Systemic Effects of Experimental Spinal Cord Injury on Bone Healing in Rabbit

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Abstract
Bone loss after spinal cord injury leads to increased fragility of bone and subsequent risk for low-trauma fractures in the sublesional parts of the body. Although in such injuries upper limbs are normally innervated, bone loss may occur in the upper extremities. The present study was designed to determine the systemic effects of spinal cord injury on the fracture healing of upper limbs in rabbits. Twenty nine skeletally mature New Zealand white rabbits received a transverse mid-humeral open osteotomy in the left upper limb with the use of a standardized technique and spinal cord injury was done using forceps model at T8 level. The animals were divided into three groups: experimental (laminectomy, spinal cord injury, and osteotomy), sham (laminectomy and osteotomy), and control (osteotomy alone). The bone healing score was calculated using modified Sandhu system by two independent pathologists. The mean (±SD) of healing scores in experimental, control, and sham groups were 7.22 (± 3.6), 8.6 (± 3.3), and 8.5 (± 4.3) respectively (P=0.68). The percentage of mesenchymal (20%) and cartilaginous tissue (35%) showed a slightly higher value in the experimental group compared with the sham group (15% and 20% respectively). A reverse pattern was seen concerning the percentage of trabecular bone, though as a whole there was no significant difference regarding the percentage of selected components of bone healing between the three trial groups. Fracture healing in innervated upper limbs is not influenced by the systemic effects of spinal cord injury.

Keywords ● Healing ● spinal cord injury ● fracture ● bone

Introduction
Spinal cord injury (SCI) leads to motor and sensory loss in the sublesional parts of the body. Osteoporosis is a common sequel of SCI, occurring primarily at sublesional cancellous-rich sites. Bone mineral content (BMC) decreases by as much as 70% following SCI. The bone loss leads to increasing fragility of bone and subsequent risk for low-trauma fractures.

According to Eser et al., in most of the patients with SCI the fracture occurred in long bones of the lower extremities. Reduction of mechanical stress thus inhibits osteoblast-mediated bone formation and accelerates osteoclast-mediated bone resorption.

Fracture healing in denervated limbs has been studied in paraplegic rats. Osteogenic cells were less proliferated and less differentiated resulting in scant callus formation or...
delayed union. The environment of paralytic limbs was evidently altered after 2 to 3 weeks of the injury, because thereafter fracture healing seemed to become poor.\(^7\)

In supraspinal level, fracture-healing area may be changed by an associated traumatic spinal cord injury. Although upper limbs are normally innervated, bone loss may occur in the upper extremities in patients with paraplegia.\(^8\) An intriguing question is that whether SCI has systemic effects on fracture healing of non-paralyzed limbs. The present study was designed to determine the systemic effects of SCI on the bone fracture healing of upper limbs in rabbits.

**Materials and Methods**

**Experimental Design**

Twenty-nine skeletally mature (one year old) New Zealand white rabbits, ranging in weight from 4-5 kg, received a transverse midhumeral open osteotomy in the left limb with use of a standardized technique,\(^9\) and partial transsection lesions of the spinal cord (at T8 level) with forceps model 23. The animals were divided into three groups: Experimental [laminectomy, SCI, and osteotomy (N=9)], sham [laminectomy and osteotomy (N=10)], and control [osteotomy alone (N=10)]. All the animals were killed four weeks after the index procedure and the healing process was evaluated by two pathologists who were blind to the animals allocation.

**Surgical protocol**

The animals were anesthetized with Ketamine (50 mg/kg, intramuscularly) and Xylazine (10 mg/kg, intraperitoneally). During the surgery, the animals were placed on a heating pad to help maintaining the body temperature.

The dorsal region and the left upper limb were shaved, prepared and draped under aseptic conditions. A 3-cm longitudinal skin incision was made sharply in the midline and carried to the level of the dorsal fascia. This was opened using electrocautery and the paraspinal muscles were dissected off the spinous process of the vertebrae above and below the T8 level.

Further dissection of the muscles off the lamina could be accomplished using periosteal elevators. The inferior edge of the superior lamina was dissected using a microdissector, then by using a Kerrison punch, T8 laminctomy was performed. After exposing the dura, the blades of modified coverslip forceps (4 mm wide \(\times\) 0.5 mm thick) were inserted into the spinal canal between the lateral aspects of the spinal cord and vertebrae (under high power surgical microscope). A SCI was induced by compressing the spinal cord between the blades of the forceps, to a pre-set separation of the blades, for a period of 15 seconds. The dura in the levels upper the injury site remained intact. The paravertebral muscles and fascia were approximated and the skin was closed. Then the left upper limb was prepared and draped under aseptic conditions. Prior to the osteotomy, four steinmann transfixation pins (2 mm in diameter and 6 cm in length) were positioned in the frontal plane of the humerus diaphysis, two proximal and two distal to the planned osteotomy site, with use of a surgical pin driver. To ensure accurate and reproducible placement of the pins, a template was used. A 2-cm longitudinal skin incision was made over the anteromedial aspect of the limb. The humerus was isolated from the surrounding soft tissue with two mini-Hofmann retractors. An osteotomy was performed at the middle third of the humerus with the use of an oscillating power saw (blade thickness 0.23 mm) under continuous irrigation. Four Steinmann pins were incorporated into a uniplanar double-bar external fixator, creating a 3-mm gap between the osteotomized bone ends. The osteotomy site was thoroughly irrigated with saline solution by a syringe, making sure that no tissue (bone, muscle, or fascia) was left in the gap. The soft tissue was then reapproximated and the skin was closed. After the operation, the lower limbs at the sublesional of spinal cord were paralyzed. The surgical wounds were covered with sterile dressings for three to four days. The pin tracks and operation sites were cleaned with povidone-iodine solution every other day in order to prevent infection. The animals were provided with food and water and were allowed free activity in standard laboratory cages during the study period. Enrofloxacin (5 mg/kg) was administered preoperatively and for 2 days postoperatively. Morphine (5 mg/kg) was administered for 2 days postoperatively. The animals were killed with an overdose of pentobarbital four weeks after index procedure and 1.5 cm segment of the humerus bone including 3 mm gap was evaluated histologically.

**Histological study**

The upper extremity was evaluated for reparative healing tissue. The specimens were placed in 10% formalin and then in 10% nitric acid solution for decalcification. A 4-mm slice from defect (fractured) region was prepared by tissue processor. After paraffin embedding, 5-micrometer sections were achieved by microtome. Hematoxylin and eosin stain were used for the staining. Each slide was evaluated for
the extent of repairing component as percent of granulation tissue, neutrophilic exudate, cartilaginous tissue, trabecular and compact bone in a transverse section from healing region. The extent of each repairing component was determined by Motic microscope and related software. The healing score was also calculated using Sandhu system,\textsuperscript{10} by two independent pathologists who were blind to all groups.

**Statistical analysis**

Two experienced independent pathologists scored the smears according to the modified Sandhu system. Inter–rater reliability was calculated using intraclass correlation coefficient (ICC) using two way random effects model (intraclass correlation coefficient = 0.89). ANOVA was used to test the differences between mean scores among the three groups. To compare non-parametric data (percentages) between the three groups Kruskal Wallis test was used. P<0.05 was considered to indicate statistical significance. SPSS software version 12.0 was used for data analysis.

**Results**

Table 1 provides the median (range) values of the extent of various healing components as percent of mesenchymal tissue, cartilaginous tissue, and trabecular bone in the transverse section of the healing region in the three trial groups. The percentage of mesenchymal and cartilaginous tissue showed a slightly higher value in the experimental group compared with the sham group. A reverse pattern was seen concerning the percentage of trabecular bone, though as a whole there was no difference regarding the percentage of selected components of bone healing between the three groups.

The mean (±SD) of healing score in experimental, control, and sham groups were 7.22(±3.6), 8.6(±3.3), and 8.5 (±4.3), respectively (P=0.68).

**Discussion**

The present study shows that SCI does not change fracture-healing in innervated limb. The effects mediated by SCI have been reported in a wide variety of tissues. They may occur in intestine, kidneys, gonads, bones, fat, and parathyroid glands.\textsuperscript{8}

A recent study suggests that bone remodeling is regulated by nerve derived signals, such as vasoactive intestinal polypeptide, calcitonin gene-related peptide, pituitary adenylate cyclase activating peptides (PACAPs), neuropeptide Y, and substance P, as well as classical neuromediators such as noradrenaline, serotonin, and glutamate.\textsuperscript{11}

It has also been demonstrated that there is no obvious change in the core binding factor alpha-1 (Cbfa-1) and AKP mRNA expression in cultured osteoblast-like cells from the rats with SCI, 3 weeks after injury. This indicates that SCI may have no effect on osteoblast function at the early stage of injury.\textsuperscript{12} These data explain why bone healing was not influenced by SCI.

In addition to neuronal mechanisms, mechanical changes should be considered. During remodeling, alignment of new bone is along the dominant local loading direction, suggesting local regulation of bone formation by mechanical stimulus.\textsuperscript{13} Mechanical loading is known to be a crucial stimulus for bone formation and resorption, thereby controlling bone mass, structure, and strength. The skeleton possesses an inherent biological control system that directs bone formation in response to high mechanical stresses (or strains), thus strengthening the skeleton in highly stressed regions. This system, sometimes called the ‘mechanostat’, involves the resident cells within bone tissue that detect and respond to mechanical loads.

It has become clear over the past several years that the osteocytes are the professional mechanosensory cells of the bone. Osteocytes situated in the bone matrix respond to mechanical load signals,\textsuperscript{14} and the gap junction of the long processes of osteocytes transmits mechanical load signals via intracellular and extracellular signal transmitters induce bone formation by osteoblasts, inhibition of bone resorption by osteoclasts, or a combination of the two.\textsuperscript{15,16} The effect of mechanical loading on bone tissue is an increase in bone formation in the periosteal bone surfaces, thus improving bone strength and reducing bone turnover and bone porosity. Consequently, mechanical loading can improve both the bone size and the shape as well as strengthening the bone tissue by improving tissue density.\textsuperscript{17}

<table>
<thead>
<tr>
<th>Repair component (%)</th>
<th>Experiment (n=9)</th>
<th>Control (n=10)</th>
<th>Sham (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesenchyme</td>
<td>20(10)</td>
<td>10(10)</td>
<td>15(20)</td>
<td>0.44</td>
</tr>
<tr>
<td>Cartilage</td>
<td>35(30)</td>
<td>35(40)</td>
<td>20(40)</td>
<td>0.25</td>
</tr>
<tr>
<td>Trabecular bone</td>
<td>40(80)</td>
<td>55(70)</td>
<td>65(50)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*Italic figures represent median (range)*
In the present study, due to using external fixator, the bone was unloaded relatively but healing occurred normally. It shows that, at least, bone healing was influenced by innervations much more than the loading. Therefore, from the pathophysiological viewpoint, unloading may not be the key factor in the fracture healing, and other factors should be taken into account.

We conclude that fracture healing in innervated upper limbs is not influenced by the systemic effects of spinal cord injury. Further understanding of this systemic response can provide important insights in systemic therapeutic strategies for the enhancement of skeletal repair.

Conflict of Interest: None declared

References