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TECHNICAL NOTE

Corynebacterium parvum versus tetracycline as pleural sclerosing agents in rabbits

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Corynebacterium parvum versus *tetracycline as pleural sclerosing agents in rabbits. F.S. Vargas, N-S. Wang, L.R. Teixeira, A.O. Carmo, L.M.M.F. Silva, R.W. Light. ©ERS Journals Ltd 1995.*

ABSTRACT: Tetracycline has been one of the most commonly used agents for producing a pleurodesis. However, it is no longer available due to more stringent requirements on the manufacturing process. The objective of this project was to determine whether *Corynebacterium parvum* is an effective sclerosant in an experimental model in rabbits.

The following medications were instilled intrapleurally in anaesthetized male rabbits: tetracycline 35 mg·kg^{-1} or *C. parvum* 4 or 8 mg, all diluted with bacteriostatic saline solution. Twenty eight days after the instillation, the animals were sacrificed and the pleural spaces assessed macroscopically for evidence of pleurodesis and microscopically for evidence of fibrosis and inflammation.

The intrapleural injection of *C. parvum* was ineffective in creating pleural fibrosis. The mean degree of pleurodesis in the 10 rabbits who received tetracycline was 3.5±0.7 (scale 0–4) whilst in the 10 rabbits that received 4 mg *C. parvum* it was 0.0±0.0, and in the 10 rabbits that received 8 mg *C. parvum* it was 0.5±0.8.

Based on this study, we recommend that *C. parvum* should not be used as a pleural sclerosant in patients with normal pleura.

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Tetracycline has been the agent most frequently used for creating a pleurodesis for the past 10 yrs. It became widely used because it is the most effective agent in producing pleurodesis in rabbits [1], and it is relatively inexpensive [2, 3]. However, tetracycline is no longer available due to stricter regulations governing the production of parenteral antibiotics.

Corynebacterium parvum has been used at least in the treatment of malignant pleural effusions, because of its effects on the progression of the cancer [4], and because it appeared comparable in effectiveness to tetracycline in the treatment of malignant pleural effusions [5, 6].

The purpose of the present study was to compare the effectiveness of tetracycline and *C. parvum* in producing a pleurodesis in rabbits after intrapleural injection. We have previously shown that in the animal model, minocycline is comparable in effectiveness to tetracycline [7], and that bleomycin is ineffective in creating pleural fibrosis, either macroscopically or microscopically [8]. We wished to determine whether *C. parvum* would be effective in this animal model.

Methods

New Zealand white rabbits weighing 2.5–4.0 kg were lightly anaesthetized with Ketamine hydrochloride 35 mg·kg^{-1} plus xylazine hydrochloride 5 mg·kg^{-1} *i.m.* The thorax was prepared for aseptic surgery by shaving the right chest wall and then cleaning it with povidone and alcohol. A 3 cm skin incision was made midway between the spine and the sternum. The muscles in the seventh or eighth intercostal space were bluntly dissected to allow exposure of the parietal pleura. Under direct vision of the pleura, a 25 G needle was inserted into the pleural space and the sclerosing drug was injected. In sequence, the muscle and skin were sutured. After the surgery, the rabbits were closely monitored for clinical evidence of pain (vocalization, tachypnoea and restlessness).

Three groups of rabbits were studied. The sclerosing agents used were tetracycline hydrochloride at a dose of 35 mg·kg^{-1} diluted to a total volume of 2 mL with bacteriostatic saline solution, or *C. parvum* at doses of 4 or 8 mg (dry weight) of the killed organisms diluted to a total volume of 2 or 4 mL, respectively, with bacteriostatic saline solution. The *C. parvum* was purchased from Pharmaceutics Laboratory of Pernambuco in Sao Paulo and was verified independently by the Biochemistry Department at the University of Sao Paulo.

Rabbits were sacrificed 28 days after the injection. Rabbits that died within the first 24 h after the injection were replaced. The rabbits were sacrificed by the injection of euthanasia solution into a vein in the ear. The thorax was removed from the remainder of the rabbit en bloc. Attempts were made to expand the lungs by the injection of 10% formalin into a plastic catheter (6 mm

diameter), which had been inserted into the exposed trachea. The entire thorax was then submerged in 10% formalin solution for at least 48 h.

The necropsy was performed by two of the investigators (FSV and LRT), who were blinded as to which sclerosant the animal had received. Each pleural cavity was carefully exposed by making bilateral incisions through the diaphragm 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 macroscopically was graded according to the following scheme: 0 = normal pleural space; 1 = no adhesions but pleural space inflamed (evidence of redness and fibrin deposition); 2 = few scattered adhesions; 3 = generalized scattered adhesions; and 4 = complete obliteration of the pleural space by adhesions.

At the time that the pleura was evaluated macroscopically, samples of the parietal pleura, visceral pleura, and lung from each hemithorax were obtained and placed in neutral buffered 10% formalin. Samples were obtained from the lower lobes with the contiguous parietal pleura. Attempts were made to sample the most representative region. These tissue samples for histological examination were processed routinely and stained with haematoxylin and eosin (H&E). The microscopic slides were evaluated blindly by two of the investigators (NSW and LMMFS) for the presence of inflammation and fibrosis, and subjectively assessed and graded (0–4) as absent (0), slight (1), mild (2), moderate (3) or marked (4).

Statistical analysis

All data are expressed as the mean±standard deviation. The scores for the gross pleurodesis and the microscopic examination in the different treatment groups were compared using unpaired t-tests.

Results

The intrapleural instillation of either 4 or 8 mg *C. parvum* produced virtually no gross pleurodesis (table 1). All 10 rabbits (100%) that received 4 mg, and 7 of the 10 rabbits (70%) that received 8 mg, had completely normal pleural spaces macroscopically at necropsy, whilst of the other three rabbits, two had few scattered adhesions and one only mild inflammation. The degree of pleurodesis in the tetracycline group was significantly greater ($p<0.001$) than in either of the *C. parvum* groups.

Table 1. – Macroscopic examination of the right side

Agent	Dose	n	Score
Tetracycline	35 mg·Kg^{-1}	10	3.5±0.7
C. parvum	4 mg	10	0.0±0.0*
	8 mg	10	0.5±0.8*

The score is presented as mean±SD. Pleurodesis was graded 0 (normal pleural space) to 4 (complete obliteration of the pleural space by adthesions). *: $p<0.001$ when compared to teteracycline.

The results with the microscopic examination for pleural fibrosis were similar to those for the macroscopic examination (table 2). The degree of pleural fibrosis after the administration of tetracycline was significantly greater ($p<0.001$) than that after either dose of *C. parvum.* The degree of pleural inflammation on the injected side was significantly greater in the tetracycline group ($p<0.01$). There were no significant differences in the pleura on the control side in three different groups.

The degree of microscopic changes in the underlying lungs were minimal in all three groups. The degree of fibrosis in the underlying lung was significantly greater ($p<0.01$) in the tetracycline group (1.4±1.2) than in either of the *C. parvum* groups (0.0±0.0) on the injected side. The degree of inflammation was similarly mild in all groups, but it was significantly greater ($p<0.01$) in the tetracycline group (1.5±0.8) than in the *C. parvum* groups (0.0±0.0).

Discussion

The present study demonstrates that *C. parvum,* at a dose of 4 or 8 mg, does not produce significant pleurodesis when injected into the pleural spaces of normal rabbits.

C. parvum, a Gram-positive anaerobic bacillus, is known to be a potent immunological stimulant that can inhibit the growth both of primary and metastatic tumours [9-11]. For this reason, it is considered an anti-cancer agent [12]. In 1978, WEBB *et al* [13] observed that the intrapleural instillation of *C. parvum* produced a reduction in the size of the effusion (in most cases the effusions stopped completely) and a significant decrease in the number of malignant cells. Since that time, there have been multiple reports concerning the treatment of malignant pleural effusions with this agent.

LEAHY *et al.* [6] reported that the effectiveness of 7 mg *C. parvum* (effective in 14 out of 16, 88%) was similar to that of 500 mg tetracycline (effective in 6 out of 7,

Table 2. – Macroscopic examination of the pleural changes

Agent	Dose	n	Fibrosis score		Inflammation score	
			Right	Left	Right	Left
Tetracyline	35 mg·Kg^{-1}	10	3.9±0.3	0.9±0.6	1.9±0.8	0.8±0.6
C. parvum	4 mg	10	0.9±0.7*	0.6±0.5	1.2±0.8#	0.6±0.7
	8 mg	10	0.4±0.5*	0.5±0.4	0.8±0.8#	0.5±0.3

The scores are presented as mean±SD. Fibrosis and inflammation were graded 0 (absent) to 4 (marked). *: $p<0.001$; #: $p<0.0l$ when compared to tetracycline.

86%) in patients with malignant pleural effusions. HILLERDAL *et al.* [14] compared bleomycin 60 U with 7 mg *C. parvum* in a randomized study, and concluded that *C. parvum* was better because it was effective in 11 out of 15 patients (73%) whilst bleomycin was only successful in 2 out of 15 patients (13%). OSTROWSKI *et al.* [15] more recently randomized patients to receive *C. parvum* or 60 IU bleomycin. They reported that the response rate with bleomycin (18 out of 25, 72%) was superior to that with *C. parvum* (9 out of 19, 47%). Finally, we demonstrated that the intrapleural instillation of bleomycin in normal rabbits does not produce pleurodesis [8].

In view of the above studies, there is no doubt that intrapleural *C. parvum* is an effective treatment for at least some patients with malignant pleural effusions. Why did *C. parvum* not produce a pleurodesis in our animal model?

There are several possibilities for the discrepancy between the results in rabbits and in patients with malignant pleural effusions. Firstly, the dose of *C. parvum* used in our rabbits may have been insufficient. This explanation appears unlikely to us. Although the range in the dose of *C. parvum* has been wide, varying from 4 mg used by CASALI *et al* [16] to 21 mg used by CURRIE *et al.* [17], the majority of the authors have used a dose of 7 mg [4, 18–20]. Therefore, if *C. parvum* was going to be effective, one would consider that a dose of 8 mg should be sufficient.

A second possibility is that the pleura of the patient with a malignant pleural effusion is altered, such that the *C. parvum* does produce a pleurodesis. The induction of a pleurodesis seems to be dependent upon the balance between the procoagulant state and the thrombolytic state, with pleurodesis occuring only if the fibrinogenic state is predominant [21]. Malignant pleural effusions (like other exudates) are characterized by increased procoagulant and decreased fibrinolytic activity [22]. The balance of these activities is reversed and favours fibrin clearance in congestive heart failure [22]. In addition, the stimulation of normal human mesothelial cells with tumour necrosis factor-α or transforming growth factor-β does not alter the balance between these two systems [23]. Therefore, if the necessary balance between the procoagulant and the fibrinolytic state is not achieved in the normal pleural space (including that of the rabbit) after *C. parvum* protein injection, pleurodesis might not result. Another factor that could be operative is increased clearance of the *C. parvum* protein from the normal pleural space. A sclerosing agent, such as *C. parvum* protein, might have to remain in the pleural space for a certain duration to create a pleurodesis. Previous studies have demonstrated that in patients with malignant pleural effusions, the clearance of the fluid is diminished when compared with patients with congestive heart failure, who presumably have normal pleura [24].

A third possibility is that the mechanism of action of *C. parvum* in producing a pleurodesis is directly related to its anti-tumour action. If intrapleural *C. parvum* controls the pleural effusion by reducing or eliminating the tumour cells [13], then no pleurodesis will result for it in patients with no malignant pleural effusions.

A possible criticism of the present paper is that the data obtained in rabbits concerning pleural sclerosis may not be directly applicable to the human. In general, since SAHN and GOOD [1] wrote their initial paper on the effect of common sclerosing agents in rabbits, most studies of pleurodesis in experimental animals have been conducted in rabbits. Studies during the past 18 months have also used rabbits to examine pleurodesis [25, 26]. In general, the results with the various sclerosing agents in rabbits have paralleled those in humans. It is possible that the killed *C. parvum* might have a different effect in rabbits than it does man. We believe that this is unlikely, however, since killed *C. parvum* has been used as an immunostimulating agent in various animal models [27–29] including the rabbit [30]. The results found with *C. parvum* in models of tumour growth or hepatic damage are thought to be relevant to the human.

Based on the results of the present study, *C. parvum* cannot be recommended as a sclerosing agent in patients who have normal pleural surfaces, such as those with pneumothorax, cirrhosis with pleural effusion, congestive heart failure with pleural effusion, *etc.* The mechanism by which *C. parvum* produces pleurodesis in patients with malignant pleural effusions remains to be defined.

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