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Evidence that mesothelial cells regulate the acute inflammatory response in talc pleurodesis

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ABSTRACT: Intrapleural instillation of talc is used to produce pleurodesis in cases of recurrent malignant pleural effusions. The mechanisms by which pleurodesis is produced remain unknown but may involve either injury or activation of the mesothelium. The aim of the current study was to assess the inflammatory response of pleural mesothelial cells to talc in an experimental model in rabbits.

A group of 10 rabbits were injected intrapleurally with talc (200 mg·kg^{-1}) and undiluted pleural fluid was collected after 6, 24 or 48 h for measurement of interleukin (IL)-8, vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1. Samples of pleura were studied to assess the inflammatory infiltrate and mesothelial cell viability.

The pleural fluid IL-8 concentration peaked at 6 h, whereas VEGF and TGF-β1 concentrations increased steadily over 48 h. Immunohistochemistry for cytokeratin showed a preserved layer of mesothelial cells despite the intense inflammatory pleural reaction.

In conclusion, it is proposed that the mesothelial cell, although injured by the talc, may actively mediate the primary inflammatory pleural response in talc-induced pleurodesis.

KEYWORDS: Inflammatory mediators, pleural effusions, pleurodesis

Malignant pleural effusion is a common complication of advanced cancer [1] and, despite the fact that patients with this malignant complication have a short life expectancy, the prompt control of the effusion is imperative to obviate discomfort of persistent cough and dyspnoea. Drainage of the pleural cavity and instillation of a sclerosing agent is the standard method to produce pleural symphysis and prevent fluid re-accumulation [2–4]. Talc, instilled either by slurry or by thoracoscopy [5–7], has been shown to produce more than 90% success in the control of recurrent malignant pleural effusion [8, 9]. The mechanism by which talc produces a pleurodesis remains unclear. Talc may injure the mesothelial layer or may stimulate monocytes or mesothelial cells to produce a local reaction mediated by major inflammatory cytokines [10, 11]. Among the cytokines involved in the acute inflammatory response, interleukin (IL)-8 and vascular endothelial growth factor (VEGF) are the main mediators of leukocyte activation and vascular capillary response to inflammation, respectively [12–14], whereas transforming growth factor (TGF)-β1 and fibroblast growth factor (FGF) are involved in the activation of fibroblasts to produce and deposit collagen [15, 16].

In animal and clinical models of pleurodesis, it is difficult to isolate the response of the mesothelial cells to the sclerosing agent because of the active multicellular population found in the pleural space. The purpose of the present study was to determine the inflammatory response of the pleural space and assess the viability of the mesothelial cells as part of the inflammatory pleural infiltrate produced by talc used for pleurodesis. The current report indicates that mesothelial cells have the potential to be the major contributor to the inflammatory response in the pleural space in talc-induced pleurodesis.

METHODS

This study was approved by the Ethics Committee of the Heart Institute (InCor), University of Sao Paulo Medical School, which oversees research involving both animals and humans.

Pleural injection

Ten New Zealand white male rabbits weighing 2.5 kg were anaesthetised and injected intrapleurally with 3 mL of talc (200 mg·kg^{-1}), which is currently used to produce pleurodesis in clinical practice (Magnesita, Bahia, Brazil; mean length 25.4 μm, range 6.4–50.5 μm). The talc was

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suspended under sterile conditions in an endotoxin-free saline solution. The protocol of pleural injection has been described in detail previously [17, 18]. After 6, 24 or 48 h, the animals were sacrificed and, after exposure of the diaphragm *via* a mid-abdominal incision, the pleural fluid was aspirated and processed for cytokine measurements.

Cytokine analysis

IL-8 (OptEIA, rabbit IL-8 set; Pharmingen, San Diego, CA, USA), VEGF (R&D System, Inc., Minneapolis, MN, USA) and TGF-β1 (R&D System, Inc.) were measured by ELISA as described previously [13].

Tissue samples

Samples of lung including the visceral pleural tissue were fixed in 10% formalin for 48 h and processed for histological analysis. The slides were stained by immunohistochemistry for cytokeratin (AE1/AE3: Dako Cytomation, Produktionsgej, Denmark) following the manufacturer's directions.

Statistical analysis

Data are expressed as mean±SD. One-way ANOVA was used to compare differences among subgroups and the Tukey test was used to perform multiple comparison procedures. A p-value <0.05 was accepted as significant.

RESULTS

Cellular response

WBC

Total white blood cell counts (WBC) were significantly greater at 6 and 24 h in comparison with 48 h (19,430±1,750 and 18,810±5,660 *versus* 11,270±1,190 cells·mm^{-3}, respectively; $p<0.05$; table 1).

Neutrophil percentage

Similar to WBC levels, neutrophil percentages were increased in the first 6 h (74±6) in comparison to 24 h (60±5; $p<0.05$) and 48 h (24±8; $p<0.001$).

IL-8

Concentrations of IL-8 increased as soon as 6 h (797±335 pg·mL^{-1}) after the pleural space was exposed to talc, and remained elevated until 24 h (665±125 pg·mL^{-1}), decreasing significantly after 48 h (134±33 pg·mL^{-1}; $p<0.001$; table 2).

VEGF

Unlike IL-8, VEGF levels increased with time. At 48 h, the VEGF concentration (689±115 pg·mL^{-1}) was significantly >6 h (164±64 pg·mL^{-1}; $p<0.001$) and 24 h (336±188 pg·mL^{-1}; $p<0.05$).

TGF-β1

Similarly to VEGF, the concentrations of TGF-β1 also increased over time. At 48 and 24 h, the levels were significantly >6 h (1,135±223 and 931±60 *versus* 385±104 pg·mL^{-1}; $p<0.001$ and $p<0.05$, respectively).

Correlations of the WBC and neutrophil percentages with cytokine IL-8

Although the WBC and neutrophil percentage showed the same behaviour as IL-8 in the pleural fluid after talc instillation, no correlation was found among these parameters (data not shown).

Microscopic analysis of the pleural tissue

Microscopic samples of the visceral pleura showed an acute inflammatory reaction as early as 6 h after intrapleural talc injection. The histological analysis of the slides stained by immunohistochemistry for cytokeratin revealed, in several zones throughout the slides, a preserved brown-stained monolayer of pleural mesothelial cells underlying an intense inflammatory process characterised by a multi-cell population (fig. 1).

DISCUSSION

The mechanisms by which talc produces pleurodesis remain poorly understood. The regulation of the pleural acute inflammatory response following the injection of a sclerosing agent is crucial to understanding the mechanism of pleurodesis. The findings of this study indicate that pleural mesothelial cells may actively contribute to modulate the inflammatory process in talc-induced pleurodesis.

The current findings are in accordance with previous reports, which have shown that mesothelial cells exposed to talc can actively produce pro-inflammatory IL-8 and monocyte chemotactic protein-1 [11, 13], VEGF [13], TGF-β [15] and bFGF [16] cytokines.

TABLE 1 Levels of pleural fluid white blood cells (WBC, cells·mm-3) and neutrophils (% N) in talc-injected (200 mg·kg^{-1}) rabbits after 6, 24 and 48 h

	WBC	% N
6 h	19430±1,750¶	74±6$^{\#,+}$
24 h	18810±5,660¶	60±5^{+}
48 h	11270±1,190	24±8

Data are presented as mean±SD. $^{\#}$: significantly >24 h, $p<0.05$; ¶: significantly >48 h, $p<0.05$; $^{+}$: significantly >48 h, $p<0.001$.

TABLE 2 Levels of pleural fluid interleukin (IL)-8, vascular endothelial growth factor (VEGF) and transforming growth factor (TGF)-β1 (pg·mL^{-1}) in talc-injected (200 mg·kg^{-1}) rabbits after 6, 24 and 48 h

	IL-8	VEGF	TGF-β1
6 h	797±335§	164±64	385±104
24 h	665±125§	336±188	931±60$^{\#}$
48 h	134±33	689±115$^{¶,+}$	1135±223¶

Data are presented as mean±SD. $^{\#}$: significantly >6 h, $p<0.05$; ¶: significantly >6 h, $p<0.001$; $^{+}$: significantly >24 h, $p<0.05$; §: significantly >48 h, $p<0.001$.

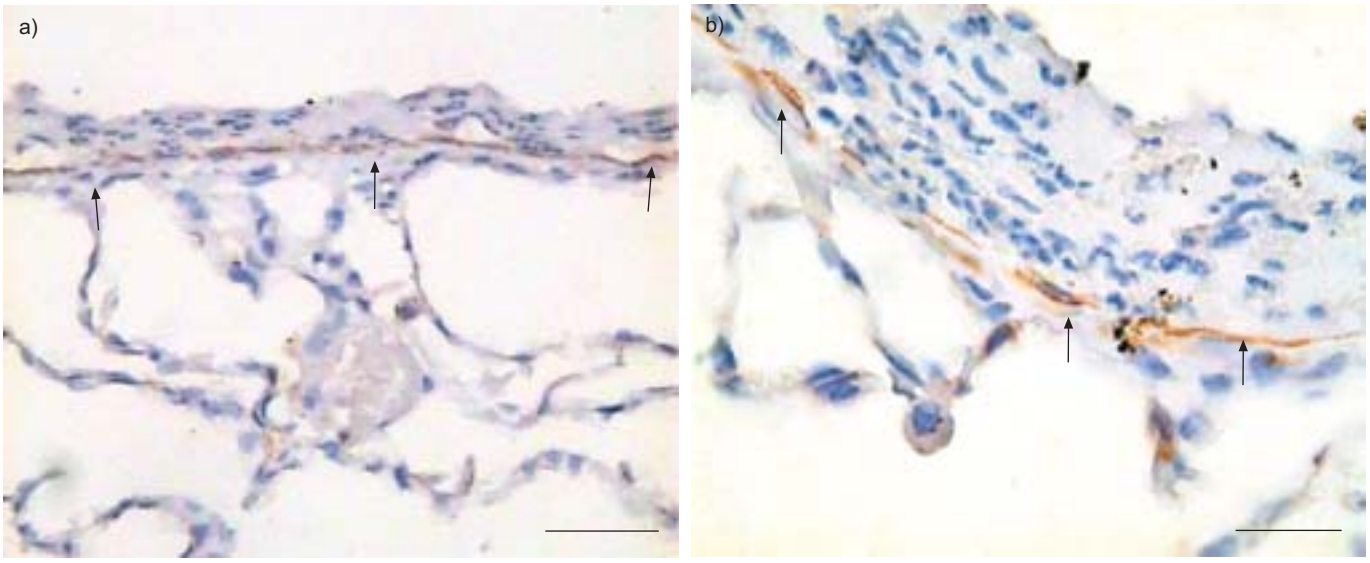

FIGURE 1. Acute pleural inflammatory reaction in rabbits, 24 h after talc (200 mg·kg^{-1})-injection, showing a preserved brown-stained cytokeratin positive monolayer of pleural mesothelial cells (arrows). Scale bars: a) 30 μm and b) 10 μm.

In addition, a recent study evaluating submicroscopic features of active pleural remodelling associated with talc pleurodesis stated that talc acutely induces a prominent injury to the mesothelial cells and mesothelial cell–mesothelial basement membrane union. However, focal remesothelialisation of the denuded areas was documented [19], showing an active role of mesothelial cells in the healing process in talc pleurodesis.

The present results indicate that WBC, neutrophil percentage and IL-8 levels were increased in the first 24 h, whereas VEGF and TGF-β1 levels were initially lower and increased with time. As the population of active inflammatory cells found in the pleural space in talc-induced pleurodesis is diverse, the specific contribution of the mesothelial cell response in pleurodesis induced by talc still poses a challenge. The current results indicate that mesothelial cells may account for the inflammation seen *in vivo*.

The dose of talc used in this study was comparable to that used in human pleurodesis. If the surface of both pleural membranes in the rabbit (200 cm^2) and in humans (2,000 cm^2) is considered, the amount of talc used *in vivo* is approximately the same: in rabbits, 200 mg × 2.5 kg·200 cm^{-2}=2,500 μg·cm^{-2} and in humans 5 g·2,000 cm^{-2}=2,500 μg·cm^{-2}.

The experimental finding that mesothelial cells may play a major role in the mechanism of pleural inflammation and contribute to an effective sclerosis in talc-induced pleurodesis may help explain why malignant pleural effusions with a high tumour burden and a low pleural fluid pH and glucose concentration may be less responsive to talc-pleurodesis than malignant effusions with less malignant involvement of the pleural space [18–22]. However, this is not absolute because effective pleurodesis can be achieved using talc by thoracoscopy despite the findings of low pleural fluid pH [23]. In conclusion, the present findings indicate that the acute inflammatory process in talc pleurodesis may have the active contribution of the mesothelial cells.

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