes lethal, lung injury occurring shortly after intrapleural administration of talc, either as a slurry (10, 14-16) or as an insufflated powder (15, 17-19) in up to 9% of patients (15). Moreover, whereas talc particles have been detected in all bronchoalveolar lavage (BAL) fluid and lung tissues analyzed from patients developing post-talc ARDS (17, 19), no talc particles have been found either in BAL samples (20, 21) or lung tissues (22) from patients with no respiratory complications after talc

administration. However, the hypothesis that this acute inflammatory lung disease is due to pulmonary talc deposition has not been proved.

It has been suggested that extrapleural talc dissemination could be a phenomenon dependent on both particle size and the talc dose used. With respect to size, we recently reported that, in the rabbit model, the probability of extrapleural talc dissemination is particle diameter-dependent (23). Following intrapleural instillation of two talc preparations of different-size distribution, both the percentage of animals affected and degree of deposition were substantially greater with the lowest talc particle size. This is remarkable, since most patients who developed post-talc ARDS received talc from the United States (10, 14, 15) which, among talc preparations, has the smallest mean particle size (24).

Little is known, however, regarding dose influence on extrapleural talc dissemination. The single experimental study on this issue revealed that, in the rat model, neither pulmonary nor extrapulmonary talc deposition was dependent on the talc dose used (25). Nevertheless, in clinical practice, most cases of post-talc ARDS have been associated with high talc doses (10, 14). In any event, the side effects and safety of talc pleurodesis require further study. To this end, the present study was designed to ascertain, in a rabbit model, the influence of different-dose talc pleurodesis on extrapleural talc dissemination and its local and distant inflammatory effects.

## METHODS

Methods word count: 759

### Study Design and Procedures

Sixty-five white male New Zealand rabbits weighing 1.5-2.0 kg were used in this study. The protocol was approved by our Ethics Committee on Animal Experimentation. Initially, 60 rabbits were randomly assigned to the following three experimental groups: Low-Dose (LD), High-Dose (HD) and sham treatment (sham). Whereas animals belonging to LD and HD groups respectively received 50 and 200 mg/kg of talc (mean Dmax = 8.36 ± 0.20 μm; Distalc, Barcelona, Spain) suspended in 2 ml of saline solution, sham-treated rabbits received only the saline solution. The doses were chosen for comparative purposes with human pleurodesis, since they are equivalent to 3 and 12 g in a 60-kg patient, and thus lie within the dose range (1 to 14 g) reported to be used in clinical practice (3, 4).

Right thoracotomy was performed at the seventh intercostal space, as previously described in detail (23), and either talc slurry or saline solution was instilled into the pleural cavity. Five animals of each group were euthanized at 4 hours, 1 day, 1 week and 1 month post-instillation. Moreover, 5 additional unoperated rabbits were used as controls (C) for the injury caused by surgical procedures. At autopsy, tissue samples from both lungs were resected and processed for histopathologic, immunocytochemical, immunoblotting and zymographic analysis following standard procedures. None of the results obtained from these animals have been reported elsewhere.

**Blood Samples**

Prior to euthanasia, a blood sample was drawn from the central ear artery of each rabbit and the following blood parameters were determined: erythrocyte and platelet numbers, leukocyte differential counts, hemoglobin, fibrinogen and plasma protein concentration, hematocrit and red blood cell indices. Blood samples from controls were used as baseline.

**Histopathologic Analysis**

Histopathologic analysis was carried out by two of the investigators who were blinded to the treatment group.

*Pulmonary Deposition.* Pleural and parenchymal talc deposition was examined by polarizing microscopy on ten uniform, randomly-selected H&E-stained sections from both lungs of each talc-treated rabbit. The degree of deposition was graded semiquantitatively following our previously described score (23): I, talc particles incorporated into the pleura but no parenchymal deposition; II, individual or small aggregates of talc particles randomly distributed in parenchyma; and III, diffuse deposition of particles involving a variable percentage of pulmonary parenchyma.

*Pleural Thickening.* Visceral pleural thickening was estimated 1 week and 1 month post-instillation in six uniform, randomly-selected sections from each rabbit as described elsewhere (23).

**Immunofluorescence**

Immunofluorescence was performed on cryostat lung sections from all rabbits. The following monoclonal antibodies were used at the indicated dilutions: mouse anti-rabbit macrophages (1:30) (RAM11; Dako, Glostrup, Denmark), mouse anti-rabbit T lymphocytes (1:40) (Serotec, Kidlington, UK) and mouse anti-human B cells (1:50) (Dako). Bound monoclonal antibodies were visualized by incubation with FITC-conjugated goat anti-mouse Ig (Dako).

**Electron Microscopy**

Ultrastructural and immunocytochemical studies of granulomatous pleural samples from lung and diaphragm were conducted following standard techniques. For immunocytochemistry, grids were incubated with the antibodies against macrophages (1:30 dilution). Bound monoclonal antibodies were visualized following incubation with 10 nm colloidal gold-conjugated goat anti-mouse Ig (British Biocell International, Cardiff, UK).

**Immunoblotting**

For immunoblotting, ipsilateral lung tissues were homogenized by Dounce homogenization. Denatured samples were then separated by reducing SDS-PAGE and transferred to nitrocellulose membranes. The membranes were probed with the monoclonal antibody anti-macrophages (1:100 dilution). Bound monoclonal antibodies were detected with

peroxidase-conjugated goat anti-mouse Ig (Dako) followed by enhanced chemiluminescence. Five independent repetitions of densitometric measurements were performed prior to statistical comparison of group means.

**Zymography**

Metalloproteinase activity was assessed in samples from the ipsilateral and contralateral lung lower lobe by gelatin zymography. Briefly, tissue homogenates were applied to a 9% SDS-polyacrylamide gel copolymerized with gelatin and subjected to non-reducing electrophoresis. Thereafter, gels were rinsed, incubated in developing buffer and stained with Coomassie blue as described elsewhere (26). Following destaining, gelatinolytic activity was detected in the form of clearing bands on a blue background. Densitometric measurements were performed as above-mentioned.

**Statistics**

Data of hematologic parameters, pleural thickening and densitometry were expressed as mean ± SEM and Student's *t* test was used to compare group means. Pulmonary and extrapulmonary talc deposition data were expressed as the proportion of affected animals versus total group and the score of pulmonary talc deposition was considered as a classification of animals according to their stage of involvement (I to III). A hypothesis test was used to compare the proportions obtained from pulmonary and extrapulmonary talc

deposition and score of pulmonary talc deposition, since the data followed a binomial distribution (presence versus absence of talc) and time was not a true discriminatory factor. Data were considered statistically significant at $p < 0.05$.

## RESULTS

### Pulmonary Talc Deposition

*Ipsilateral lung*. Polarized light microscopy revealed that while 70% (14/20) of rabbits treated with a 200-mg/kg talc dose showed talc deposition, only 10% (2/20) of animals receiving a 50-mg/kg dose had talc particles in ipsilateral lung parenchyma ($p < 0.001$). Furthermore, whereas 11 of the 14 animals showing pulmonary talc deposition in the HD group were classified as stage III, the only 2 rabbits with ipsilateral lung deposition in the LD group were classified as stage II (Table 1). In both experimental groups, ipsilateral pulmonary deposition was an early event occurring in the first 4 hours post-instillation (Figure 1A).

On the other hand, polarization revealed that, early after instillation, some affected animals classified as stage III additionally showed both individual particles and small talc aggregates within some alveoli. Histopathologic analysis of lung specimens showed that this early talc deposition resulted in edema and mild necrosis of the adjacent lung parenchyma. Moreover, at 1 month, rabbits from the HD group developed pneumonitis foci in areas of great talc deposition.

*Contralateral lung*. Polarization revealed that while no rabbit of the LD group had talc particles in the contralateral lung, 55% (11/20) of animals treated with a high dose showed talc deposition ($p < 0.001$). The deposition was already detected 4 hours post-instillation and no apparent differences in the percentage of affected rabbits were observed among experimental times. In all cases, contralateral deposition was classified as stage II and consisted of a reduced number of small-sized particles (Dmax < 20 μm). These

particles were seen inside either interstitial macrophages (Figure 1B) or multinucleated giant cells. Talc particle-containing cells were not observed in the pleura.

**Extrapulmonary Talc Deposition**

*Mediastinum and mediastinal pleura.* Macroscopic examination of the mediastinal space revealed that while 20% (4/20) of rabbits of the LD group showed talc in the mediastinum, the percentage of animals of the HD group with talc masses within this anatomical space was 90% (18/20) ($p < 0.01$). Talc masses were mainly located on the mediastinal side of the ipsilateral mediastinal pleura.

*Pericardium.* At autopsy, inspection of the mediastinal cavity revealed that while no rabbit from the LD group showed talc on the heart surface, 30% (6/20) of rabbits of the HD group had visible talc masses in the pericardium ($p < 0.05$). Pericardial talc deposition was always associated with the presence of talc masses on the mediastinal side of the ipsilateral mediastinal pleura. Similar percentages of involvement were observed when the four time points studied after instillation were compared.

*Liver.* Macroscopically, 25% (5/20) of rabbits treated with a high talc dose, but none of those receiving a low talc dose, showed talc deposition on the liver surface ($p < 0.05$). Talc masses ranging from 1 to 3 mm in diameter profile were mainly located on the right hepatic lobe adjacent to the falciform ligament. In addition, visible collections of talc adhering to the abdominal side of the diaphragm were detected in the 2 rabbits which, at 4 hours, showed liver deposition. In both animals, a large amount (8.4 and 12.3 ml) of peritoneal exudate accompanied this abdominal deposition. Hepatic deposition was an early phenomenon

occurring in the first 4 hours post-talc instillation.

On all occasions, the mineral nature of the extrapleural deposits was confirmed by polarized light microscopy.

**Hematologic Parameters**

When the hematologic profile of all experimental groups was compared, differences in lymphocyte, monocyte, neutrophil and platelet counts were detected in the first 24 hours post-instillation. No significant differences were observed among groups when the other eleven hematologic parameters determined were considered.

Comparison of peripheral white cell counts of the sham-treated group with the baseline value of unoperated control animals revealed that surgical trauma itself was responsible for early (4 hours), but transient, lymphocytosis (Figure 2A), monocytosis (Figure 2B) and neutrophilia (Figure 2C). Rabbits of the LD group showed similar hematologic variations, and only the differences observed in neutrophil counts reached statistical significance compared with the sham group (Figure 2C). In contrast, in the HD group, lymphocyte (Figure 2A), monocyte (Figure 2B) and neutrophil (Figure 2C) counts were significantly lower than those obtained in the sham group 4 hours post-instillation.

With reference to platelet count, this parameter exhibited a different evolution in comparison with the above-mentioned white blood cells. On the one hand, reactive thrombocytosis following the surgical procedure was not observed, since platelet counts of sham-treated animals never significantly differed from the baseline value of unoperated control rabbits (Figure 2D), and on the other, platelet number was the only blood parameter

that was significantly higher in talc-treated than sham-treated animals, with the greatest counts corresponding to rabbits of the HD group (Figure 2D). At 1 day post-instillation, differences observed between the HD and sham groups achieved statistical significance.

**Pleural and Pulmonary Inflammatory Response**

*Acute response.* Four hours after talc slurry instillation, light microscopy revealed focal inflammatory responses in the ipsilateral lung of rabbits treated with either a low or a high talc dose. These inflammatory foci expanded centrifugally from points of the visceral pleura where talc masses were located. The main histological changes observed were mesothelium denudement and disorganization of basal lamina and underlying connective tissue. In addition, the subjacent pulmonary parenchyma showed capillary vasodilation and hyperemia, two phenomena associated with interstitial edema and intense leukocyte infiltration. At 4 hours, neutrophils and monocytes were the main infiltrating cell types (Figure 3).

*Pleural repair.* Between 1 day and 1 week following talc administration, repair responses in the form of fibrosis and pleural reepithelialization developed at the injured pleural areas. Consequently, at 1 week and 1 month, both talc-treated groups showed patchy pleural thickening. At these time points, the degree of fibrotic visceral pleural thickening was markedly greater ($p < 0.001$) in the HD (72.3 ± 2.6 μm at 1 week and 61.8 ± 4.5 μm at 1 month) than in the LD group (26.0 ± 6.7 μm at 1 week and 22.3 ± 5.9 μm at 1 month). Polarized light microscopy showed the presence of both individual particles and talc aggregates within the thickened submesothelial space and foreign-body granulomas developed as a consequence of these pleural talc depositions.

*Cellular effectors.* Histologic and immunocytochemical studies (Figure 4) revealed that, at 1 day, the cellular composition of the pulmonary infiltrate had changed. From this time point, lymphocytes and macrophages were the main infiltrated cell types. Redistribution of these infiltrated cells was observed late following instillation, and most lymphocytes and macrophages were situated in association with granulomatous lesions. Whereas T lymphocytes were located at the periphery of the granulomas, some B lymphocytes were further located in the inner zones of these reactive structures. Electron microscopy revealed that macrophages, epithelioid cells and multinucleated giant cells in the granulomatous lesions contained numerous talc particles (Figure 4E). Talc particles were observed within endocytic compartments, which were often located in the vicinity of the nucleus. By immunogold techniques, reactivity to the RAM11 antibody was only detected in the membrane of these compartments (Figure 4E inset).

Western blot analysis using the monoclonal antibody RAM11 against rabbit macrophages revealed a single band of an apparent molecular weight of 125 kDa in homogenate lung samples from both talc- and sham-treated groups (Figure 5). Densitometric analysis of the blots showed that, at all time points studied, intensity of the band was higher in talc-treated than in sham-treated animals. However, the temporal profiles of both talc-treated groups differed, since the higher relative mass of the macrophage marker in the LD group was detected 1 week post-instillation, but was recorded at 1 day in the HD group. These relative masses were, respectively, three and five times significantly greater ($p < 0.001$) compared with the corresponding sham group (Figure 5).

*Matrix metalloproteinase (MMP) activity*. By zymographic analysis, four major gelatinolytic activities of around 92 (latent MMP-9), 86 (active MMP-9), 72 (latent MMP-2) and 62 (active MMP-2) kDa were identified in both ipsilateral (Figure 6A) and contralateral

(Figure 6B) lung tissue homogenates from all experimental rabbits. Densitometric analysis of these gelatinolytic bands revealed a substantial increase in metalloproteinase activity in both lungs from talc-treated animals compared with the sham group, with the most significant variations being recorded soon after talc slurry instillation. At 4 hours, gelatinolytic activity of the latent form of MMP-9 increased by either 178% (ipsilateral) or 82 % (contralateral) in the HD group and 91% (ipsilateral) or 31 % (contralateral) in the LD group compared with the sham-treated group (Figure 6). Further, at this same time point, the active form of MMP-2 increased by either 48% (ipsilateral) or 36 % (contralateral) in the HD group and 21% (ipsilateral) or 15 % (contralateral) in the LD group versus the sham-treated group (Figure 6). In both lungs, the differences observed between HD and sham groups achieved statistical significance ($p < 0.05$).

## DISCUSSION

The present study provides experimental evidence that both pulmonary and extrapulmonary talc deposition can follow talc slurry pleurodesis in rabbits. Further, our results have allowed us to demonstrate for the first time that this phenomenon is dose-dependent and occurs during the first 4 hours after instillation. The use of a low dose of talc, whose particle size (mean Dmax = 8.36 μm) is similar to others low-sized talc preparations extensively used in clinical practice (24), significantly reduces extrapleural talc dissemination.

With respect to pulmonary dissemination, histopathologic analysis of the ipsilateral lung samples revealed that both the number of affected rabbits and the degree of parenchymal deposition were markedly lower in animals treated with a 50-mg/kg talc dose (10% of animals affected) than in those receiving a 200-mg/kg dose (70% of animals affected). These results concur essentially with the two previous studies examining pulmonary deposition after talc pleurodesis in the rabbit model. Thus, the pioneer study of Kennedy and coworkers (27) reported no talc deposition in pulmonary parenchyma following a 70-mg/kg dose instillation. Moreover, our recent study assessing the influence of particle size on extrapleural talc dissemination showed that, when a 200-mg/kg dose of the same talc used here was administered, 60% of rabbits showed talc in lung parenchyma (23).

Nevertheless, our observations are in disagreement with the findings of Werebe and coworkers (25), who stated that in the rat model, pulmonary talc deposition is not dose-related. Those authors reported that all rats receiving talc doses of either 67 or 36 mg/kg showed talc particles in lung parenchyma. This apparent discrepancy must be attributed to the doses tested by those investigators. These doses may have been excessively large considering

the extremely thin pleura of the rat (~ 3 μm [28, 29]). In any event, these results, taken together, seem to suggest that pulmonary talc dissemination should occur more readily in animals with a thin pleura, such as the rat, than in rabbits with a not-so-thin pleura (~ 10 μm [23, 30]) or humans with a thick pleura (~ 100 μm [31]).

On the other hand, a striking result of the current study is the finding that around half the rabbits treated with a high dose, but none of those receiving a low dose, also showed talc particle deposition in contralateral pulmonary parenchyma. Although translocation of mineral fibers, including chrysotile and crocidolite, to the contralateral lung following intrapleural injection has been reported in rats (32), to our knowledge, this is the first report revealing talc particle dissemination to contralateral pulmonary parenchyma after talc pleurodesis. Although no dissemination route can be ruled out, the diameter (Dmax < 20 μm) and reduced number of particles, distribution of the latter and the fact that our previous study (23) showed hematogenous dissemination to extrathoracic organs such as spleen and kidney to be almost nonexistent suggest hypothetical dissemination through lymphatic stomata. Stomatal openings, up to 30 μm in diameter in rabbits (33), are known to be distributed in the parietal pleura of both rabbits (33, 34) and humans (35, 36), and evidence seems to indicate that they constitute the major route for the drainage of particulate matter (32, 37-40), including talc (27), from the pleural cavity into mediastinal and parasternal lymph nodes. In this way, Monchaux and coworkers (32) pointed out that asbestos fibers intrapleurally injected into rats could reach the contralateral lung parenchyma by retrogression from the mediastinal lymph nodes.

As to extrapulmonary talc dissemination, necropsy evaluations revealed that talc pleurodesis can also result in the deposition of talc particles in both mediastinal and abdominal cavities. Extrapulmonary talc deposition has also been demonstrated in other

animal models, such as rat (25, 41) and sheep (42), and in humans (18, 43). As observed in the lungs, extrapulmonary dissemination of talc particles was an early and dose-related phenomenon. Thus, whereas numerous rabbits treated with a high dose had talc in the mediastinum (90%), pericardium (30%) and liver (25%), when a low dose was administered this involvement was drastically reduced in the case of the mediastinum (20% of rabbits affected), or even avoided in the pericardium and liver. These results confirm and extend our previous observations (23) and are in keeping with the findings of Kennedy and coworkers (27) who, administering a low dose of 70 mg/kg, reported mediastinal dissemination in 17% of rabbits and no liver involvement. Cardiac dissemination was not examined by those authors.

There is currently a paucity of information on the systemic effects of talc pleurodesis. Available data suggest that a systemic inflammatory response could develop following talc pleurodesis. Thus, whereas hyperleukocytosis (34 x $10^9$/L) following talc pleurodesis has been reported in a patient (44), the intrapleural administration of talc in the rabbit model has been associated with both elevated angiotensin-converting enzyme activity in serum (45) and hyperplasia of the white periarteriolar substance of the spleen (23). Results of the present study are consistent with these findings, revealing, in addition, that this systemic inflammatory response occurs in the early hours after instillation and is dose-related.

Indeed, comparison of peripheral white cell counts of the three experimental groups with baseline values of unoperated control animals revealed that, early after instillation, all experimental rabbits developed a transient increase in the number of lymphocytes, monocytes and neutrophils. However, although talc-treated animals were subjected to a double inflammatory stimulus, surgical trauma and sclerosing agent, the highest leukocyte counts corresponded, unexpectedly, to the sham-treated group and the lowest to rabbits treated with a

high talc dose. These findings are consistent with the hypothesis that talc pleurodesis results in a redistribution of leukocytes from peripheral blood to talc-inflamed tissues and lymphatic organs (23), thus causing a drop in peripheral counts.

In addition to the above-mentioned hematologic variations, data of platelet counts revealed that talc pleurodesis was also associated with early, short-lived reactive thrombocytosis, with the highest platelet numbers corresponding to rabbits treated with a high talc dose. These results reinforce the argument that a systemic inflammatory response develops shortly after talc pleurodesis, since secondary thrombocytosis has been described as a typical acute-phase response (46) and has been identified in a variety of inflammatory pulmonary disorders (47, 48).

Concerning local inflammatory response, histopathologic analysis of lung samples showed neutrophils and macrophages to be the main cell types involved in the acute inflammatory response developing in lung tissues soon after talc instillation. These results add to previous observations revealing a neutrophilic reaction (49) and perivascular mononuclear infiltrates (27) in lung parenchyma of talc-treated rabbits and are in line with several studies describing a massive influx of neutrophils and macrophages in high-volume exudative pleural effusions shortly after talc pleurodesis both in rabbits (27, 50) and humans (51, 52). Moreover, our current histopathologic, immunocytochemical and zymographic findings permitted us to determine that the tissue accumulation of these two types of inflammatory cells was considerably greater in the lungs of animals receiving a high talc dose than in those treated with a low dose.

In addition to the aforementioned acute inflammatory responses, talc pleurodesis was also associated with undesirable chronic pulmonary disorders consisting of fibrotic visceral pleural thickening and foreign-body granulomatous reactions. Nevertheless, microscopic

examination of lung specimens revealed that the lower the dose of talc, the lower the development of both visceral pleural thickening and granulomas. These results concur essentially with the findings of Lee and coworkers (53) who, 1 week after a 400-mg/kg talc dose instillation in the rabbit model, reported a greater visceral pleural thickening (85 μm) than that obtained with 200 mg/kg in this study (72.3 μm).

For all these reasons, and given that, in clinical practice, no difference in efficacy is observed between low (2-5 g) and high (10-14 g) talc doses (3, 4) and that most patients developing ARDS received high talc doses (10, 14), we agree with the opinion that only the lower dose should be used (7). Likewise, we agree that talc pleurodesis should not be performed after procedures that would enable more rapid and increased concentrations of talc to enter the ipsilateral lung and systemic circulation, such as pleural abrasion or multiple biopsy procedures (7, 17).

In conclusion, the present study demonstrates that pleurodesis performed with a high dose of talc is associated with an increased risk of both pulmonary and extrapulmonary talc deposition, which may originate undesirable acute and chronic inflammatory responses.

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**FIGURE LEGENDS**

***Figure 1.*** Pulmonary talc deposition. H&E staining. (*A*) Ipsilateral lung. Polarized light microscopy of individual and small aggregates of talc particles located at pulmonary parenchyma after 4 hours of high-dose instillation. Bar = 200 μm. (*B*) Contralateral lung. Small talc particle (*arrow*) phagocytized by a parenchymal macrophage from the HD group at 1 day. 45° polarization. Bar = 50 μm.

***Figure 2.*** Peripheral blood parameters. Circulating lymphocyte (*A*), monocyte (*B*), neutrophil (*C*) and platelet (*D*) counts from the sham, LD and HD groups. Values from unoperated control group are used as baseline (0 hours). Data are presented as mean ± SEM. $^{*}p < 0.05$ compared with unoperated control group. $^{\dagger}p < 0.05$ compared with sham group at 4 hours. $^{\ddagger}p < 0.05$ compared with LD group at 4 hours. $^{\S}p < 0.05$ compared with HD group at 4 hours. $^{-}p < 0.05$ compared with sham group at 1 day.

***Figure 3.*** Pleural and pulmonary inflammation 4 hours after a high-dose instillation. H&E staining. Neutrophils and monocytes are the main infiltrating cell types. *Arrows* indicate the elastic layer of the visceral pleura. Bar = 25 μm.

***Figure 4.*** Macrophages. (*A-D*) Indirect immunofluorescence with the monoclonal antibody RAM11 against rabbit macrophages. Bars = 50 μm. (*A*) Representative lung section from a sham-treated rabbit showing both pleural and pulmonary macrophages (*arrows*). (*B*) Two aggregates of macrophages neighboring parenchymal vessels (*v*) at 4 hours post-instillation.

LD group. (*C*) Numerous heavily-stained macrophages distributed throughout peripheral lung parenchyma. The pulmonary limit is delineated by a discontinuous line. HD group at 1 day. (*D*) Labeled cell-containing granuloma (*gr*) located in visceral pleura. Note the disruption of the elastic layer (*e*) by some of the labeled cells (*arrow*). HD group at 1 month. (*E, E inset*) Transmission electron microscopy. (*E*) Talc particle-containing epithelioid cell located in a pleural granuloma. Talc particles, characteristically electron-dense, are seen inside endocytic compartments (*arrows*). LD group at 1 week. Bar = 1 μm. (*E inset*) Immunogold staining. Talc particle-containing endocytic compartments are labeled with RAM11 antibody. x52000.

***Figure 5.*** Immunoblotting. Western blot analysis of lung homogenates using the monoclonal antibody RAM11 against rabbit macrophages reveals a single band of an apparent molecular weight of 125 kDa. The experimental groups illustrated include LD 1 day (*Lane 1*), LD 1 week (*Lane 2*), HD 1 day (*Lane 3*), HD 1 week (*Lane 4*) and sham (*Lane 5*).

***Figure 6.*** Gelatin zymography. Ipsilateral (*A*) and contralateral (*B*) lung homogenates zymograms from the LD group at 4 hours (*Lane 1*) and 1 week (*Lane 2*), HD group at 4 hours (*Lane 3*) and 1 week (*Lane 4*), and sham group (*Lane 5*) are shown. Positions of latent forms of MMP-9 and MMP-2 are indicated.

**TABLE 1. SCORE OF TALC DEPOSITION IN THE IPSILATERAL LUNG**

| | LD | | | | HD | | | |
|---|---|---|---|---|---|---|---|---|
| Score | 4 h | 1 d | 1 wk | 1 mo | 4 h | 1 d | 1 wk | 1 mo |
| I | 4 | 5 | 4 | 5 | 2 | 1 | 1 | 2 |
| II | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 |
| III | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 2 |

Definition of the score: I, talc particles incorporated into the pleura but no parenchymal deposition; II, individual or small aggregates of talc particles randomly distributed in parenchyma; and III, diffuse deposition of particles involving a variable percentage of pulmonary parenchyma.