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An innovative animal experimental model of rib fracture with bone displacement

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Abstract

Background Rib fractures, the most common blunt chest trauma worldwide, have been evaluated in multiple retrospective cohort studies. Histology laboratories using innovative techniques are crucial to improving patient outcomes; animal models are especially valuable in this regard. Currently, the creation of animal models of fractures involves cutting to avoid lethal pleural injury and induce linear fractures incompatible with real-world morphology. Suitable models with bone displacement, which are common surgical targets, are limited. Therefore, we aimed to develop an animal experimental model that aligns with real-world clinical conditions and allows for more in-depth research on blunt chest trauma.

Methods We conducted an animal study using New Zealand rabbits to create fractures with displacement, without injuring the pleura. A facial bone drill was used to create several fracture holes in the ribs that were carefully dissected using a rongeur and scissors. After reaching the border between the bone and pleura, they were separated through blunt dissection with scissors. Fractures were created without injuring the pleura, and a rongeur was used to displace the bones.

Results Seven rabbits successfully underwent surgery, with each awakening without pleural injury and pneumothorax. Surgery effectiveness was primarily assessed using chest computed tomography (CT) to evaluate types of rib fractures. CT was performed in the second week and second and third months postoperatively. All rabbits exhibited visibly displaced rib fractures, and no additional chest complications were observed.

Conclusions This innovative animal model allowed us to study pathophysiological changes following rib fracture. Future studies investigating new medical materials and surgical procedures will benefit from this animal model.

Keywords Rib, Fracture, Bone displacement, Rabbit, Animal model

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Background

Rib fractures are the most common type of blunt chest injuries. Ribs maintain the anatomical structure of the chest wall, protect vital organs in the chest cavity, and assist in respiration. Fractures to the ribs usually indicate a high-force injury, and are often accompanied by contusion of the heart and lungs, pneumothorax, or hemothorax [1]. Compromised respiration can result in subsequent pulmonary complications such as pneumonia and empyema that can adversely affect the prognosis of trauma patients [2, 3]. Some patients may even develop chronic restricted lung disease, which may shorten life expectancy [4, 5]. The severe pain caused by rib fractures can require varying periods of bed rest and use of painkillers [6–8], and the time to return to work can be as long as 3 months [9, 10].

Currently, the conservative treatment remains the standard procedure for single or non-dislocated rib fractures. In recent years, the treatment of rib fractures has progressed from purely conservative to surgical reduction and fixation with titanic plates [6, 11, 12]. Reducing pain caused by fractured ends, restoring the normal anatomical structure, and assisting in respiration have been beneficial, and these surgical approaches have been adopted as routine treatments [13, 14]. Moreover, research on the treatment of rib fractures has progressed from previous retrospective studies focusing on epidemiology to studies integrating anatomical biomechanics, surgical materials, and respiratory physiology [15, 16]. However, because patients with trauma typically arrive at the emergency department, conducting double-blinded randomized controlled trials is not ethically permissible. Therefore, animal experiments are crucial in this field. Previous studies have used rabbits or pigs to investigate the histology of bone healing after internal fixation with steel plates [17–19]. However, the current experimental animal literature on rib-related research involves the use of sharp instruments to create linear fracture patterns [20, 21]. This differs significantly from the clinical presentation of rib fractures, which are mainly caused by blunt trauma. Fracture surfaces are usually irregular and, more importantly, bone displacement may occur.

Moreover, if large animals such as pigs or sheep were chosen as subjects and pneumothorax occurred in previous animal experiments, chest tube placement was recommended [19, 22]. However, limited data exist on selection of chest tube size and its placement and removal. If rabbits or mice were used, the use of chest drainage tubes would not be possible owing to their size, and during pneumothorax, the only option would be to use a syringe to aspirate air, which is not safe. Therefore, the occurrence of pneumothorax may increase mortality risk in small animals. Establishing an experimental model of rib fractures in small animals that is consistent

with clinical conditions and maintains the integrity of the pleura will be highly beneficial. This model in small animals would simplify the experimental process, comply with Animal Research and Ethics principles, and increase the relevance and applicability of research in the fields of thoracic trauma anatomy and respiratory function. This can greatly improve the biomechanical aspects of rib fracture research.”

Methods

Animal

Both male and female adult New Zealand White rabbits aged ≥ 2 years, weighing an average of 1.5–2 kg, were selected, primarily because they are easy to breed and relatively inexpensive. Previous studies have also indicated that the use of New Zealand White rabbits to evaluate rib repair is feasible and accurate [20, 23].

Surgery

The preoperative steps were as follows: (1) anesthesia was induced using ketamine (35 mg/kg) and xylazine (5 mg/kg) via an intramuscular injection; (2) fur was shaved at the surgical site; (3) the rabbit was transferred to the surgical table and maintained under anesthesia with 1–3% isoflurane inhalation. Thereafter, a lateral thoracotomy was performed on the right side of the chest wall to expose the ribs. We created one fracture in two different ribs, randomly selected from the 4th to the 8th ribs.

The following steps describe our technique to introduce displacement rib fractures without injuring the pleura. When the ribs were exposed, we first used a bone drill commonly used to introduce facial fractures to create multiple small holes in the ribs. This drill technique has already been implemented in human surgical practice. The size of the drill is 0.8 mm width, and the size of the rabbit rib is about 5–6 mm width. Our goal is to achieve a precise disruption of the first layer of the bone cortex, which cannot be accomplished using standard tools. Therefore, the number and percentage of drill holes are determined during the actual procedure. Once the entire width of the bone cortex has been drilled, this step is considered complete.

It was not necessary to drill completely through the ribs, and reaching the posterior cortical layer was sufficient. Tissue scissors were used to carefully connect the holes, after which bone forceps were used to separate the fractured ends of the ribs. A blunt downward dissection technique was then employed to disrupt the relatively thin posterior cortical layer of the bone via scissoring until it was near the pleural layer (Fig. 1). The bone was carefully separated from the pleural layer, and the dorsal side of the knife and bone Rongeur was used to create a displacement fracture in the ribs (Fig. 2). In this way, we created only a displacement about one cortex thickness

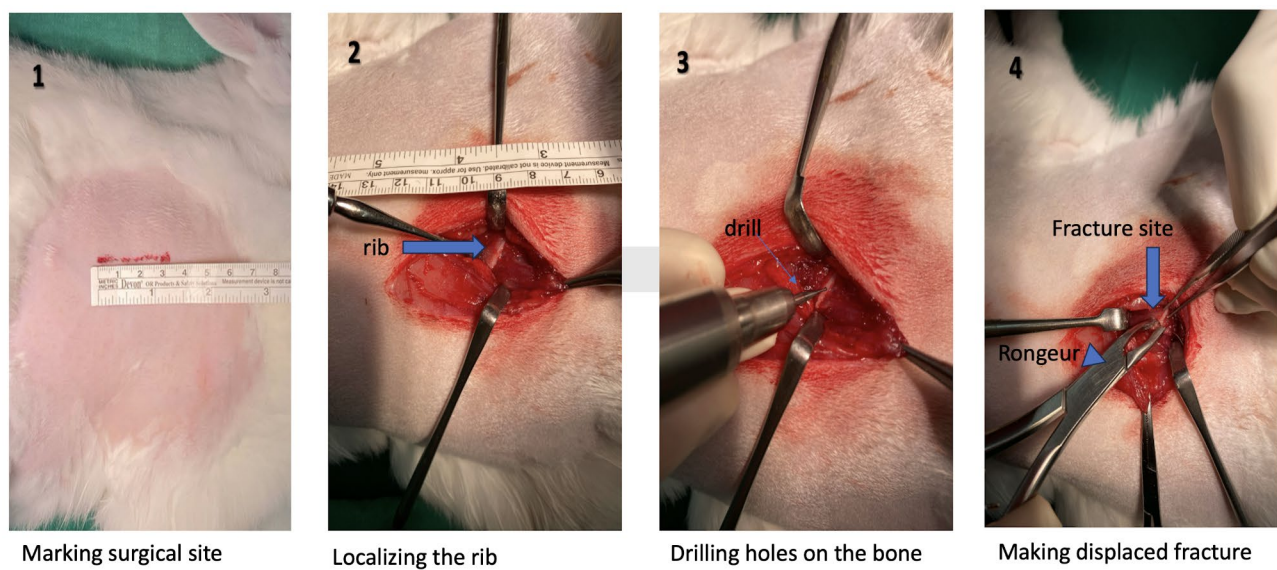


Fig. 1 Surgical procedure for creating rib fractures. (1) Marking. (2) Localizing the rib. (3) Drilling holes on the bone (4) Making displaced fracture

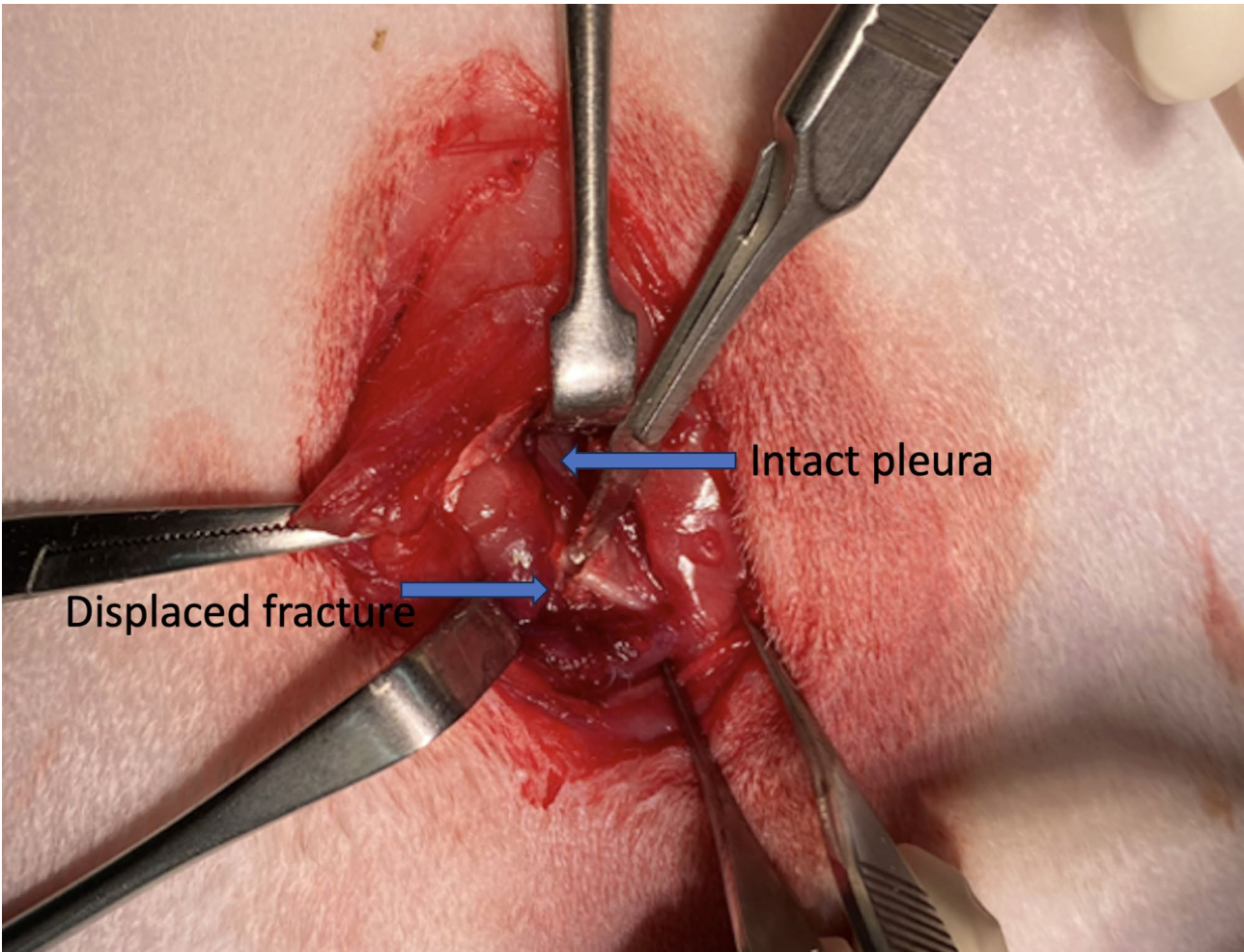


Fig. 2 Creating displaced blunt fractures by dissecting the bone with the dorsal side of the knife

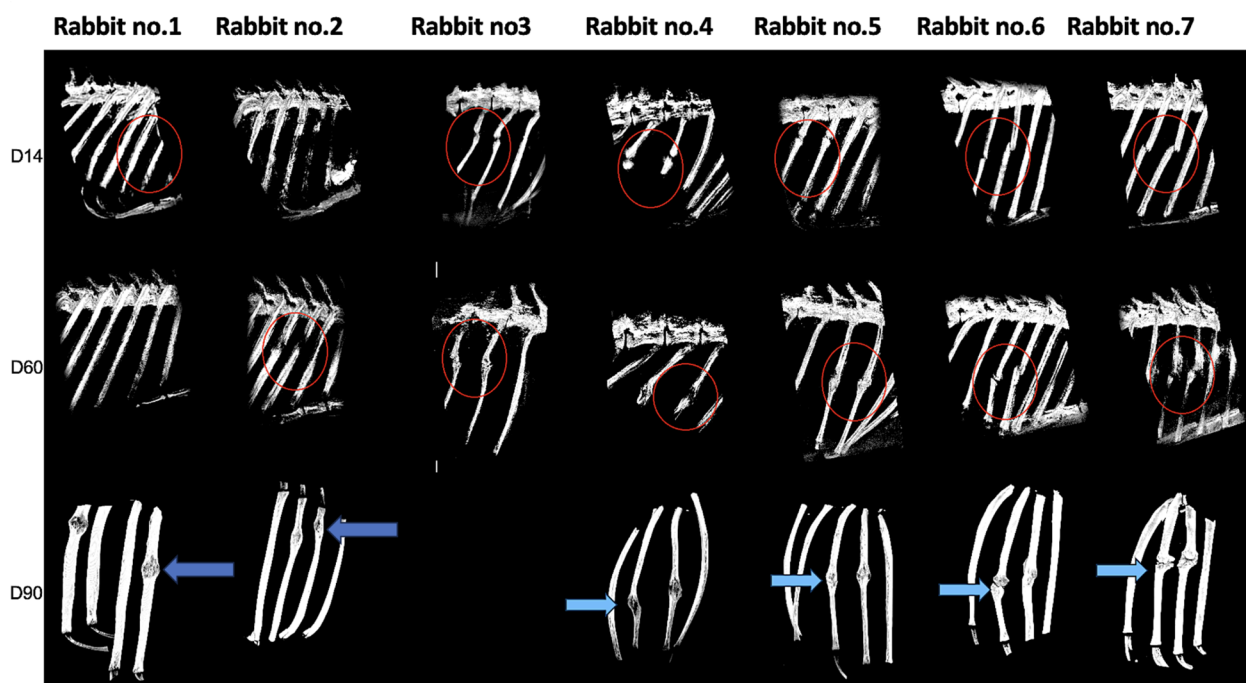


Fig. 3 Computed tomography of the bone days 14, 60, and 90 postoperatively

The red circle represents the displaced fracture site found on computer tomography on day 14 or 60, and the arrow represents the healed bone on day 90

in the rabbit's ribs, which is approximately 2–3 mm in width.

After creating the displaced fracture, the rabbit's breathing patterns and sounds were observed for approximately 1–2 min. If the pleura broke, an air leak was observed.

Postoperative care

The wound was closed using absorbable sutures for the muscle and non-absorbable sutures for the skin. A neomycin antibiotic ointment was applied, and ketoprofen (2–5 mg/kg) was injected subcutaneously. The rabbit was placed in a clean cage with a heating pad or heat lamp until consciousness was regained after anesthesia. The breathing patterns were monitored. If the pleura were injured, a disturbed breathing pattern was observed. During our procedure, since the pleura remained intact, no clinically significant air leakage was observed."

All animal procedures received institutional approval from the Institutional Animal Care and Use Committee at the Animal Center of Chang Gung Hospital (approval number 2021113002). And all the animals used in this study were owned by the Animal center of Chang Gung hospital. All animals were cared for in accordance with the regulations of the National Institute of Health of Taiwan.

Results

All seven rabbits successfully underwent surgery, with each rabbit awakening without pneumothorax. No abnormal respiratory behavior was observed in any of the rabbits during the initial 30 min after surgery.

To observe any potential impact on growth resulting from the surgery, weights of the rabbits were recorded on the day of surgery and in the first, second, and third postoperative months. No significant weight loss was observed in any rabbit.

Surgical effectiveness was primarily assessed using chest computed tomography (CT) to evaluate the type of rib fracture. CT was chosen over conventional radiography because radiography primarily projects the chest wall as a flat plane, which may not accurately capture the three-dimensional nature of the dislocated fractures that we aimed to create.

CT was conducted in the second week and second and third months postoperatively. In the second week, the focus was on observing acute phase fracture manifestations, whereas in the second month, the focus was on observing subacute phase fracture manifestations. In the third month, as the rib fractures healed, another chest CT was conducted to assess healing and evaluate the formation of calluses, with any visible signs of nonunion. All rabbits exhibited visible dislocated rib fractures, and no additional chest complications were observed (Fig. 3).

However, the CT machine was undergoing its annual maintenance when Rabbit 3 was scheduled for a scan.

As a result, we were unable to perform the CT scan for approximately two weeks, and therefore, no imaging results were available for Rabbit 3. As for the result of healing, all fracture sites were fully healed according to the results of the 90-day CT scan. Regarding Rabbit 3, the CT scan performed on day 60 showed a satisfactory healing condition.

Discussion

In clinical practice, rib fractures are often associated with dislocations, with more severe cases involving flail chest or multiple rib fractures with dislocation. However, a review of most experimental animal studies related to rib fractures revealed that investigators often create linear fractures using sharp instruments, which is quite different from rib fractures encountered clinically [24, 25]. Therefore, we aimed to develop an experimental animal model that aligns with real-world clinical conditions and allows for more in-depth research on blunt chest trauma.

Based on our findings, it is feasible to create dislocation fractures in small-to-medium-sized animals while maintaining pleural integrity for basic research on blunt chest trauma. Additionally, maintaining the integrity of the pleura prevents occurrence of pneumothorax and provides a humane experimental method for animals that aligns with the ethical standards of modern medical research.

And further applying this model to larger animals to clarify the utility of surgical fixation in realistic fractures can greatly help us better understand the clinical course of rib fractures.

Limitation

Although this animal model creates a realistic fracture close to practical fracture conditions, our choice of rabbits still presents significant differences compared to humans. The next step will be to apply this model to larger animals, such as pigs, which will be crucial for future research.

Conclusion

In this study, we described a rib fracture technique that can be expanded to larger animals to create animal models for medical research. This technique will allow us to understand the impact of different types of rib fractures on respiration, pain response, and anatomical stress in animals. When applied to current rib fracture treatments, this technique may improve our ability to understand whether surgical intervention is helpful and may help develop better surgical techniques and materials.

Abbreviations

CT Computed tomography

Supplementary Information

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Supplementary Material 1

Author contributions

CAL: conceptualization, investigation, writing—original draft; JFH: methodology; CPH: data analysis; CYF: data collection; SAC: data curation; CHL: software; CHH: validation; CTC: writing—review, and editing;

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (approval number 20220-114480), and the need for informed consent was waived.

The use of laboratory animals in this study is all approved by their owner, the Animal Center of Chang Gung Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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