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Original Contribution

ACUTE PLEURAL CHANGES AFTER INTRAPLEURAL INSTILLATION OF IODPOVIDONE IN THE RABBIT

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ABSTRACT

Pleurodesis is intended to achieve a symphysis between parietal and visceral pleura in order to prevent accumulation of air or fluid. The aim of this research was to assess the acute gross and microscopic pleural findings, after intrapleural administration of *iodpovidone* as a sclerosing agent. 22 New Zealand White rabbits were used for the study. After instillation of 2 ml 10% iodpovidone into the right pleural cavity, the animals were sacrificed in groups of three on days 1, 2, 3, 4. Gross pathology, pleural fluid analysis and histology analysis were performed. It was found that jodpovidone caused pleural effusion with characteristic exudates and appearance of first fibrinous strands on day 3. It was found also that there were mesothelial abnormalities and dilatation of subpleural vessels, in response to intrapleural instillation of iodpovidone. The inflammatory cell infiltrates changed from polymorphonuclear to mononuclear, with fibroblast appearance on day 3. The study has shown that iodpovidone, as a pleural sclerosing agent, caused pleural inflammatory reaction. Mesothelial cell abnormalities and dilatation of subpleural vessels are triggers of the pleural inflammatory reaction. It showed that the 3- to 4-day period represents the time of acute pleural changes caused by intrapleural injection of iodpovidone.

Key Words: iodpovidone, pleurodesis, pleural effusion

INTRODUCTION

Pleurodesis, from the Greek *pleura* and *desis* (binding together), is intended to achieve a symphysis between parietal and visceral pleura, in order to prevent accumulation of either air (pneumothrax) or fluid (pleural effusion) in the pleural space The methods of pleurodesis include surgical abrasion with a dry gauze sponge through thoracotomy or video-assisted thoracoscopic surgery (VATS), or intrapleural instillation of a sclerosant agent through tube thoracostomy or through VATS.

The ideal pleural sclerosing agent for chemical pleurodesis should be easily administered, inexpensive and widely available. It should induce minimum morbidity and be 100% effective. Many different agents have been used for creating a pleurodesis, but none of them meets all of these criteria. The search for the ideal agent for pleurodesis continues and the choice of a particular agent depends on the local availability and experience.

On the other side, the mechanisms by which various agents produced a pleurodesis are incompletely understood. In studying the mechanisms of pleurodesis, the objective of our present study was to assess the acute gross and microscopic pleural findings in rabbits that receive *iodpovidone* as a sclerosing agent.

MATERIAL AND METHODS

Twelve New Zealand male rabbits weighing 2, 5-3, 5 kg were used for this study. The animals were in groups of three, sacrificed after 1, 2, 3, 4 days of the intrapleural instillation of Iodpovidone.

Experimental procedure

The animals were lightly anaesthetised with 35 mg/kg ketamine hydrochloride injected intramuscularly. The thorax was prepared for aseptic surgery by shaving the right chest and then cleaning it with t-ra Jodi and alcohol. A

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3-cm skin incision was made midway between the spine and the scapular tip. The muscles in the sixth or seventh intercostals space were bluntly dissected to allow exposition of the parietal pleura. A specially prepared multi-perforated catheter (from the 22-G angiocatheter) was introduced into the pleural space and 2 ml 10% Iodpovidone was injected. In sequence, the catheter was removed and the muscles and skin were sutured.

Gross pathology and pleural fluid analysis

The animals were sacrificed with lethal dose of 50 mg/kg of thiopental injected into the marginal ear vein. In the supine position, the sternum and the median portions of the ribs were removed so the two hemithoraces could be evaluated macroscopically. The left hemithorax served as a control. The pleural changes were documented by digital photography. Pleural fluid from the right hemithorax was collected at the time of sacrifice for analysis of the lactic acid dehydrogenase (LDH), protein levels and glucose.

Tissue specimen preparation

For the microscopic studies, tissue specimens of the right visceral pleura and lung were obtained. The specimens were placed in a 10% formalin solution for two days and fixed in paraffin. Histological sections were prepared, stained with haematoxylin and eosin. The microscopic slides were evaluated by light field microscopy.

Animal care

In this study we adhered to the "Guide to the Care and Use of Experimental Animals".[1]

Statistical analysis

The data of pleural fluid analysis were estimated by variable analysis. Data were expressed as the mean ± SEM.

RESULTS

Gross pathology: The intrapleural instillation of 2 ml Iodpovidone produced pleural effusion only in the right pleural cavity (in the side of injection). The left pleural cavity and the left lung, which served as control, did not change (**Figure 1**).

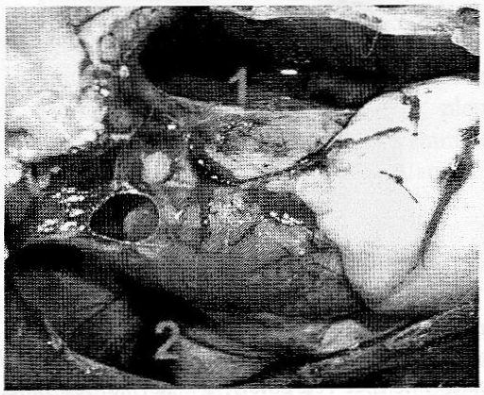

Figure 1: *Digital photography on 1 day. The sternum and the median portions of the ribs are removed. (1- pleural effusion into the right pleural cavity, 2- left pleural cavity without effusion and normal left lung)*

The pleural effusion was represented as serous exudates on days 1 and 2. On day 3 the effusion was determined as sero-fibrinous exudate, with the first fibrin strands between the pleural surfaces. More fibrin strands were examined on day 4 (**Figure 2**).

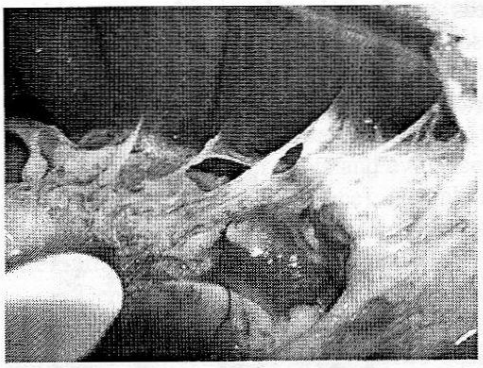

Figure 2: *Digital photography on 4 day. Fibrin strands between the visceral and parietal pleural surfaces are represented*

Visceral pleura at Days 1 and 2 demonstrated erythema and loss of the normal glistening pleural sheen, as a result of hyperaemia of the subpleural vessels (**Figure 3**). There was no pleural erythema on days 3 and 4.

Pleural fluid analysis: The mean levels of LDH in the pleural fluid were 1670 UI/L (± 86). The mean pleural fluid glucose levels were 1.46 mmol/l (0,12±) and the protein levels were 56, 0/l (±0,48).

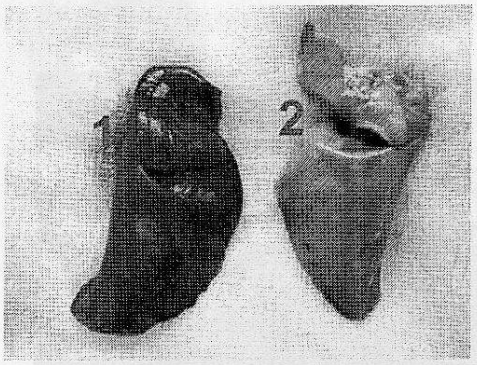

Figure 3: *Photography of dissected lungs on 1 day. (1-right lung with erythema of the visceral pleura /intensive red color/, 2 – normal left lung, serving as a control)*

There was no pleural erythema on days 3 and 4.

Histology

Specimens obtained on day 1 after Iodpovidone instillation demonstrated denudement of mesothelial cells. Visceral pleural surface with marked mesothelial desquamation was observed. Hypertrophia of mesothelial cells was established in small areas of pleural surfaces without desquamation. Mesothelial cells became cuboidal with nuclear enlargement. Separation between cells was apparent (**Figure 4**).

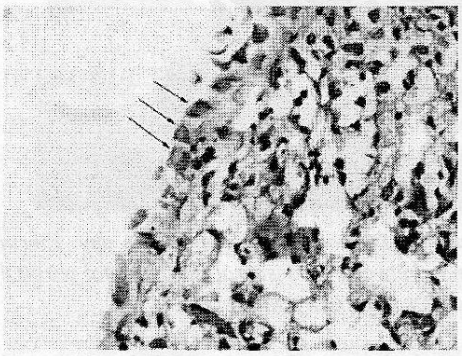

Figure 4: *Photomicrograph on day 1 at x400. Note the oedematous visceral pleura, with denudement of mesothelial cells. The arrows point to the resting cuboidal mesothelial cells with separation between them.*

Microscopically, oedema of the visceral pleura and subpleural connective tissue, with dilated subpleural venules and arterioles was noted on day 1. It was observed that dilated vessels were filled with erythrocytes. Many erythrocytes were visible not only in the dilated vessels but also around them, as a result of incensement of their permeability (**Figure 5**).

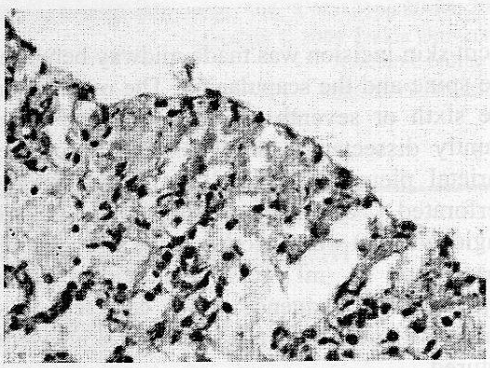

Figure 5: *Photomicrograph on day 1 at x400. Note the dilated subpleural vessels, filled with erythrocytes. There are many erythrocytes around the vessels.*

Mesothelial abnormalities were persistent on day 2. Large areas with denudement of mesothelial cells were persistent. Proliferation (hyperplasia) of the preserved mesothelial cells was observed on day 2. The cells were well-arrangement in two and three layers onto the pleural surface. Specimens on day 2 also demonstrated inflammatory infiltrates into the oedematous, thickened pleura and subpleural connective tissue, most marked around subpleural vessels. The inflammatory infiltrates were polymorphonuclear and consisted of neutrophils, plasma cells, and eosinophils (**Figure 6**). Subpleural vessels on day 2 decreased in sized.

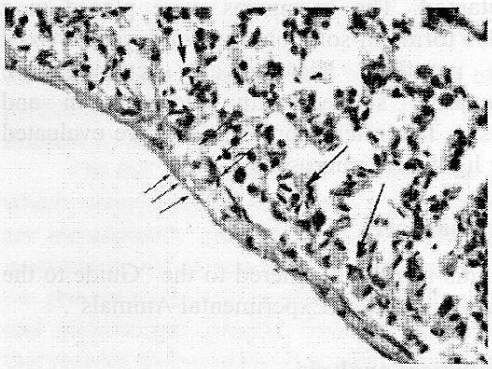

Figure 6: *Photomicrograph on day 2 at x400. Small arrows point to the oedematous visceral pleura, without mesothelial layer. Large arrows point to the subpleural polymorphonuclear cell infiltrates.*

Proliferation of the preserved mesothelial cells was very visible on day 3. On day 3 we established that mononuclear cells (macrophages and lymphocytes) presented as a component of inflammatory infiltrates into the thickened pleural and subpleural connective tissue (**Figure 7**). At first, fibroblasts appeared in the inflammatory infiltrates on this day (**Figure 7**).

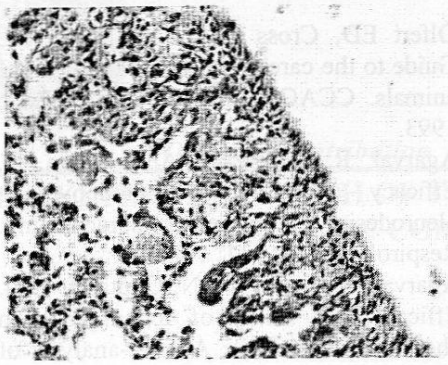

Figure 7: Photomicrograph on day 3 at x200. Note the mononuclear cells as a component of inflammatory infiltrates. Note the first fibroblasts in infiltrates.

Specimens obtained on day 4 demonstrated inflammatory infiltrates which consisted predominantly of mononuclear cells. There was no enlargement of the subpleural vessels. More fibroblasts have been shown in the thickened visceral pleura and subpleural tissue (**Figure 8**).

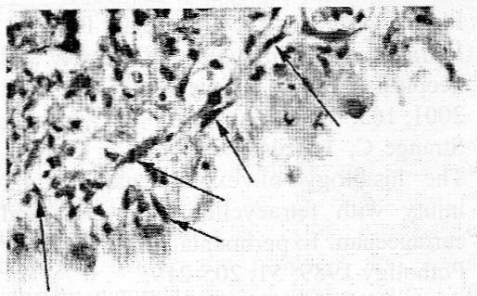

Figure 8: *Photomicrograph on day 4 at x400. The arrows point to the fibroblasts in the visceral pleura and the supleural tissue.*

DISCUSSION

Iodpovidone is an iodine-based topical antiseptic. Some studies have reported iodpovidone as a promising agent for pleurodesis [2,3,4,5]. Even though the authors have reported its success, few controlled trial on its efficacy exists. On the other hand, there are no studies about the mechanism of iodpovidone-induced pleurodesis. We have been studying mechanism of iodpovidone-pleurodesis with the goal of using it as a pleural sclerosant in our clinical practice. Our aim was to study only the acute pleural changes that have been caused by this agent. That was why this research about efficacy of iodpovidone as a sclerosing agent is a form of preclinical testing for us.

Steven Idell has reported that the mechanism of pleurodesis closely approximates the pathogenesis of wound healing [6, 7]. According to C. Strange and associates, the mechanism of pleurodesis is based on pleural irritation in order to create an inflammatory reaction leading to fibrogenesis [8]. We decided to study the acute pleural changes as a result of the inflammatory reaction, after intrapleural instillation of iodpovidone.

Pleural effusion, erythema of the visceral pleura and dilated subpleural vessels, demonstrated in the study, are the results of inflammatory pleural reaction caused by iodpovidone. .Microvascular permeability was increased. Increase of the vascular permeability caused plasma extravasation into the injured tissue. This initiates the inflammatory response after intrapleural iodpovidone injection and contributes to the formation of exudative effusion. We established that pleural effusion has characteristics of exudates by performing pleural fluid analysis, using the Light's criteria [9]. It has confirmed our hypothesis that iodpovidone caused acute pleural inflammatory reaction. Steven Idell pointed that in pleural inflammatory processes extravascular fluids are rich in clotting substrates and local coagulation is initiated by tissue factor with formation of transitional fibrin neomatrix [6]. We have demonstrated fibrin strands between pleural surfaces on day 3 and day 4. They are results and evidence of increased local (pleural space) coagulation and formation of fibrin matrix in exudate, after intrapleural iodpovidone injection.

Denudement of mesothelial cells, demonstrated on the day 1, showed that intrapleural injection of iodpovidone caused pleural injury. On the other hand, this is evidence and confirmation of the hypothesis of J. Huggins and S. Mutsaers that the mesothelial layer must be desquamated before pleurodesis occurrence[10, 11]. J. Huggins and S. Mutsaers have established that not only the injury and the following mesothelial regeneration appear to be the key of pleurodesis occurrence [10, 11]. In the study we have demonstrated hypertrophy and hyperplasia of the preserved mesothelial cells. This is an evidence of mesothelial regeneration and reactivation in response to pleural injury, caused by iodpovidone.

The mechanism of action of iodpovidone in the pleural space remains unclear. Denudement of mesothelial cells and pleural and subpleural vasodilatation, which were demonstrated in the study, may be related to the low pH (2, 97) of the

iodpovidone solution. According to R Agarwal and associates iodine has strong oxidative and cytotoxic properties, which can induce inflammatory response in the wall of any fluid-containing structures [3,4].

We have shown that the duration of acute pleural changes is 3 days, with some evidence. At first, fibrin strands between pleural surfaces appeared on day 3. On day 4 more strands were visible. On the second, inflammatory infiltrates of the visceral pleura and the subpleural tissue, on day 1 and day 2 were polimorphonuclear cells. On day 3, the first mononuclear cells in the infiltrates appeared. On day 4 infiltrates were predominantly mononuclear. Finally, the first fibroblasts as a component of the inflammatory infiltrates appeared on day 3. More of the fibroblasts in the thickened visceral pleura and subpleural tissue were demonstrated on day 4. The fibroblasts in the pleura are as a result of fibroblast influx, theoretically stimulated by the pleural production of fibroblast growth factor [12]. During the process of pleurodesis, the fibrinous neomatrix will undergo continuous remodelling. It will be caused by invasion of fibroblasts into the fibrinous strands and collagen activated fibroblast production. According to the fibroblast invasion into the visceral pleura, demonstrated in the study, we have concluded that day 4 represents the time point at which the first signs of early fibrosis of visceral pleura begin to occur microscopically.

CONCLUSION

Intrapleural injection of iodpovidone, as pleural sclerosing agent has caused pleural inflammatory reaction.

Mesothelial cell abnormalities and dilatation of subpleural vessels is trigger of the pleural inflammatory reaction, after intrapleural iodpovidone instillation.

The 3- to 4-day period represents the time of acute pleural changes, caused by intrapleural injection of iodpovidone.

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