Distribution of Calibrated Talc After Intrapleural Administration: An Experimental Study in Rats

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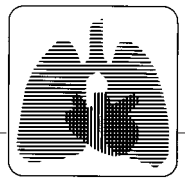

laboratory and animal investigations

Distribution of Calibrated Talc After Intrapleural Administration*

An Experimental Study in Rats

Anne Fraticelli, MD; Andrée Robaglia-Schlupp, MD; Helene Riera, BS; Suzanne Monjanel-Mouterde, MD; Pierre Cau, MD, PhD; and Philippe Astoul, MD, PhD

Study objective: **Many reports have shown that talc is the most effective and least expensive agent for the creation of a pleural symphysis. However, its use still remains controversial due to severe acute respiratory side effects possibly related to the systemic dissemination of talc particles. The purpose of this study was to assess the distribution of calibrated talc after intrapleural administration in rats.**

Material and methods: **Thirty-seven Wistar male rats were randomly assigned to undergo pleurodesis by talc slurry (33 rats) or by simple chest tube drainage (control group; 4 rats). Forty milligrams of calibrated talc suspended in 1 mL sterile saline solution was injected into rats in the treated group. The animals were randomly assigned for autopsy at 24 or 72 h after pleural injection. Lungs, parietal pleura, diaphragm, liver, kidneys, spleen, pericardium, brain, and blood were assessed by polarized light for birefringent talc particle detection and counting.**

Results: **No deaths were observed. The autopsies showed no pleurodesis at 24 and 72 h. Despite high doses of talc (extrapolated from the dose of 10 g in a 70-kg adult man), few talc particles were found in the liver of two rats, in the spleen of one rat, and only one particle of talc was observed at the brain surface of the rat studied by scanning electron microscopy. No particles were found in the other organs, in particular in the contralateral lung and blood, contrasting with previously published results using noncalibrated talc particles.**

Conclusions: **The lack of systemic dispersion of talc particles, with the packaging talc we currently use in our clinical practice, is probably due to the size of the talc particles, which are larger than the other talc preparations. Calibrated talc is required in case of intrapleural administration for pleurodesis to avoid systemic dissemination and potential secondary acute respiratory failures.**

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Key words: malignant pleural effusions; pleurodesis; respiratory failure; talc; thoracoscopy

Patients with recurrent, symptomatic malignant pleural effusions require local therapy for the relief of breathlessness, unless they have a tumor cell type that is sensitive to chemotherapy. Based on experimental and clinical data, many reports[1–5] have shown that asbestos-free talc is the most effective agent for pleural symphysis. It stimulates pleural mesothelial cells to release chemokines and to express adhesion molecules that may play a critical role in pleurodesis.[6,7] However, its use still remains controversial due to adverse effects.

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Among these effects is the association of cases of acute respiratory failure with talc poudrage or slurry, which has prevented talc from being largely accepted

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as the ideal pleurodesis agent.[8,9] The highest incidence was reported recently in a retrospective review with a 33% rate of respiratory complications and death.[10] Consequently, the safety of talc administered intrapleurally is questionable despite the zero reported incidence of respiratory complications in some large series.[5,11,12]

The mechanism by which talc produces acute lung injury is unknown. Although it has been suggested that respiratory failure might be more common after the administration of large doses of talc, acute respiratory failure has been described[13] after the administration of 2 g talc. However, if talc is unique with regard to chemistry, the size of the particles in the final talc preparation may vary, which may determine the local or systemic distribution of intrapleural talc and, consequently, potential respiratory failures.

Previous experimental studies[4,14] in animals have shown systemic distribution of talc after intrapleural slurry administration. The study carried out in rats by Werebe and collaborators[14] has shown talc particles in all organs in every rat, independent of the dose of talc and the time of necropsy. However, there is no mention of the size of talc particles in the talc preparation used by these authors.

The hypothesis of the present study is that instances of acute respiratory failure encountered after the intrapleural administration of talc are related to its systemic dissemination, which depends on the diameter of the particles. This experimental study was designed to assess the distribution of calibrated talc after intrapleural administration in rats.

Materials and Methods

Animal Subjects

The protocol was approved by the institution's Committee on Investigations Involving Animal Subjects of the University of the Mediterranean (Marseille, France). All animals were housed and procedures were performed in the facilities of the Laboratory of Pharmacokinetics (University of the Mediterranean). Animal care was provided in accordance with the European Guidelines (European Community publication 86/109).

Operative Design

Thirty-seven Wistar male rats (mean [± SE] body weight, 351 ± 21 g) were randomly assigned to undergo pleurodesis by talc slurry (33 rats) or by simple chest tube drainage (4 rats) on day 1. The pleurodesis procedure was performed using 40 mg asbestos-free talc (Steritalc; Novatech; Aubagne, France) suspended in 1 mL sterile saline solution. This talc preparation (Luzenac; Toulouse, France) is currently used in our clinical practice.[12]

General anesthesia was induced in the rats with an intraperitoneal injection of 9% chloral hydrate (body weight, 0.5 mL per 100 g) [E. Merck; Darmstadt, Germany], which allows sedation for a period of 20 to 30 min with spontaneous breathing. The animals were placed in the right lateral decubitus position with all four limbs restrained. An antimicrobial skin preparation was employed prior to all invasive procedures, each of which was performed using an aseptic technique. For thoracoscopic procedures, a small lateral incision on the left-lateral chest of the rat via the fourth or fifth intercostal space was performed. Another incision on the same site provided access to the pleural space and resulted in total lung collapse. A 1.9-mm rigid telescope (Richard Wolf; Knittlingen, Germany) then was introduced, and a visual examination of the left pleural cavity was performed. The telescope then was replaced by a 2-mm pleural catheter (Plastimed; Saarbruken, Germany) for talc administration mimicking the clinical technique of blind administration through a chest tube. At the end of the procedure, the chest wall incision was closed with a 7.0 nylon suture, as previously described.[15] If there was any evidence of air leakage through the incision, additional sutures were placed until the incision was completely closed. A sterile 25 G 1.5-gauge needle (angiocatheter) with an attached 5-mL syringe then was inserted into the pleural cavity. The Teflon catheter was threaded over the needle, and the needle was removed to avoid lung injury during re-expansion. Using the attached syringe, air was removed from the closed pleural cavity, and the lung was reinflated. Complete lung re-expansion was verified by observation of a decrease in respiratory rate and visualization of the lung through the chest wall of the rat. Chest muscles and skin were closed with a single layer of 6.0 silk sutures. After recovering from the intervention, the animals were allowed to return to their cages and were monitored daily after the procedure for signs of pain or discomfort, wound integrity, and vital signs. Twenty-four hours or 72 h later, each animal was killed by intraperitoneal chloralhydrate injection.

Tissue Preparation and Macroscopic Evaluation

Lungs, parietal pleura, diaphragm, liver, kidneys, spleen, pericardium, and brain were dissected out. The presence of talc particles at the surface of the organs was assayed by macroscopic examination. Then tissues were frozen into liquid nitrogen and stored at −86°C, all tissues from the same animal in a single cryotube (Nalgene; Nalge Nunc International Corporation; Rochester, NY). Then each sample was defrosted and dried (2 to 5 days in an oven at 56°C), was crushed using a mortar, and was digested in 40 mL pure bleach. After centrifugation at 3,000 revolutions per minute for 15 min, the supernatant was stored for further control (see below), and the pellets were rinsed in distilled water in order to discard the bleach, centrifuged, then resuspended in 1 mL distilled water. One hundred microliters of the vortexed suspension were layered into Kova slide 10 (Hycor Biomed; Garden Grove, CA).

About 1 mL blood was sampled trough cardiac puncture. A buffy coat was obtained by centrifugation (cell preparation tube, Vacutainer; Becton Dickinson; Franklin Lakes, NJ) and smeared. Slides were stained using May-Grünwald-Giemsa stain and were observed in polarized light microscopy.

Talc Particles Detection and Counting

Coded and randomized slides were evaluated by a single observer by polarized light microscopy (Diaplan; Leica; Solms, Germany) [magnification, ×6.3/×20], and the birefringent talc particles were numbered. This analysis was designed to check the amount of talc deposition among the following organs: homolateral (left) and contralateral (right) lungs; pericardium; parietal pleura; diaphragm; kidneys; spleen; liver; brain; and blood sam-

pled from the heart. The data on talc particle deposition for each entire organ at 24 h and 72 h was expressed as the mean (± SE) number of talc particles and was compared using the Mann-Whitney test.

Controls Performed in the Study

Dry talc particles were layered onto a pure carbon microscopic stub, sputter coated with 40 nm carbon (Balzers; Balzers AG; Balzers, Liechtenstein), and viewed in a scanning electron microscope (PSEM 515; Philips; Amsterdam, the Netherlands). The elementary composition of talc particles was determined by x-ray microanalysis (EDX4 device; EDAX; Mahwah, NJ) using the facilities of the Center Pluridisciplinaire de Microscopie Electronique et de Microanalyse X, Faculté des Sciences et des Techniques de Saint Jérome, Marseille, France (Fig 1).

The tissues sampled from one rat (rat 2) were dried, talc particles were detected, and their elementary composition was analyzed using the same scanning electron microscope.

The talc suspension, prepared just before the intrapleural injection, also was observed by polarized light microscopy in order to check the morphology of the injected product.

One milliliter of talc suspension was crushed in a mortar, digested using pure bleach, rinsed in distilled water, then observed by polarized light microscopy in order to check that all the procedures did not change the talc particle size distribution.

The supernatant obtained after the centrifugation of crushed and bleach-digested tissue samples did not contain talc particles. Therefore, the protocol used did not result in a significant lack of talc particles deposited in tissue samples after pleural injection.

Results

All animals tolerated both the anesthesia and the procedure well, and no animals suffered any side effects from the surgical procedure.

Macroscopic Analysis

At the time of the autopsy, no pleural symphysis was observed at either 24 h or 72 h after the intrapleural administration of the talc slurry. Except for the organs of the left hemithorax (*ie*, lung surface, pericardium, diaphragm, and parietal pleura), where white spots of talc were noted, there were no abnormalities on macroscopic examination of the surface of the organs sampled for the study.

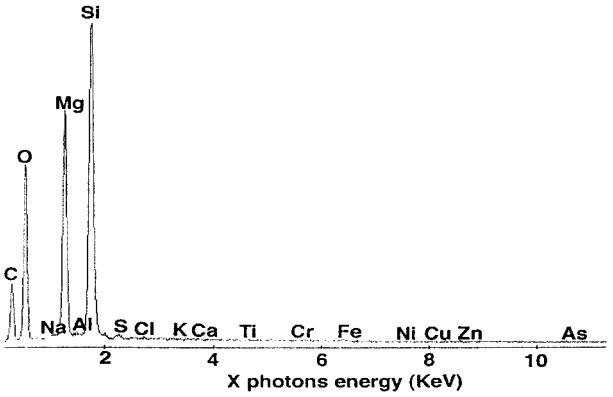

FIGURE 1. Elementary composition of talc particles determined by x-ray microanalysis.

Table 1—*Comparison Between Talc Deposition at 24 and 72 h After Intrapleural Slurry Injection in the Left Pleural Cavity

Organs	Time of Autopsy		p Value
	24 h	72 h	
Lung and visceral pleura	280.88 ± 112.33	467.87 ± 176.56	0.499
Parietal pleura	79.29 ± 61.42	136.23 ± 63.45	0.450
Diaphragm	63.50 ± 46.49	13.33 ± 8.27	0.554
Pericardium	6.47 ± 2.45	14.60 ± 9.94	0.933

*Values given as mean ± SE, values otherwise indicated.

Index of Talc Particle Deposition

Table 1 compares the score for talc deposition in the homolateral side 24 h and 72 h after intrapleural injection. Talc deposition was consistently higher for the lung and visceral pleura (which was not dissociated for analysis) than for the parietal pleura, the diaphragm, and the pericardium. This score was 371.4 particles (± 102.90) for the lung and visceral pleura, 106.70 particles (± 43.6) for parietal pleura, 39.20 particles (± 24.40) for the diaphragm, and 10.30 particles (± 4.8) for the pericardium (Fig 2).

The systemic distribution of talc particles 24 and 72 h after injection was assessed in the right lung, brain, liver, spleen, kidneys, and blood, representing a total of 198 samples. Talc particles were found in four samples (2%) [Table 2].

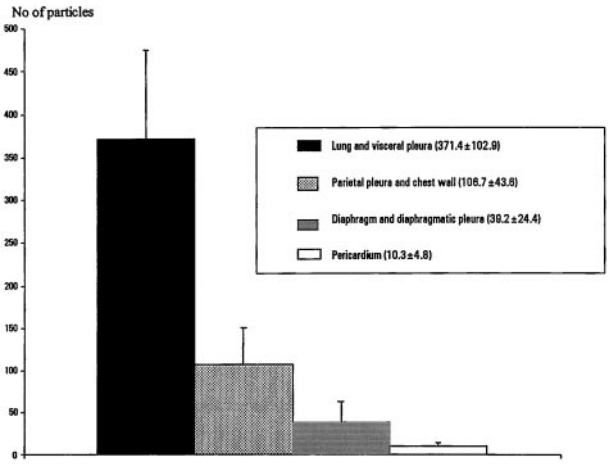

FIGURE 2. Talc deposition in the homolateral lung after slurry administration of 40 mg calibrated talc in 32 rats. Values are given as the mean ± SE.

Table 2—*Systemic Dissemination of Talc Particles 24 h (18 rats) and 72 h (15 rats) After Intrapleural Slurry Injection on the Left Side

Autopsy	Right Lung	Brain	Liver	Spleen	Kidneys	Blood
24 h	NP	1 (1†)	5 (1)	2 (1)	NP	NP
72 h	NP	NP	75 (1)	NP	NP	NP

*Values given as No. of talc particles (No. of rats with talc). NP = no particles of talc.
†Studied with an electron microscope.

Discussion

Sterile asbestos-free talc is the most inexpensive and efficient agent to use for pleurodesis.[16,17] As for other agents, chest pain and fever are the most common minor adverse effect. Among acute serious adverse effects, including empyema and arrhythmia, which are rarely reported, respiratory failures and death after talc poudrage or slurry injection have been described.[10,13,18,19]

In a recent review of the literature, Sahn[20] found acute respiratory failures in 0.71% of patient receiving talc slurry or poudrage for the treatment of pleural effusions and in 0.15% of patients treated with talc poudrage for pneumothoraces.[3] However, these acute respiratory failures, which usually are of rapid onset, vary from series to series between a very high incidence of 33%[10] and no case of ARDS in very large series.[11,12]

If there are multiple possible causes for the development of such respiratory failure after pleurodesis that is related to or is not related to talc particles, it was postulated that intrapleural talc moves into the parietal pleural lymphatic system and is transported to the mediastinal lymph nodes and thoracic duct, where it enters the systemic circulation.

This systemic absorption of talc must be considered, as was shown by Werebe and collaborators[14] in an experimental animal study. However, the size distribution of talc particles (as well as the talc elementary composition) is different from one talc mine to another, thus resulting in differences in pleural permeability and the systemic dispersion of particles after intrapleural injection.

Our study was designed to assess the distribution of talc particles in all organs and the blood after the intrapleural injection of 40 mg calibrated talc. Figure 1 shows the elementary composition of talc particles determined by x-ray microanalysis. The particle size distribution and the specific surface area of this talc preparation were recently determined.[21] The mean and median particle sizes were 33.6 μm and 31.3 μm, respectively, which varies markedly by more than a factor of three in the physical characteristics of several talc preparations that are used intrapleurally for the production of pleurodesis in the United States, South America, Taiwan, and the rest of Europe.

The dose represents an extrapolated dose of 10 g for a 70-kg adult human, taking into account the variability of clinical practice with the use of 5 to 10 g talc for pleurodesis. The time of autopsy was chosen based on the results of a previous experimental study[14] showing that absorption was very rapid through the pleura, reaching systemic circulation and being deposited in the organs as soon as 24 h after the pleural injection. Moreover, acute respiratory failure is an early complication after pleurodesis.

A small number of talc particles was found in organs with a very low incidence. No talc particles were found in the blood, kidneys, and contralateral lung. For one rat, 75 talc particles were found in the liver. The size of the talc particles was similar to those from the intrapleurally injected calibrated talc. The lack of difference between the talc particles suggests a contamination of the tissue by other talc-containing tissue. Indeed, as is the case for the previous studies published, we cannot rule out a contamination of tissues by other talc-containing tissue of the same rat, because all tissues from the same animal were frozen and stored together in the liquid nitrogen container.

The local distribution of talc did not show a difference between necropsies performed 24 h after the injection and those performed 72 h after the injection. This result may indirectly mean that no absorption of the talc from the pleural cavity occurred. As was the case for others authors, we were unable to dissociate the visceral pleura and the homolateral lung for talc analysis. Thus, we cannot discuss the passage of talc through the visceral pleura to the lung tissue, as previously described.[8,13] However, no previous correlation has been made between the presence of talc particles in BAL fluid and acute respiratory failure, and conversely talc would be expected in the BAL fluid of patients who have not experienced respiratory failure.[20]

We are worried about the difference between our data and those in the literature, in particular the article by Werebe and collaborators.[14]

In our study, a careful thoracoscopic inspection of the pleural cavity of the rats was made to check the absence of small lacerations of the lung provoked by the surgical manipulation. Indeed, such superficial lesions of the lung might allow for the entry of some talc particles directly into the lung or the vessels. The lack of such information in previous studies cannot rule out this kind of dissemination.

Moreover, no information is available concerning the origin of and the size distribution (and the elementary composition) of talc particles used in

study by Werebe et al,[14] which is questionable. The same group recently has reported a 15-year experience with thoracoscopy talc poudrage.[22] They mentioned that the pleurodesis is usually produced using asbestos-free talc with particle sizes of 5 to 70 μm, without other relevant details.

Although we do not know the difference in permeability between normal and inflamed pleura, based on the results of our study we can hypothesize that the local and systemic dissemination of talc after intrapleural administration as a slurry injection is related to a systemic absorption and strongly depends on the size of talc particles. Taking into account the experimental data and clinical practice, calibrated talc is required for pleural symphysis. The ideal diameter of talc particles must be assessed by further studies using progressively smaller talc particles.

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