









Research paper

The influence of tramadol and parecoxib on erythromycin or bleomycin-induced pleurodesis in rabbit: A pilot study

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ABSTRACT

Introduction: Recurrent pleural effusion secondary to advanced malignant diseases can lead to poor quality of life, recurrent hospital stays and increased hospital costs, which has yet to be extensively explored.

Aim: To compare the effectiveness of erythromycin and bleomycin in producing pleurodesis in rabbits and to determine the influence of different analgesic drugs namely tramadol sodium and parecoxib sodium intramuscular on experimental pleurodesis induced by erythromycin or bleomycin intrapleural on the aforementioned rabbits.

Material and methods: This was an experimental animal pilot study involving 28 white New Zealand rabbits which were divided into 4 groups of 7 specimens. They received different agents as follow: group A (erythromycin and parecoxib sodium), B (erythromycin and tramadol sodium), C (bleomycin and parecoxib sodium), and D (bleomycin and tramadol sodium) at the right hemithorax. The control was marked at the contralateral left hemithorax. After 30 days the rabbits were euthanized to allow for evaluation of macroscopic and microscopic pleural and parenchymal adhesions by a blinded respective pathologist.

Results and discussion: The degree of pleurodesis induced by the intrapleural injection of erythromycin indicated that it was superior to bleomycin as a sclerosing agent in the rabbit sample ($P = 0.003$). The concomitant use of analgesics revealed that tramadol sodium reduces the degree of pleurodesis to a greater extent than parecoxib sodium ($P = 0.009$).

Conclusions: The use of intrapleural erythromycin as a potent agent of chemical pleurodesis that is insensitive to the concomitant analgesic effect of parecoxib sodium has important clinical implications in relation to the effectiveness of chemical pleurodesis as a procedure.

1. INTRODUCTION

Recurrent spontaneous pneumothorax and malignant pleural effusions are commonly encountered, especially in those with advanced malignant diseases. These debilitating complications can lead to a worsening morbidity. The cancers that are more frequently prone to malignant pleural effusion are pleural mesothelioma, lung and breast cancers.¹ Cusumano et al. emphasized that these conditions typically result in a poor prognostic outcome and a reduced quality of life.² Being the number one tumor in women, breast cancer is the second leading cause of malignant pleural effusions requiring therapeutic chest drainage and sclerotherapy.^{3,4} These issues are crucial, but have yet to be extensively explored and investigated. The treatments range from clinical observation and simple thoracocentesis to more invasive techniques, namely chemical pleurodesis, pigtail pleural drainage, pleuroperitoneal shunting and pleurectomy.⁵ Previous research has shown that significantly greater symptomatic relief can be achieved with the introduction of chemical pleurodesis using fibrin glue, pleuroperitoneal shunt or blood products as compared to other methods.^{6,7}

It is postulated that the intrapleural administration of sclerosing agents induces the production of pleural mesothelium of primary fibroblast growth factor (bFGF).⁸ Subsequently, the pleural mesothelial damage develops due to acute phase reaction and inflammatory activity that culminates in the process of fibrosis causing permanent cessation of the fluid collection.^{9,10} In addition to infiltrating cells including neutrophilic infiltration, mesothelial cells have been demonstrated to actively participate in pleural inflammation via release of various mediators and proteins, including platelet-derived growth factor (PDGF), interleukin-8 (IL-8), monocyte chemotactic peptide (MCP-1), nitric oxide (NO), collagen, antioxidant enzymes and the plasminogen activation inhibitor (PAI).¹¹

At present, many sclerosant agents have been tested and found to be useful in chemical pleurodesis. Agents such as autologous blood products, tetracycline derivatives, anti-neoplastic antibiotics and macrolide antibiotics have similar potency as sclerosing agents in pleurodesis but are not widely used clinically. Both bleomycin (anti-neoplastic antibiotic) and erythromycin (macrolide) are effective sclerosing agents with minimal side effects.¹² The mechanism of action of erythromycin gives consequences of good inflammatory effects by releasing different immune cells that given bactericidal actions and less complications with documented efficacy as sclerosing agent.¹³ The production of IL-8, vascular endothelial growth factor (VEGF), and transforming growth factor β 1 (TGF- β 1) that may actively mediate the primary inflammatory pleural response in pleurodesis.¹⁴

During the early stage of pleurodesis, the inflammatory reaction produced is regulated by anti-inflammatory agents, thus attenuating the fibrotic changes. Other analgesics used for pain management after pleurodesis include non-steroidal anti-inflammatory drugs (NSAIDs). Experimental studies have proven that corticosteroids and NSAIDs can lessen the

pleurodesis-induced inflammatory reaction.^{15–17} However, studies on the interaction by opioid drugs are lacking. Consequently, the effect of parecoxib sodium as a selective cyclooxygenase-2 (COX-2) inhibitor and as a newer generation of NSAIDs was assessed in this research. Tramadol sodium, an atypical opioid which is a centrally acting analgesic on chemical pleurodesis has not been studied before. These results suggest that the use of selective COX-2 inhibitors can be considered and recommended in human pleurodesis.¹⁸

2. AIM

This study aimed to compare the effectiveness of erythromycin and bleomycin in producing pleurodesis in rabbits and to determine whether tramadol sodium or parecoxib sodium intramuscular could influence the effectiveness of the procedure.

3. MATERIAL AND METHODS

3.1. Samples

In addition, the Code of Practice for the Care and Use of Animals for Scientific Purposes and The Principle of Humane Animal Experiment Technique devised by Russell and Burch, 2002 were followed as guidelines for this study.¹⁹ Twenty-eight New Zealand white rabbits (*Oryctolagus cuniculus*) were divided into 4 groups of 7 specimens. All specimens were about 2 months old and weighed 2–4 kg. They were assigned to groups A, B, C and D, and scheduled to receive different agents as follows: group A (erythromycin and parecoxib sodium), B (erythromycin and tramadol sodium), C (bleomycin and parecoxib sodium) and D (bleomycin and tramadol sodium). The right hemithorax was designated an experimental area, whereas the left hemithorax was labeled as the control. The rabbits were anesthetized with intramuscular zoletil 50 mg (recommended dosage 25 mg/kg).¹⁹ When they were in deep anesthesia, a 10 × 10 cm square section of the right anterior chest wall was shaved with an electric shaver (Figure 1A). The working area was cleaned with antiseptic solution chlorhexidine (10%) and povidone-iodine (1%).

3.2. Chemical pleurodesis and intramuscular analgesia

Under aseptic technique, the right hemithorax area was draped routinely. On the operating table, the upper limbs of the rabbits were immobilized with soft ropes. The right 5th intercostal at the midclavicular line was identified with a marker. At the marking site, the right pleural space was entered using a syringe 10 mL BBraun with 20 gauge needle, after feeling a 'give away' sensation. The syringe was substituted for a sclerosing agent-filled type by maintaining the needle in situ. Chemical pleurodesis was induced by administering 25 mg/kg of a sclerosing agent (either erythromycin or bleomycin) following the group schedule. The dilutions

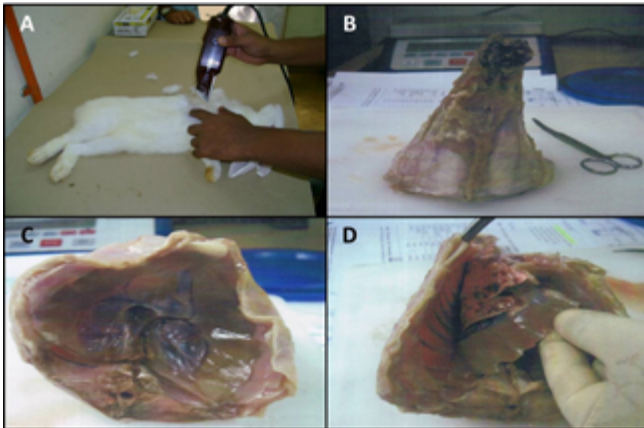


Figure 1. (A) The right anterior chest wall was shaved. (B) The soft tissue of the chest wall was dissected to expose the whole thorax. (C) Inspection of the diaphragm from the inferior view. (D) Inspection of the appearance of pleural cavity and lung fields.

of respective sclerosing agents were followed in accordance with the standard formulations given. A pulse oximeter was used to ensure oxygen saturation throughout the procedures. After 10 minutes of instillation, both syringe and needle were removed together gently. The puncture wound was layered with chloramphenicol ointment. The right thigh of the lower limbs was injected using either tramadol sodium or parecoxib sodium 1 mg/kg intramuscularly. Both sclerosing and analgesic agents were given according to the guidelines devised by Russell and Burch.¹⁹ The procedure lasted approximately 20 minutes.

3.3. Post-procedural and daily monitoring

All the rabbits were placed in a recovery room to allow spontaneous recovery to be observed by the laboratory animal house technicians. They were placed in separate labeled cages in a ventilated room under similar conditions. In accordance with the Housing and Husbandry Guidelines for Laboratory Animals Used in Biomedical Research, each rabbit was allocated a 2787 cm² floor area, 35 cm in height with food and water containers. The animal rooms were kept in a clean, sanitary environment with adequate diffuse lighting (range 130–325 lux), and with a standard ventilation rate of 10 to 15 air changes per hour maintained daily. All rabbits were fed with standard commercially available pellets that supplied all the required nutrients. General daily observations and monitoring included general activity, health condition, oral intake and room conditions. The shaved skin and wounds were regularly inspected for any signs of infection or inflammation. A prophylactic antibiotic was not given in this study unless the rabbits revealed early symptoms of infection, such as snuffle, nasal discharge, reduced oral intake or wound infections. The rabbits received a once-daily injection of the respective anti-inflammatory agent to the thighs of the lower limbs in week 1, followed by once-weekly injection until day 30.

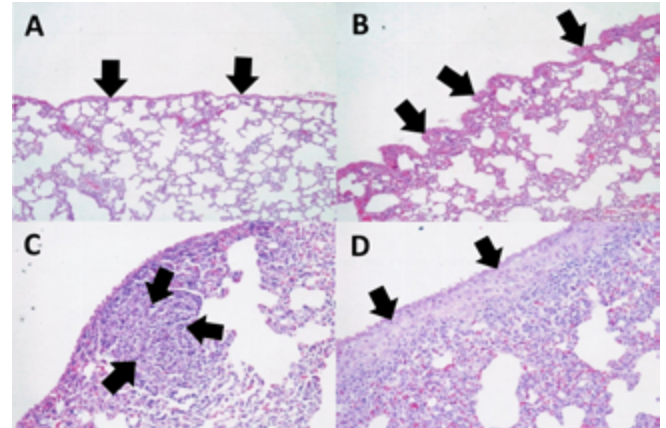


Figure 2. (A) Appearance of mild pleural thickening (black arrow) with scattered chronic inflammatory cells. (B) Moderate pleural thickening and fibrosis (black arrow). (C) Marked pleural thickening with numerous chronic inflammatory cells (black arrow). (D) Marked pleural thickening and fibrosis (black arrow).

3.4. Tissue dissection

After 30 days, the rabbits were euthanized via administration of a 100 mg/kg injected overdose of phenobarbital intraperitoneal as deemed acceptable in Guidelines for Animals Used in Biomedical Research.¹⁹ The soft tissue of the chest wall was dissected to expose the whole thorax (Figure 1B). When adhesions were identified at the dissection sites, the samples were collected carefully to maintain the integrity of the visceral pleura. After en-bloc dissection of the thoraxes, the lungs were expanded and submerged with 60 mL of neutral buffered formalin solution (10%).

3.5. Histological evaluation

The purpose of the evaluation by the blinded respective pathologist was to look for macroscopic adhesions in the obtained specimens of the tissues of pleura and lung parenchyma. The macroscopic evaluation was done by using a scoring system (0 – normal, 1 – 1 to 3 small adhesions, 2 – more than 3 adhesions, 3 – generalized adhesions, 4 – complete obliteration of the pleural space by adhesions). The dissected thorax was inspected generally, starting with the diaphragm appearance (Figure 1C). The sternal bone was separated from the thorax by cutting it open gently, followed by both diaphragms (Figure 1D). These tissues were processed for microscopic examination by using hematoxylin-eosin stain to detect the inflammatory and fibrotic reaction. The specimens were examined under routine light microscopy (magnification $\times 40$ and $\times 100$). Presence of inflammation and fibrosis was graded by the same pathologist on the scale of 0 to 4, according to Teixeira et al.¹⁶ The selected microscopic slides that represent each group were analyzed via microscope (Olympus BX51 with cellF image analyzer multi-fluorescence software) in order to get better view and assessment of the inflammation and fibrosis: normal pleural from control, mild changes (Figure 2A), moderate changes (Figure 2B), marked changes (Figure 2C) and pleural thickening (Figure 2D).

3.6. Statistical analysis

All data from the evaluation form were entered in the appropriate software following the guidelines laid down in the Advanced Bio-Statistics and Research Methodology for Medical Research published by Universiti Sains Malaysia. Statistical Package Social Science software version 12.0.1 was used to analyze the data statistically with the help of fellow lecturers from the Biostatistics Department of Community Medicine Health Campus, Universiti Sains Malaysia. The data were analyzed using non-parametric methods (Mann-Whitney test) to compare each group (intergroup comparison), whereby a *P* value of less than 0.05 was considered as statistically significant. The results were reported as median with interquartile range (IQR) between 5 groups.

4. RESULTS

A total of 28 male rabbits underwent experimental pleurodesis in accordance with the group schedule. All specimens tolerated the procedures without event. However, among them 10.71% developed infection and 7.14% experienced bleeding. There were 2 cases of mortality (7.14%): one on Day 1, and one, on Day 4, post procedures. Postmortems showed that the first rabbit had experienced lung collapse secondary to iatrogenic lung injury, while the second rabbit had lung infection secondary to lung injury and hemorrhage. Results were constant in group A compared to other groups macroscopic and microscopic in grade 4 adhesion (Tables 1 and 2). There were significant differences between group A and C, and between group A and D, whereas other groups showed no statistically significant differences (Table 3). Significant differences were observed between the group control and A, B and D, except in the case of group C, where there was no statistically significant difference (Table 4).

5. DISCUSSION

This pilot study demonstrated that intrapleural injection of erythromycin was superior to bleomycin as a sclerosing agent to initiate chemical pleurodesis in rabbits (*P* = 0.003). The sustained systemic administration of concomitant analgesics in this study revealed that the centrally acting opioid, tramadol sodium reduces the degree of pleurodesis as compared to the selective COX-2 inhibitor, parecoxib sodium (*P* = 0.009). However, inconsistent results were noted in the groups when comparing erythromycin with parecoxib sodium. Although the distribution of data revealed an inconsistency in these groups, it is clear that pleurodesis has a beneficial effect on mild to moderate cases of disease.

Furthermore, all groups - except for the one administered the combination of bleomycin and tramadol sodium - experienced an excellent chemical pleurodesis effect compared to the control group. Furthermore, no changes were observed in the control group (score of 0). Similar findings were noted by Balassoulis et al. showing the effective-

ness of erythromycin as a sclerosing agent for pleurodesis in patients with recurrent malignant pleural effusions.³ It was concluded that 79.4% had a complete response, 8.8%, a partial response and 11.8%, no response.³ Another study by Teixeira et al. showed that there was no interaction between systemic parecoxib sodium injection and talc or silver nitrate pleurodesis.¹⁷ The results suggest that selective COX-2 inhibitors (parecoxib sodium) can be considered and recommended in human pleurodesis.

However, in contrast to these findings, the results involving the ordinary anti-inflammatory agent indicated that the degree of pleural adhesion is reduced in animals with talc-induced pleurodesis. These animals received a systemic administration of anti-inflammatory agents (steroidal or non-steroidal) in contrast to those specimens with silver nitrate-induced pleurodesis who were also administered the anti-inflammatory agents.¹⁶ A study by Teixeira et al. has demonstrated that the administration of corticosteroids triamcinolone within 24 h of a pre-intrapleural doxycycline injection lessens the efficacy of pleurodesis in rabbits.²⁰ The injections of corticosteroids decrease the effectiveness even more if given on a weekly basis.

Based on these findings, it can be postulated that erythromycin is better than bleomycin in stimulating mesothelial cells to activate the inflammatory response. Each of these two sclerosant agents probably modulates by utilizing different mechanisms to produce inflammatory reactions. It is also possible that the use of a concomitant agent in correspondent clinical doses of selective COX-2 inhibitors (parecoxib sodium) gives slightly more positive results than opioid (tramadol sodium) that reduce the degree of pleurodesis typically seen in erythromycin pleurodesis.

Antony et al. conducted a significant study with similar findings that showed intrapleural administration of tetracycline hydrochloride is effective in achieving pleural fibrosis.²¹ They demonstrated that tetracycline hydrochloride could stimulate mesothelial cells to release a growth-factor-like activity for fibroblasts. Baumann et al. added that intrapleural tetracycline resulted in pleural macrophage influx, hence the formation of pleural fibrosis, whereby intrapleural carrageenan induced macrophage influx without fibrosis.²² Alan et al. reported lately that the use of NSAIDs as adjunct therapy to analgesia will increase the risk of recurrence by about 40% following surgical pleurodesis for pneumothorax.²³ The authors suggested that the use of NSAIDs should be avoided routinely after surgical pleurodesis.

The main limitations of this research are that it is a pilot study in experimental animal research which was conducted with limited funding and resources. Difficulty was experienced in getting supplies of genetically identical animals of average weight of 2–3 kg in Malaysia. The experiments had to be delayed until it was possible to recruit sufficient groups of 7 animal models per group. Hormonal and immunologic characteristics were the confounding factors in this experimental animal study. They were controlled and minimized by tackling the genetics (same syngeneic animal models), nutrition (same diet), environment (same living

and cage conditions) and exposure to disease (disease-free, as monitored by laboratory animal research unit technician on a daily basis). Biases in the evaluations of the macroscopic and microscopic results were offset by blinding the respective pathologist. In addition, the freshly prepared samples were evaluated macroscopically by a pathologist on the same day.

6. CONCLUSION

The results of the current study indicate that the sclerosant agent erythromycin is better at inducing chemical pleurodesis than bleomycin as indicated by the significant statistical differences. Furthermore, the concomitant use of analgesia with selective COX-2 inhibitors (parecoxib sodium) give more positive results than the centrally acting opioid (tramadol sodium) that reduces the degree of pleurodesis simultaneously. Consequently, the author recommends the utilization of intrapleural erythromycin as a potent agent of chemical pleurodesis and the concomitant use of analgesics in the form of parecoxib sodium in accordance with the clinical implications of this study.

Conflict of interest

None declared.

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Ethics

The experimental study was approved by the Research Committee and Animal Ethical and Research Committee of Universiti Sains Malaysia (USM/Animal Ethics approval/2008/(36)(118).

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