Rib fractures: Could we accelerate the healing?

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Abstract
Aim: We thought about “How fractures are improving more rapidly.” For this purpose, we made an experimental model to investigate the effect of zinc supplementation on the development of new bone in young rabbits. Material and Method: Six weeks of age, male New Zealand white rabbits were evaluated in four groups, each including five subjects. To simulate the RFs, subperichondrial costal cartilage resections beginning from the third costal cartilage were carried out in the right hemithorax according to groups. Rabbits in Group 1 and 2 underwent partial resection of the two ribs; rabbits in Group 3 and 4 underwent total resection of those. Zinc was administered by intraperitoneal injection of 6 mg/kg/day for four weeks after the surgery for group 2 and 4. The animals were followed-up at the twenty-fourth week of their life. Results: We analyzed histologic changes in the bone. There were statistically significant differences for osteoblasts and osteoclasts among all subgroups. Histologic consolidation was significantly increased by zinc supplementation. According to the literature, in our study, while zinc stimulates osteoblastic bone formation, suppresses osteoclastic bone resorption. Discussion: Although the proper treatment of rib fracture (RF), long-term disability and persistent chest wall pain frequently develop and may take several months to recover, leads to the high hospital, medicine, labor, and social burden. Our findings indicate that zinc supplementation accelerates the consolidation of ribs. Zinc can be used to increase the bone maturation such as the site of new bone formation in RF.

Keywords
Zinc; Supplementation; Rib Fracture; Rabbits

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Introduction
Mortality due to trauma ranks third after cardiovascular diseases and cancers among the causes of adult death worldwide [1]. Rib fractures (RFs) are a common injury affecting greater than 145,000 people each year in the United States, with at least one-third of patients requiring hospital admission [2]. It is clear that this number is higher, considering that 77% of patients with rib fractures are not applying to emergency departments. Historically RF's treatment is symptomatic, based on pain control with analgesics including narcotics and non-steroidal anti-inflammatory drugs (NSAIDs), respiratory physiotherapy, and specific treatment of associated complications [3]. Approximately 51% of them will encounter complications including nosocomial pneumonia, prolonged respiratory failure, prolonged hospitalization, or death, due to coexisting injuries, age, and comorbidities [2-4].

Operation technique
Subjects were placed in the right lateral decubitus position on the operating table with spontaneously breathing. Then, chests were shaved and cleaned with a povidone-iodine solution (10% povidone-iodine, Betadine, Kansuk, Istanbul, Turkey); lateral thoracotomy incision was carried out after infiltrating with 1% lidocaine and with 1:100000 epinephrine (JETOKAIN SIMPLEX ampule; Adeka Pharmaceutical Company, Istanbul, Turkey). The pectoral muscles were divided along the laterally to allow exposure of the costal cartilages and thoracic wall. A horizontal incision was made over the third and fourth costal cartilage level. Subperichondrial costal cartilage resections were carried out in the right hemithorax according to groups. In each subject, bleeding and pneumothorax control was performed with sterile serum saline. After all controls, the chest wall was closed with continuous sutures by anatomical layers without any grafting.

Postoperative Care and Follow-Up
Pain control in animals was provided by Tradomol HCl (Contramal, 100 mg 2 ml, Abdi Ibrahim Ltd., Istanbul, Turkey) 1-2 mg/kg/day i.m. for five days during the postoperative period. The animals were followed-up until the rabbits were accepted to go into adulthood at the twenty-fourth week of their life. All animals were euthanized with a lethal IV dose of Non-barbiturate anesthetic (Ketamine/Xylazine) painlessly according to the existing instructions established by the latest report of the AVMA Panel on Euthanasia. Three times the anesthetic dose was used for euthanasia [9].

Zinc protocol
Zinc sulfate (ZnSO4) (Merck, CAS number 7446-20-0 Darmstadt, Germany) was in crystalline form. The solution was prepared by dissolving 1 mg/ml in distilled water to make the stock solution. It was stored at 4°C at which it could remain stable for at least a month. The solutions to be injected were prepared by diluting stock solutions using 0.9% NaCl (0.1 mg/ml) on a daily basis. Working solutions were used within 1 hour following the preparation. Zinc was administered with 0.5 ml of saline by intraperitoneal injection of 6 mg/kg/day at 10-12 every day for four weeks after the surgery for Groups 2 and 4 [10].
Pathological Evaluation

All materials were decalcified in 10% buffered formaldehyde for 48 hours after the fixation period until they were tempered enough to be cut with a microtome. Tissue specimens from appropriate sites were then taken for the autotechnical follow-up, embedded in paraffin and stained with Hematoxylin & Eosin (H&E) and sectioned with a microtome to calculate the osteoclast, osteoblast, lymphocyte count and the area of new bone formation (Figure 1). All stained preparations were examined with Nikon Coolpix 5000 digital camera (Nikon Corporation. Minato-ku, Tokyo Japan). Care was taken to select as possible the same areas for each case in evaluating. The selected areas were scanned with a Nikon Coolpix 5000 digital camera (Nikon Corporation. Minato-ku, Tokyo Japan) with a microscope mounted at the same microscope magnification. At the same time, Nikon Stage Micrometer (MBM11100, Nikon Corporation. Minato-ku, Tokyo Japan) images were also taken for calibration with the same microscope magnification. All images were transferred to a PC environment for analysis with Clemex Vision Lite 3.5 (Clemex Technologies inc. Longueuil, Quebec Canada) (Figure 2). First, the length was calibrated with Nikon Stage Micrometer (MBM11100, Nikon Corporation. Minato-ku, Tokyo Japan). After the calibration, the area to be examined was determined as 38732.7 μm². The osteoblasts, osteoclasts, and lymphocytes on the 38732.7 μm² areas selected on the digital images of H&E stained preparations were marked and automatically counted by the mentioned image analysis program. The damaged cells were excluded from the evaluation during the examination.

Statistical analysis

All data were assessed using the Statistical Package for Social Sciences (SPSS) 18.0 portable for Windows (SPSS Inc, Chicago, Illinois, USA). In every subgroup, we analyzed histologic changes in the bone. The effect of zinc treatment between groups was evaluated by using the Kruskal-Wallis test and the Mann-Whitney U test. There were statistically significant differences for osteoblasts and osteoclasts between all subgroups (Table 1). A p-value (<0.05) was used to indicate a significant difference.

Results

Regardless of the cause of deaths animals were replaced with new ones. All rabbits have been included in our experiment lived up to the end of the study. There were no local complications such as skin reaction, wound infections, or bleeding around the operating. There were statistically significant differences for osteoblasts and osteoclasts among all subgroups (Table 1). Table 1 shows no statistically significant difference between groups in Groups 1 and 2 except fibroblasts, Lymphocytes, and VEGF when compared to in Zn (Groups 2 and 4) and non-Zn groups (Groups 1 and 3). The difference was in osteoblasts, osteoclasts, and fibroblasts between only Groups 1 and 2. Table 2 shows the mean and standard deviation values of all groups. While there was no difference in VEGF between the groups, the numerical difference was higher in Zn than in non-Zn groups. They were affected by the shape of the surgical dissection. There was no difference in lymphocytes. However, there were more lymphocytes in the Zn group than in the non-Zn groups. Zinc application increased the number of lymphocytes. Besides, the number of lymphocytes was affected by the shape of the resected ribs.

Table 1. Comparative groups’ t- and p-values

<table>
<thead>
<tr>
<th></th>
<th>Group 1-2</th>
<th>Group 3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (Vascular Endothelial Growth Factor)</td>
<td>4.8±1.48</td>
<td>4.2±1.48</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>6.4±2.96</td>
<td>4.0±2.96</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>5.8±1.30</td>
<td>4.0±1.30</td>
</tr>
<tr>
<td>Osteoblast</td>
<td>18.8±2.28</td>
<td>16.2±2.28</td>
</tr>
<tr>
<td>Osteoclast</td>
<td>5.8±0.83</td>
<td>4.0±0.83</td>
</tr>
</tbody>
</table>

Results for the Groups 1 and 2. There is only a significant difference between these groups. We believe that growth center conservation and limited resection are the causes of this difference. Although the same numbers of ribs were removed, there was no statistically significant difference between the Groups 3 and 4 in which the growth center was not preserved, and the mean and standard deviation values were higher. Fibroblasts increased in groups where the growth center was not preserved. There were more fibroblasts in the Zn group than non-Zn.

Osteoblasts were significantly higher in each group of zinc than in each group without zinc. However, the groups in which the growth center was resected (Groups 3 and 4) were lower than the non-resected groups (1 and 2). Even the positive effect of zinc did not prevent this decline. However, the partial resection in Group 1 was similar to the total resection in Group 4 with Zn. Zinc has the same effect on the conservation of the growth center.

Osteoclasts were statistically significant and low in Zn groups compared to those without zinc. However, in the groups in which the growth center was resected (Groups 3 and 4), it was more than the groups that were not resected (Groups 1 and 2). Zinc groups are less in zinc-free groups. Zinc decreases osteoclasts and increases bone mass. Osteoclasts are less in Zn groups than in non-Zn groups. It decreases osteoclasts, thus increasing bone mass and consolidation. Histologic consolidation was significantly increased by zinc supplementation. By the literature, in our study, while zinc stimulates osteoblastic bone formation, suppresses osteoclastic bone resorption.

Discussion

Traffic accidents are the most frequent cause of hospitalization and RF, and anticipated to increase by 65% until 2033 in developing countries. For this reason, it seems certain that we will encounter more RF in the coming years. Chest pain depending on RFs is caused by broken bones and injured muscles. It is generally exacerbated by any movement of the chest wall including deep breathing and coughing in even normal breathing movements. The current standard treatments especially in-patients (patients be in the hospital) are based on powerful pain control intervention such as epidural analgesia, intrapleural local anesthetics instillation, rib blocks, and intravenous narcot-
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Outpatients with RFs are most commonly treated only with relatively powerless oral analgesics such as narcotics and NSAIDs (NSAIDs). After being discharged from the hospital these powerful treatments are terminated. Patients are to be left alone with severe pain that takes several weeks to heal [4]. Despite all treatment options, 51% of the cases get into complications such as nosocomial pneumonia, prolonged respiratory failure, long-term hospitalization, or death due to age, accompanying the comorbid disease, and trauma [2-4]. Although the number of RFs and the age of the patient are designated as the factors that increase mortality and morbidity in the literature, this situation is controversial [11]. While high morbidity is defined in patients older than 65 years, a similar high mortality and morbidity tendency has been described in younger patients [12]. The number of RF is similar. Wharton and colleagues showed that high mortality and morbidity were not associated with the number of RF in 35,467 disease series. However, they did not assess whether rib fractures worsened or exacerbated existing comorbidities or had any effect on the severity of the accompanying injuries [13]. On the other hand, Jones et al. identified five and more RFs as an independent cause of mortality in 98,836 series of disease. This needs to be explained [14]. Mortality that ranges from 2 to 20% and morbidity mostly due to the accompanying injuries are reduced with the control of pain in chest trauma and especially in RFs. They are reduced by the provision of pain and bone stability [15].

In the literature, there are a lot of experimental studies relating to Zn and bone healing. To our knowledge, this is the first study about the zinc and RF. It is the only trace element affecting the basic structure or functions of osteogenic enzymes involved in bone development and healing. [6]. In many parts of medicine including cardiothoracic surgery, oral surgery and orthopedics, controlled and guided bone growth is based as well on bone metabolism consisting of osteogenesis, bone modeling and bone remodeling [16]. According to the significant effect on the bone formation by zinc, we hypothesized that the in the treatment of rib fractures after median sternotomy the oral surgery can be used to accelerate healing. Our experimental model was similar to a rib fracture model, in addition, the growth center of the ribs was resected. [11]. In the literature, minimal cartilage resection and saving the growth center of the ribs at costochondral junctions has been suggested in order to produce faster healing [17]. Nevertheless, histologic consolidation was significantly increased by zinc supplementation even in the group where growth centers were resected (Table 2). In accordance with the literature, in our study, while zinc stimulates osteoblastic bone formation, it suppresses osteoclastic bone resorption. Like in every study, there are some limitations in our study. Firstly, as an experimental animal study cannot entirely be applicable to humans, therefore, additional prospective human studies are needed. Secondly, the method of administration, dose, and additional cost of Zinc should be investigated in humans.

Conclusion

Although human beings have been trying to overcome chest trauma, especially rib fractures from the first people, even today, it is still painful and can be potentially disabling. The actual figures and costs are much higher than expected. Because of the inadequacy of existing or traditional treatment, 30% of patients do not return to entirely their work or normal activities. This results in social and economic costs both to the national healthcare system and to individuals in the form of lost productivity and decreased the quality of life. Any improvements to its treatment would have even had great benefit not only on the individual but also on society [4, 18]. Our findings indicate that zinc supplementation accelerates the consolidation of ribs. Zinc can be used to increase the bone maturation such as the site of new bone formation in rib fracture. Healing modulation or acceleration may alleviate pain, enhancing recovery and reduce disability on the treatment of RFs. Zinc supplementation could be a next step that we are looking for a long time.

References

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