Comparison of nitrogen mustard, cytarabine and dacarbazine as pleural sclerosing agents in rabbits

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ABSTRACT: We have previously shown that the intrapleural injection of mitozantrone but not bleomycin resulted in pleural fibrosis. Mechlorethamine hydrochloride (nitrogen mustard) was used extensively in the past to control malignant effusions, with relatively good success. The objective of this study was to determine if the intrapleural injection of nitrogen mustard would produce pleural sclerosis in our experimental model in rabbits.

We therefore evaluated sclerosing capabilities of nitrogen mustard as well as those of cytarabine and dacarbazine. Nitrogen mustard (0.4 and 0.8 mg·kg⁻¹), cytarabine (3, 6 and 20 mg·kg⁻¹) and dacarbazine (4, 8 and 20 mg·kg⁻¹) were instilled intrapleurally into anaesthetized rabbits. Twenty eight days after the instillation, the animals were killed, and the pleural spaces were assessed grossly for evidence of pleurodesis and microscopically for evidence of fibrosis and inflammation.

The intrapleural injection of 0.8 mg·kg⁻¹ nitrogen mustard was effective in creating pleural fibrosis, either grossly or microscopically. The mean degree (scale 0–4) of gross pleurodesis in the rabbits that received 0.8 mg·kg⁻¹ nitrogen mustard was 3.2±1.0 and the mean degree of microscopic pleural fibrosis was 3.5±0.8. The intrapleural injection of 0.4 mg·kg⁻¹ nitrogen mustard and the different doses of cytarabine (3, 6 and 20 mg·kg⁻¹) and dacarbazine (4, 8 and 20 mg·kg⁻¹) were ineffective in producing pleurodesis.

From this study, we conclude that the intrapleural injection of 0.8 mg·kg⁻¹ of nitrogen mustard produces clinically significant pleurodesis in rabbits. Consideration should be given to future clinical studies utilizing 0.6–0.8 mg·kg⁻¹ nitrogen mustard intrapleurally for the treatment of malignant pleural effusion.
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Pleural effusion secondary to metastatic malignant disease of the pleura is a frequent occurrence [1]. Since systemic therapy is usually ineffective in controlling the effusion, efforts have been made to manage the effusion locally. The most common method used to control the pleural effusion has been the injection of various agents into the pleural space in an attempt to effect fusion of the visceral and parietal pleura and eradicate the pleural space (pleurodesis).

Many different agents have been injected into the pleural space in an attempt to create a pleurodesis [2], but none are ideal. Talc, either insufflated or in a slurry, is probably the agent used most commonly for pleurodesis at the present time. Although it appears to be the most efficacious agent [2], there are concerns about its possible toxicity, especially the development of the acute respiratory distress syndrome (ARDS) soon after its administration [2, 3]. Tetracycline was the agent most commonly used in the 1980s, but the parenteral form of tetracycline is no longer available. The tetracycline derivatives, minocycline and doxycycline appear comparable in efficacy to tetracycline [2, 4], but they are less

effective than talc, are not available in all countries, and their administration is sometimes associated with severe pain [4]. The effectiveness of heat killed *Corynebacterium parvum* appears comparable to that of the tetracycline derivatives [2], but its availability is quite limited.

Since a tumour is responsible for the effusion, it seems reasonable to use an antineoplastic agent to control the effusion. The percentage of complete responses after the intrapleural instillation of various antineoplastic drugs after pleural drainage has ranged 25–85% [2].

The mechanisms for the pleurodesis with antineoplastic agents are still unknown. It is not clear whether the agents act *via* their antitumour effect or by injuring the pleura so that a pleurodesis results. There is good evidence that bleomycin is an effective agent for treating malignant pleural effusions in some patients [5–7], but it does not create a pleurodesis when injected into rabbits [8]. Alternatively, mitozantrone is effective in treating malignant effusion [9–11], and also produces a pleurodesis in the normal rabbit pleura [12].

Nitrogen mustard was one of the first antineoplastic agents to be used intrapleurally to treat malignant pleural

effusion [13–17]. Initially, it was used because of its anti-tumour effects. Even though it was effective in patients with tumours that do not usually respond to nitrogen mustard, its effectiveness was still attributed to its antineoplastic activity [14]. It was theorized that the local instillation produced higher drug levels, which could exert antineoplastic effects on those tumours that were usually resistant. Subsequently, LEININGER et al. [18] reported that when tube thoracostomy was used in conjunction with intrapleural nitrogen mustard, the results were superior to nitrogen mustard injection alone. These workers attributed the efficacy of nitrogen mustard combined with intercostal chest tube drainage to the production of a chemical pleuritis, that causes an effective pleural symphysis [18].

The purpose of the present study was to determine whether nitrogen mustard produces a pleurodesis when injected into the pleural space of the normal rabbit; and in addition, to determine whether the intrapleural injection of cytarabine or dacarbazine produces a pleurodesis. The latter two drugs were selected because, to our knowledge, they had not been evaluated previously in an animal model.

Methods

The methods used in the present study were similar to those used in prior studies [8, 19, 20]. New Zealand white rabbits, weighing 2.5–3.5 kg, were lightly anaesthetized with ketamine hydrochloride, 35 mg·kg⁻¹, plus xylazine hydrochloride, 5 mg·kg⁻¹ intramuscularly. The thorax was prepared for aseptic surgery by shaving the right chest wall and then cleaning it with povidone iodine and alcohol. A 3 cm skin incision was made midway between the spine and the sternum, and the muscles in the seventh or eighth intercostal space were bluntly dissected to allow exposition of the parietal pleura. Under direct vision of the pleura, a 25-gauge needle was inserted into the pleural space, and the drug was injected. When the needle had been removed from the pleural space, the muscle and skin were sutured. After surgery, the rabbits were closely monitored for clinical evidence of pain or other abnormalities.

Sixty four rabbits received intrapleural injection of 0.4 (n=10) or 0.8 (n=8) mg·kg⁻¹ of nitrogen mustard (mechlorethamine hydrochloride), 3 (n=10), 6 (n=9) or 20 (n=7) mg·kg⁻¹ of cytarabine, and 4 (n=10), 8 (n=6) or 20 (n=4) mg·kg⁻¹ of dacarbazine, in random order. A control group, which received only saline intrapleurally, was not studied because previous studies have shown that when bleomycin [12], fibrin glue, or interleukin-8

(IL-8) in saline (unpublished observations) were injected into the pleural space, the pleura was normal after 28 days. The drugs were diluted to a total volume of 2 mL with bacteriostatic saline solution. The animals were killed 28 days after the injection by means of euthanasia solution intravenously. The thorax was removed from the remainder of the rabbit en bloc. A 10% solution of formalin was injected into the exposed trachea through a 6 mm diameter catheter, in order to expand the lungs. The entire thorax was then submerged in 10% formalin solution for at least 48 h.

The necropsy was performed by two of the investigators (EM and FSV), who were blinded as to which drug the animal had received. Each pleural cavity was carefully exposed by making bilateral excisions through the diaphragms and through all the ribs in approximately the midclavicular line. In this manner, the sternum and the medial portions of the anterior ribs were removed, so that the lung and pleural cavities could be evaluated.

The degree of pleurodesis observed grossly was graded according to the following scheme: 0=normal pleural space; 1=no adhesions but pleural space inflamed as evidenced by redness and fibrin deposition; 2=few scattered adhesions; 3=generalized scattered adhesions; and 4=complete obliteration of the pleural space by adhesions. Samples of the parietal pleura, visceral pleura and lungs from each hemithorax were obtained and placed in neutral buffered 10% formalin solution. The tissue samples were processed routinely for histological examination and stained with haematoxylin and eosin. The microscopic slides were assessed and graded (0–4) as having absent, equivocal, mild, moderate, or marked inflammation or fibrosis. Specimens of the heart, liver, kidney and spleen were also obtained from each animal for microscopic analysis.

Statistical analysis

The data are expressed as the mean±SD. Since the data usually failed either the normality test or the equal variance test, the results after the different treatments were compared using the Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks. Comparisons of one side with the other side were made using the paired t-test.

Results

The intrapleural instillation of 0.8 mg·kg⁻¹ nitrogen mustard produced clinically significant gross pleurodesis in the majority of rabbits (table 1 and fig. 1). The degree

Table 1. – Gross pathology of the right side following intrapleural instillation of nitrogen mustard, cytarabine or dacarbazine (n=64)

Pleurodesis score	Nitrogen mustard		Cytarabine			Dacarbazine		
	0.4 mg·kg⁻¹ (n=10)	0.8 mg·kg⁻¹ (n=8)	3 mg·kg⁻¹ (n=10)	6 mg·kg⁻¹ (n=9)	20 mg·kg⁻¹ (n=7)	4 mg·kg⁻¹ (n=10)	8 mg·kg⁻¹ (n=6)	20 mg·kg⁻¹ (n=4)
0	4 (40)		8 (80)	8 (89)	7 (100)	10 (100)	5 (83)	3 (75)
1	2 (20)		1 (10)					1 (25)
2	3 (30)	3 (38)	1 (10)					
3	1 (10)			1 (11)			1 (17)	
4		5 (62)						
mean±SD	1.1±1.1	3.2±1.0*	0.3±0.7	0.3±1.0			0.5±1.2	0.2±0.5

values are presented as absolute number, and percentage in parenthesis. *: p<0.05, compared to all the sclerosing agents.
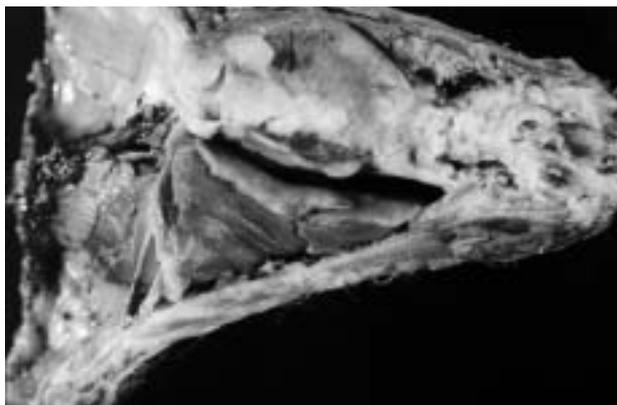
Fig. 1. – Photomicrograph of the macroscopic appearance of the thorax in one animal that received 0.8 mg·kg^{-1} nitrogen mustard. Note that the right pleural space (superiorly) is completely obliterated by adhesions (score = 4).
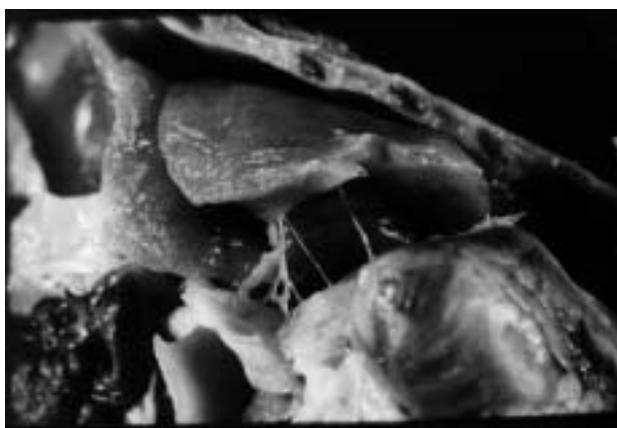

Fig. 2. – Photomicrograph of the macroscopic appearance of the thorax in one animal that received 0.4 mg·kg^{-1} nitrogen mustard. Note that there are only a couple of adhesions in the right pleural space (superiorly) (score = 2).

Table 2. – Microscopic examination of the pleural changes following instillation of nitrogen mustard, cytarabine and dacarbazine

Sclerosing agents	Fibrosis score right	left	Inflammation score right	left
Nitrogen mustard				
0.4 mg·kg^{-1}	2.4±1.1	0.3±0.5	0.3±0.5	0.2±0.4
0.8 mg·kg^{-1}	3.5±0.8	0.1±0.3	1.4±0.9	0.1±0.3
Cytarabine				
3 mg·kg^{-1}	1.3±0.8	0.5±0.7	0.7±0.9	0.4±1.0
6 mg·kg^{-1}	2.1±1.3	0.8±1.3	1.1±0.7	0.7±1.4
20 mg·kg^{-1}	1.1±1.6	0.3±0.5	0.0±0.0	0.0±0.0
Dacarbazine				
4 mg·kg^{-1}	1.8±0.6	0.8±0.4	0.9±0.3	0.3±0.5
8 mg·kg^{-1}	1.8±1.8	0.0±0.0	1.2±1.2	0.3±0.8
20 mg·kg^{-1}	1.5±0.6	0.2±0.5	0.7±0.5	0.0±0.0

Fig. 3. – Photomicrograph of the microscopic appearance of the visceral pleura in one animal that received 0.8 mg·kg^{-1} nitrogen mustard. Note that there is a thick layer of fibrous tissue over the visceral pleural (score = 4). (Haematoxylin and eosin stain; internal scale bar = 100 µm).

Fig. 4. – Photomicrograph of the microscopic appearance of the visceral pleura in one animal that received 0.4 mg·kg^{-1} nitrogen mustard. Note that there is mild thickening of the visceral pleural (score = 2). (Haematoxylin and eosin stain; internal scale bar = 200 µm).

of pleurodesis in the 0.8 mg·kg^{-1} nitrogen mustard group was significantly greater (p<0.05) than that in all of the other groups. None of the other antineoplastic agents at the doses studied produced clinically significant pleurodesis. There was no statistically significant difference in the gross pleurodesis between the groups receiving 0.4 mg·kg^{-1} nitrogen mustard (fig. 2) or 3, 6 or 20 mg·kg^{-1} cytarabine or 4, 8 or 20 mg·kg^{-1} dacarbazine.

The results of the microscopic examination for pleural fibrosis were similar to those of the macroscopic findings (table 2). The degree of pleural fibrosis on the injected side after the administration of 0.8 mg·kg^{-1} nitrogen mustard (fig. 3) was significantly greater (p<0.05) than that either after 0.4 mg·kg^{-1} nitrogen mustard (fig. 4), cytarabine or dacarbazine with different doses. No statistically significant difference (p>0.05) was observed between cytarabine and dacarbazine. With all doses of the three drugs, there was more fibrosis on the injected than on the noninjected side. There was no significant difference between the degree of pleural fibrosis on the control side between any of the eight regimens. There was relatively little pleural inflammation on the injected side in any of the groups (table 2). The highest mean level of inflammation was only 1.4, and this was seen in the 0.8 mg·kg^{-1} nitrogen mustard group.

The degree of microscopic change in the underlying alveolar portion of the lungs was minimal in all groups. In none of the groups did the mean score for either inflammation or fibrosis exceed 0.8.

None of the rabbits died before 28 days. At necropsy, there was no evidence of mediastinal shift or haemothorax in any of the rabbits. At necropsy, there was no evidence of heart, liver, kidney or spleen damage in any of the rabbits.

Discussion

The results of this study demonstrate that nitrogen mustard, at a dose of 0.8 mg·kg^{-1} produces clinically significant pleurodesis in the majority of rabbits. Nitrogen mustard at the dose usually recommended in humans (0.4 mg·kg^{-1}) did not produce clinically significant pleurodesis. The intrapleural injection of cytarabine (3–20 mg·kg^{-1}) or dacarbazine (4–20 mg·kg^{-1}) did not induce clinically significant pleurodesis.

Nitrogen mustard is an alkylating agent, which is used intravenously in the therapy of several neoplastic diseases. It was one of the first agents used to induce pleurodesis in the early 1950s [13, 15]. With the intrapleural injection without tube thoracostomy efficacy ranged 28–87% with doses ranging 10–30 mg [16]. When nitrogen mustard was subsequently used in conjunction with tube thoracostomy, the results tended to be better. KINSEY *et al.* [21] reported that the intrapleural injection of 30 mg nitrogen mustard through the chest tube to 62 patients controlled the effusion completely in 54 (87%). LEININGER *et al.* [18] administered 10 mg of nitrogen mustard through the chest tube to 18 patients and reported that the treatment was effective in 17 patients (94%). These workers attributed the efficacy of the instillation of nitrogen mustard to the production of a chemical pleuritis that resulted in an effective pleurodesis. ANDERSON *et al.* [22], however, reported much poorer results in 60 patients who received 0.4 mg·kg^{-1} nitrogen mustard through a chest tube. In this report, only about 40% of the patients were free of pleural effusions after 90 days. The results with nitrogen mustard summarized above are comparable to those with the tetracycline derivatives, particularly when the nitrogen mustard is used in conjunction with tube thoracostomy. It is noteworthy that, in a recent review of the agents used to treat malignant pleural effusions, nitrogen mustard was not mentioned [2].

There has been limited previous work evaluating the ability of intrapleural nitrogen mustard to create a pleurodesis in an animal model. SAHN *et al.* [23], using a rabbit model comparable to the one used in the present study, reported that the administration of 0.2 mg·kg^{-1} of nitrogen mustard intrapleurally did not result in a pleurodesis. These results are compatible with the results of the present study; 0.4 mg·kg^{-1} intrapleurally in the present study did not result in a pleurodesis.

The present study strongly suggest that there is a relationship between the dose of nitrogen mustard and whether a pleurodesis will be produced. A dose of 0.4 mg·kg^{-1} was ineffective in producing a pleurodesis, whilst a dose of 0.8 mg·kg^{-1} was effective. In human studies, the doses used have ranged up to 0.4 mg·kg^{-1} or 30 mg. The toxicity of nitrogen mustard intrapleurally is relatively mild. Symptoms are primarily systemic in origin, except for local chest pain, and include fever, nausea, vomiting, and very mild depression of the haematopoietic system. The systemic effects are much less severe than those which occur when the drug is administered intravenously at the same dose [14, 24]. This raises the possibility that higher doses can be used to treat maignant pleural effusions. WEISBERGER [15] suggested that nitrogen mustard could be given at doses of 0.6 mg·kg^{-1} or higher, with the expectation of improved results. One reason that nitrogen mustard can be given at high doses intrapleurally

is that it is rapidly metabolized to an inactive form when it is in contact with body fluids [24].

In the present study, the dose of 0.8 mg·kg^{-1} appeared to be well-tolerated by the animals. None of the rabbits died and examination of the heart, liver, kidney and spleen microscopically revealed no abnormalities.

There have been numerous reports evaluating the effectiveness of other antineoplastic agents intrapleurally for the treatment of malignant pleural effusions, since the early reports using nitrogen mustard for this purpose. Agents evaluated have included: thiotepa [25], 5-fluorouracil [26], bleomycin [27–29], doxorubicin [30, 31], etoposide [32], and mitozantrone [9–11]. The overall success rates with these agents has ranged 24–66%, and most have been less than 40% [2]. The results with cytarabine and dacarbazine in the present study do not provide encouragement for further studies with either of these drugs.

One possible exception is the drug mitozantrone. MAICHE *et al.* [9] treated 15 patients with 30 mg mitozantrone in 30 mL saline, and reported that the effusion was controlled in 10 (67%). KELLY *et al.* [10] administered 20 mg·m^{-2} to 15 patients with metastatic sarcoma and reported that complete resolution of the effusion was achieved in 76% of the patients. GROTH *et al.* [11] treated 54 patients with 30 mg mitozantrone and reported that 71% of the patients had complete disappearance of the effusion for 2 months. We have previously shown that the injection of 1.5 mg·kg^{-1} mitozantrone in our rabbit model produces a pleurodesis [8, 19].

It is interesting to compare the histological pictures 28 days after injection in rabbits given 1.5 mg·kg^{-1} mitozantrone and in rabbits given 0.8 mg·kg^{-1} nitrogen mustard. The mean degree of microscopic pleural fibrosis on the injected side was greater in the nitrogen mustard group (3.5±1.0) than in the mitozantrone group (2.6±1.5). In contrast, the degree of pleural inflammation on the injected side in the nitrogen mustard group (1.4±0.9) was less than that in the mitozantrone group (2.0±0.7). Another difference was that the underlying lung on the injected side and the contralateral lung and pleura had much more inflammation and fibrosis in the mitozantrone group. These observations suggest that the mechanism by which these two different drugs produce fibrosis is different. The intrapleural injection of mitozantrone appears to have a much greater systemic effect than does the intrapleural injection of nitrogen mustard.

The mechanism by which the intrapleural injection of an antineoplastic drug produces pleural fibrosis remains unknown. The observation in the present study that nitrogen mustard produces pleural fibrosis in normal rabbits and our previous observation that mitozantrone also produces pleural fibrosis in rabbits suggest that these agents act by inducing a chemical pleuritis rather than through their antineoplastic actions. However, a direct antitumour mechanism is suggested by the fact that bleomycin is effective in treating malignant pleural effusions [5–7], but it does not produce pleural fibrosis in rabbits [8]. Perhaps the mechanism varies from drug to drug. Interestingly, the microscopic picture after nitrogen mustard instillation was much more similar to that after the injection of minocycline [19] than that after mitozantrone.

From this study, we conclude that the intrapleural injection of 0.8 mg·kg^{-1} of nitrogen mustard produces

clinically significant pleurodesis in rabbits. Consideration should be given to future clinical studies utilizing 0.6–0.8 mg·kg⁻¹ nitrogen mustard intrapleurally for the treatment of malignant pleural effusions.

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