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ORIGINAL ARTICLE

Malignant pleural effusion and talc pleurodesis. Experimental model regarding early kinetics of talc particle dissemination in the chest after experimental talc pleurodesis

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Summary

Purpose: *Talc remains a commonly used agent for pleurodesis malignant pleural effusion. Nevertheless, it is associated with a 3-9% incidence of pulmonary reactions ranging from simple pneumonitis to acute respiratory distress syndrome (ARDS). The underlying lung pathology and the size and rate of talc particle dissemination have been implicated as the cause of these complications. There seems to be an acknowledged lack of evidence regarding detailed very early intrathoracic talc particle migration.*

Materials and methods: *Thirty white male New Zealand rabbits underwent experimental pleurodesis and were randomly assigned to 3 (A, B, C) study groups (10 in each group). Rabbits were sacrificed 6, 12 and 18 h after talc administration. Samples from both lungs, mediastinum and parietal pleura were obtained. The number of talc crystals (m) deposited was counted and averaged along all slices of the various tissue samples.*

Results: *A high degree of early talc deposition and subsequent epithelial injury in all examined tissues was observed. Diffuse talc deposition occurred in both lungs, but in a different manner. On the side of talc administration, talc particles were deposited in a time-dependent fashion. On the contralateral side, talc was rapidly deposited during the first hours after the procedure, then the rate of deposition decreased, and increased again between 12 and 18 h after the procedure.*

Conclusion: *Large-sized talc particles are deposited on both lungs very early after pleurodesis. At the same time inflammatory pulmonary changes appear bilaterally. Despite contradicting data in the literature, these findings should always be kept in mind when performing this procedure in high risk patients.*

Key words: ARDS, malignant pleural effusion, side effects, talk particles systemic distribution, talc pleurodesis

Introduction

Since the beginning of the last century, pleurodesis had become the most frequent therapeutic modality to address recurrent malignant pleural effusion or pneumothorax. A variety of agents have been used over the years. The preference and popularity of these agents, such as talc, tetracycline, minocycline, doxorubicin, bleomycin, interferon-β, and more recently transforming growth factor-β (TGF-β), have fluctuated, due to concerns over potential complications, patient's tolerance, paucity of data for agent effectiveness, need for repeated applications, and cost-effectiveness [1,2].

Talc remains the most commonly used agent for pleurodesis. Nevertheless, talc pleurodesis is associated with a 3-9% incidence of pulmonary inflammatory reactions ranging from simple pneumonitis or low grade pulmonary edema, to full blown ARDS [3-5]. The underlying lung pathology, as well as the size and rate of talc particle migration and dissemination with its resultant inflammatory reaction, have been implicated by many investigators as the main cause of these complications. There seems to be an acknowledged lack of evidence and paucity of data regarding detailed early (less than 24 h) intrathoracic talc particle migration [2-4].

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In view of the above, we conducted an experimental talc pleurodesis protocol in a rabbit model to specifically obtain, a: detailed quantitative data on the early talc particle migration and deposition rate in the ipsilateral and contralateral lung; and b: to correlate these migration and deposition data with the severity and time of appearance of pathological pulmonary findings usually associated with acute pulmonary inflammatory states.

Materials and methods

Talc characteristics and preparation

Talc is pulverized, natural, foliated, hydrated magnesium silicate, with chemical formula $Mg_3Si_4O_{10}(OH)_2$ [6]. The talc used in our study was an asbestos-free formula commercially available for pleurodesis (Merck K GaA, Germany). This formula consists of a mixture of large (50-100 microns) talc particles. The experimental procedure was performed as previously described [4,7-9]. Each animal received 200 mg/kg of talc, suspended in 2 ml of saline solution. Talc was sterilized by autoclaving at 121° C and 1 Atm for 30 min.

Animal groups

Thirty white male New Zealand rabbits weighing 1.5-2.5 kg were randomly assigned to the 3 study groups: group A, in which rabbits were sacrificed 6 h after talc administration; group B, in which rabbits were sacrificed after 12 h; and group C, in which rabbits were sacrificed after 18 h. Approval of the animal experimentation protocol was obtained from the Athens Division of Veterinary Experimentation.

Pleurodesis

Pre anesthesia was accomplished with xylazine hydrochloride 5 mg/kg and ketamine hydrochloride 35 mg/kg, administered i.m. Then, all animals were intubated with a polyethylene tube (No 2.5) and connected to a small animal ventilator.

Under aseptic conditions, a very small right thoracotomy was performed and a catheter in the 5th intercostal space was placed, in order to administer talc (200 mg/kg, suspended in 2 ml of saline solution) as slurry into the pleural cavity, under direct view. After that, the catheter was removed under positive pressure from the ventilator and the incision was closed in layers. Following incision closure, the animals were turned over to ensure homogeneous talc distribution, and were then extubated. The animals were maintained in adequate cages and fed according to the protocol of the animals' quarters.

After 6, 12 or 18 h, the animals were sacrificed with 40 mg/kg i.v. of phenobarbital solution into the marginal ear vein. The dissection was performed by one of the investigators, who was blinded to the treatment group. Samples from both lungs, mediastinum and parietal pleura, were obtained.

Tissue sample processing

The obtained tissue samples were fixed in 10% neutral formalin. Then they were cut to 5 μm thick slices which were placed on gelatinized slices.

The sections were then fixed with xylol, hydrated and stained with hematoxylin – eosin. Talc particle assessment was carried out by light field and polarizing microscopy in an optic microscope for birefringent particles. All slices were coded, randomized and evaluated by a single observer with no code access.

Index of birefringence particle deposition

The number of talc crystals (m) deposited was counted in 5 fields of 100× along all slices of the various samples of the examined tissues. The numbers of deposited talc crystals were averaged for each tissue location, as previously described [8]. The amount of deposited talc crystals was defined as: m_6=mean number deposited at 6 h, m_{12}= mean number deposited at 12 h, m_{18}= mean number deposited at 18.

Statistics

The Kruskal-Wallis H statistical test was applied on each comparison in order to detect any statistically significant differences between time points. In case of a statistically significant difference, the p-value was estimated. In such a case, Mann-Whitney U was applied in order to detect the statistically significant pairs of time points (significance level $p<0.05$). Data were presented as mean ± standard deviation (SD).

Results

Number of talc crystals

In all tissue locations examined, statistical testing detected significant differences in particle deposition between 6, 12 and 18 h time points. For each tissue location, the mean value of Birefringence Particle Index of Deposition is given in Table 1.

Table 1. Talc crystals distribution

Location	*Mean number ± SD of talc crystals after pleurodesis*			
	6 hours	*12 hours*	*18 hours*	*p-value*
Pleural cavity				
Ipsilateral lung	12.6±5.0	12.6±4.1	33.4±2.4	<0.001
Contralateral lung	12.0±3.8	4.9±2.4	8.3±2.0	0.001
Ipsilateral parietal pleura	13.5±2.6	15.2±2.8	16.9±2.0	NS
Contralateral parietal pleura	12.5±2.4	6.9±2.4	14.9±2.1	<0.001
Mediastinum				
Adipose tissue of upper mediastinum	5.8±2.7	7.3±3.2	11.5±4.8	0.012
Adipose tissue of lower mediastinum	7.3±6.4	7.6±2.0	19.1±8.2	0.001
Trachea	1.5±2.0	3.4±2.6	1.6±1.9	NS
Lymph node stations 8-10	5.1±4.5	8.2±2.4	2.2±2.0	<0.001
Lymph node stations 4-7	7.0±4.3	11.3±3.1	4.0±2.0	<0.001

NS: nonsignificant, SD: standard deviation

Parietal pleura

On the parietal pleura, the lesions most commonly observed due to local inflammation were characterized by the presence of macrophages and interstitial lymphocyte infiltration. This process also induced a progressive mesothelial destruction and regression of the basal lamina and underlying connective tissue. Submesothelial capillary vasodilatation and initial local necrosis with extravasation of leukocytes and erythrocytes was also observed.

Ipsilateral parietal pleura

The time sequence of talc deposition at the ipsilateral pleura was a progressively increasing one: Ipsilateral parietal pleura: $m_6 < m_{12} < m_{18}$.

Contralateral parietal pleura

Similar pathological findings were observed at the left (contralateral) parietal pleura. These changes were more evident 6 h later than the ipsilateral side. Talc deposition decreased from the 6th to the 12th h, and then progressively increased at 18 h, to a final level higher than the initially recorded at 6 h.

Contralateral parietal pleura: $m_{12} < m_6 < m_{18}$

Lungs

Regarding lung tissue at 6 h, talc crystals were initially found as particle accumulations in the peripheral part of the organ. The inflammatory reaction at 6 h showed initially edematous areas with capillary dilatation and macrophage and eosinophil infiltration. The parenchymal architecture was progressively disorganized with talc particle aggregates accumulating in the alveolar ducts. On some slices, even at 6 h, talc crystals reached small lymphatic and blood vessels, forming small thrombi through the bronchovascular space. A progressive accumulation of fibrin matrix was observed at 12 h on the ipsilateral lung. Additional findings at the 12 h point included increased thrombus formation and eosinophil infiltration This fibrin matrix contained most of the talc particles instilled. Some contiguous airways, particularly alveoli, alveolar ducts and bronchioles, were disorganized and contained aggregates of particles. In addition to congestion, edema, focal necrosis, eosinophil infiltration and fibrin deposition, the alveolar wall became lined with waxy hyaline membranes. Such membranes were the hallmark of the 18 h specimens. They were more pronounced on the ipsilateral side and consisted of protein-rich edema fluid mixed with the cytoplasmatic and lipid membranes of damaged epithelial cells. These findings indicated substantial epithelial injury at the 12-18 h period after pleurodesis.

Ipsilateral lung

Talc particle migration on the ipsilateral lung followed a progressively increasing pattern (Table 1). Right lung: $m_6 = m_{12} < m_{18}$

Contralateral lung

On the contralateral side, talc deposition decreased from the 6th to the 12th h period, and then progressively increased at 18 h, to a final level slightly lower than the initially recorded at 6 h (Table 1).

Left lung: $m_{12} < m_{18} < m_6$

Overall, almost all animals showed substantial epithelial injury, starting with edema and cellular infiltration, progressing to thrombosis and tissue destruction and ending with fibrin deposition and membrane formation between 12-18 h after pleurodesis. As a general rule, these changes were similar in severity on both sides but appeared on the ipsilateral side 6 h prior to the contralateral side.

Discussion

Talc is still the sclerosing agent most frequently used in order to create pleurodesis in patients with either recurrent malignant pleural effusion or spontaneous pneumothorax. The reason of this worldwide preference is that talc administered either by aerosol (insufflation) or in a suspension form (slurry) is available, inexpensive and effective. However, questions have been raised over the years by several investigators regarding the safety of intrapleural talc injection. Complication rates after talc pleurodesis in the literature show considerable variations. There have been reports of ARDS [2,10], pulmonary embolism [3,11] and acute lung injury, pulmonary edema and acute pneumonitis [11] occurring after intrapleural talc administration. Early on, Gaensler [12] and Youmans et al. [13] have reported severe side effects resulting from talc pleurodesis, such as pain, fever, embolization, respiratory failure and fatal cardiac arrest. On the other hand, Weissberg and Zeev [14], gathering their experience, observed that purified talc is extremely safe for pleurodesis. Others, like Schafers and Dresler [15], reported that there were actually quite a number of reports proving talc-related side effects during pleurodesis, again raising questions about the safety of this material. The incidence of respiratory failure following talc pleurodesis has varied widely from study to study. In a retrospective analysis in Seattle, Rehse and coworkers [16] reviewing their experience reported an ARDS incidence of 9% requiring mechanical ventilation. On the other hand, Cardillo et al. [17] reported that there were no cases of ARDS or respiratory failure after thoracoscopic poudrage, using 5 g of talc insufflated intrapleurally. This difference was attributed on the hypothesis that ARDS following talc administration is either particle-size or dose-dependent, occurring in patients receiving more than 5 g of talc composed of small sized particles. The hypothesis of dose dependence is probably wrong since Xie et al. [18] reported that acute respiratory failure still developed in 5 out of a total of 550 patients (1.3%), who received only 2 g of insufflated talc for either recurrent pleural effusion or pneumothorax. As regards the influence of particle size in the resultant inflammatory reaction, several recent studies have shown that large-sized talc particle preparations disseminate less and induce less inflammatory reaction [19,20]. An important point to keep in mind is that in the study that found essentially no talc dissemination was performed in rats, used highly calibrated talc particles and examined the tissues at 24 h and 3 days after the procedure [20], in other words much later than in our study. A detailed study by Genofre and coworkers [21] compared the levels of systemic inflammatory markers after experimental pleurodesis with small and normal-sized talc preparations. They concluded that small-sized particles produce a more intense systemic inflammatory response, as measured by leucocyte counts, lactic dehydrogenase (LDH) levels, and interleukin-8 (IL-8) levels. The differences in these parameters reached statistical significance after 24 h.

Proven mechanisms by which a talc administration procedure causes lung injury are still unknown [2,18]. The prevailing hypothesis is related to the rate of lymphatic absorption [10] and the rate and degree of intrathoracic distribution [2,18] with subsequent initiation of an inflammatory mediator-dependent response [2,5]. This hypothesis is supported by studies in both animals and humans. Gary Lee and Texeira [2] reported that the very early rate of talc particle producing pleurodesis, and consequently potential side effects, is unknown. Detailed data regarding the time frame and location of talc particle migration and deposition are not available.

In view of the above, we designed this experimental talc pleurodesis study in rabbits, in order to find out (a): how rapidly lymphatic absorption of talc particles takes place very early after the procedure; (b): what is the exact early (less than 24 h) intrathoracic talc particle distribution; and (c): the associated degree and time frame of ipsilateral and contralateral pulmonary changes relating to epithelial injury.

This study provides detailed data regarding the rapid and progressive intrathoracic distribution of large-sized talc. Our results showed a high degree of early talc deposition and subsequent epithelial injury in all examined tissues. Our data complete and reinforce previous less detailed data about the lymphatic drainage and the intrathoracic distribution of talc particles. On the other hand, our findings seem to contradict other studies [20] that show much less particle dissemination. We showed that talc of large size is absorbed rapidly through the pleura, reaching the blood stream via lymphatics, and is deposited on intrathoracic organs shortly after its intrapleural administration. An interesting point derived

from our study is that diffuse talc deposition occurs in both lungs, not exclusively on the side of the procedure, but in a different manner. On the side of talc administration, talc particles are deposited in a time-dependent fashion. On the contralateral side, talc is rapidly deposited in the first hours after the procedure, then the rate of deposition decreases, and finally increases again between 12 and 18 h after the procedure.

Concerning the potential side effects, morphologically we described findings observed in pulmonary edema, acute lung injury, ARDS and pulmonary embolism. These findings occurred in both lungs, in a sequential fashion, the ipsilateral side reaching its more destructive point approximately 12-18 h after the procedure, 6 h before the contralateral side.

Our data are in accordance with clinical observations and reinforce reports regarding the potential side effects after talc pleurodesis. The rapid (6-18 h) bilateral deposition of large-sized talc and the observed associated inflammatory changes demonstrated in this study, should be kept in mind, since ARDS generally appears 12-24 h after pleurodesis. These findings should increase our caution when performing this procedure on high risk patients or patients with pre-existing pulmonary disease. Moreover, we think that these data could also further justify ongoing research on the development of agents modifying the pulmonary inflammatory reaction as well as the development of newer and safer pleurodesis agents [3,16].

References

1. Bouros D, Froudakis M, Siafakas N. Pleurodesis. Everything flows. Chest 2000; 118: 577-579.
2. Gary Lee YC, Texeira LR, Devin CJ et al. Transforming growth factor- β_2 induces pleurodesis significally faster than talc. Am J Resp Crit Care Med 2001; 163: 640-644.
3. Kennedy L, Rusch VW, Strange C, Ginsberg RJ, Sahn SA. Pleurodesis using talc slurry. Chest 1994; 106: 342-346.
4. Webere EC, Pazetti R, Milanez de Campos JR et al. Systemic distribution of talc after intrapleural administration in rats. Chest 1999; 115: 190-193.
5. Ferrer J, Montes JF, Villarino MA, Light RW, Valero JG. Influence of particle size on extrapleural talc dissemination after talc slurry pleurodesis. Chest 2002; 122: 1018-1027.
6. Zazenski R, Ashton WH. Talc occurrence, characterization and consumer applications. Regul Toxicol Pharmacol 1995; 21: 218-229.
7. Vargas FS, Texeira LR, Vaz MA et al. Silver nitrate is superior to talc slurry. Chest 2000; 118: 809-811.
8. Vargas FS, Texeira LR, Antonangelo L et al. Experimental pleurodesis in rabbits induced by silver nitrate or talc. Chest 2001; 119: 1516-1519.
9. Light RW, Wang NS, Sassoon CSH, Gruer SE, Vargas FS. Talc slurry is an effective pleural sclerosant in rabbits. Chest 1995; 107: 1702-1706.
10. Campos JRM, Werebe EC, Vargas FS, Jatene FB, Light RW. Respiratory failure due to insufflated talc. Lancet 1987; 349: 251-252.
11. Milanez R, Vargas F, Filomeno L et al. Intrapleural talc for the treatment of malignant pleural effusions secondary to breast cancer. Cancer 1995; 75: 2688-2691.
12. Gaensler E. Parietal pleurectomy for recurrent spontaneous pneumothorax. Surg Gynecol Obstet 1956; 102: 293-297.
13. Youmans CR, Williams RD, McMinn MR. Surgical management of spontaneous pneumothorax by bleb ligation and pleural dry sponge abrasion. Amer J Surg 1970; 120: 644-648.
14. Weissberg D, Zeev I. Talc pleurodesis- experience with 360 patients. J Thorac Cardiovasc Surg 1993; 106: 689-695.
15. Schafers SJ, Dresler CM. Update on talc, bleomycin, and the tetracyclines in the treatment of malignant pleural effusions. Pharmacotherapy 1995; 15: 228-235.
16. Rehse DH, Aye RW, Florence M. Respiratory failure following talc pleurodesis. Am J Surg 1999; 177: 437-440.
17. Cardillo G, Facciolo F, Carbone L et al. Long term follow up of VATS pleurodesis in malignant recurrent pleural effusions. Eur J Cardiothorac Surg 2002; 21: 302-306.
18. Xie C, Texeira LR, Wang NS, McGovern JP, Light RW. Serial observations after high dose talc slurry in the rabbit model for pleurodesis. Lung 1998; 176: 299-307.
19. Ferrer J, Montes JF, Villarino MA, Light RW, Garcia-Valero J. Influence of particle size on extrapleural dissemination after talc pleurodesis. Chest 2002; 122: 1018-1027.
20. Fraticelli A, Robaglia-Schlupp A, Rierra H, Monjanel-Mouterde S, Cau P, Astoul P. Distribution of calibrated talc after intrapleural administration: an experimental study in rats. Chest 2002; 122: 1737-1741.
21. Genofre EH, Vargas FS, Texeira LR, Acencio MM, Antonangelo L, Marchi E. Systemic inflammatory acute response in talc pleurodesis using talc of different size particles. Chest 2004; 126: 726-731.