Basic Science

Effect of Intermittent Administration of Parathyroid Hormone on Fracture Healing in Ovariectomized Rats

Jun Seop Jahng, MD
Hyun Woo Kim, MD

ABSTRACT

The effects of intermittent administration of parathyroid hormone on fracture healing in ovariectomized rats were examined to evaluate its potential use as a therapeutic agent for osteoporotic fractures. Three months postovariectomy, bilateral tibial shaft fractures were induced and stabilized by intramedullary nailing with Kirschner wires. Saline, 17β-estradiol (Sigma Chemical Corp, St Louis, Mo), or recombinant human parathyroid hormone (1-84) (Korean Green-Cross Pharm Corp, Seoul, Korea) was given once a day for 30 consecutive days during fracture healing. Fracture healing was assessed by morphometric and mechanical analysis of fracture callus. Intermittent parathyroid hormone administration increased the morphometric and mechanical parameters in a dose-dependent manner. A bone-resorption inhibiting agent, 17β-estradiol did not offer advantage in terms of fracture healing in ovariectomized rats. Findings suggest intermittent parathyroid hormone administration may benefit osteoporosis and fracture.

Osteoporosis is a health-care problem in the older population and frequently leads to orthopedic treatment for fracture repair. Drugs to treat osteoporosis can be classified according to the response of the bone. They either stimulate bone formation or inhibit bone resorption. Among the bone-forming agents, intermittent parathyroid hormone administration has anabolic effects on the bones of animals and humans. Parathyroid hormone can increase bone mass at all four envelopes: cancellous, endocortical, intracortical, and periosteal. Anabolic action of parathyroid hormone does not require previous stimulation of bone resorption induced by ovariectomy.

Fracture healing involves a complex pattern of interactions between local and systemic regulatory factors. Processes of normal and abnormal fracture healing and various factors affecting them have been studied widely. Hormones may play a substantial role in bone repair but there is lack of uniformity in the presentation of data. Many authors presume aging decreases the quality of fracture repair, and an age-related decline in the capacity for fracture repair has been reported. However, the question of impaired fracture healing in osteoporotic patients and the role of estrogen deficiency in its etiology still remain unclear.

Traditionally, parathyroid hormone has been well known for its catabolic action on the skeleton. To date, there are no studies investigating the influences of exogenous parathyroid hormone administration on fracture healing, which is essentially an anabolic process. The ovariectomized rat model causes changes in bone that resemble most of the characteristics of human postmenopausal bone loss. It is now generally accepted that ovariectomized rats should be used as the animal model for screening new agents for osteoporosis therapy.

This study compared fracture healing in normal and ovariectomized rats and examined the effect of intermittent administration of parathyroid hormone on fracture healing to evaluate its potential use as a therapeutic agent for osteoporotic fractures.

MATERIALS AND METHODS
Experimental Protocols

All animal studies were carried out with the approval of the institutional review board. A total of 75 4-month-
old mature female Sprague-Dawley rats were used. Mean and standard deviation of weight was 256±7 g. The animals were randomly divided into 5 groups and weight matched. They were then treated according to the protocol shown in Table 1. Fifteen animals underwent a sham operation and served as intact controls (group 1). Bilateral ovariecograms were performed from a dorsal approach in the remaining 60 animals in the other four groups (groups 2, 3, 4, and 5). Groups of four animals were housed in a cage kept at a constant temperature and humidity. They had free access to a standard diet of rat chow and water.

Three months postoperatively, all animals in the five groups underwent bilateral controlled tibial shaft fractures under intraperitoneal anesthesia with ketamine hydrochloride and xylazine at doses of 50 mg/kg and 25 mg/kg body weight, respectively. The desired fracture patterns were created using a modified technique of Bak and Andreassen.19,20 Both lower extremities were shaved and prepped with povidone-iodine solution. A hole was made 4 mm proximal and 2 mm medial to the tibial tuberosity percutaneously using a 20-gauge needle. The needle was driven into the medullary canal. By rotating the needle, the canal was reamed just proximal to the ankle joint allowing for the wire to seat and prevent it from perforating the cortex.

A fracture was then created 5 mm above the tibiofibular junction by three-point bending using specially designed forceps with blunt jaws. The fractures were immediately stabilized by closed intramedullary nailing using a .73-mm Kirschner wire (Zimmer Co, Warsaw, Ind) through the prepared hole. Rotation was checked by comparing the alignment of the foot and the thigh.

Radiographs were taken postoperatively to document the fracture configuration and wire placement. A satisfactory fracture pattern was defined as transverse mid-shaft fracture without obvious angulation, comminution, or displacement, and with intramedullary fixation bridging the fracture. After recovering from the procedure, the animals were allowed to resume free activities and weight bearing in the cage.

After fracture surgery, saline, estrogen, or parathyroid hormone was administered daily to rats by subcutaneous injection for 30 consecutive days: saline solution (a volume equal to the volume of the drugs given) in groups 1 and 2, 17β-estradiol (Sigma Chemical Corp, St Louis, Mo) (30 μg/kg) in group 3, low doses of recombinant human parathyroid hormone (1-84) (Korean Green-Cross Pharm Corp, Seoul, Korea) (15 μg/kg) in group 4, and high doses of recombinant human parathyroid hormone (1-84) (150 μg/kg) in group 5.

Animals were sacrificed by carbon dioxide inhalation at postfracture surgery day 30, and the success of the ovariectomy was confirmed by absence of any residual ovarian tissue at sacrifice. Healing left and right tibiae were harvested after the hindlimbs were disarticulated at the knee joints. Soft tissues and fibulae were carefully cleared, preserving the integrity of callus. Intramedullary K-wires were taken out through the original entrance. All tibiae of the left side were examined by morphometric analysis of periosteal callus (quantitative analysis of fracture healing), and all right tibiae were tested mechanically (qualitative analysis of fracture healing).

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Operation</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sham operation</td>
<td>Fracture [saline]</td>
</tr>
<tr>
<td>2</td>
<td>Ovariectomized</td>
<td>Fracture [saline]</td>
</tr>
<tr>
<td>3</td>
<td>Ovariectomized</td>
<td>Fracture [17β-estradiol]</td>
</tr>
<tr>
<td>4</td>
<td>Ovariectomized</td>
<td>Fracture [L-PTH]</td>
</tr>
<tr>
<td>5</td>
<td>Ovariectomized</td>
<td>Fracture [H-PTH]</td>
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</table>

Abbreviations: L-PTH=low doses of PTH, H-PTH=high doses of PTH.

Mechanical Measurement of Fracture Callus

For mechanical testing, all right tibiae were stored in gauze soaked in normal saline solution at −20°C until needed. All mechanical testing was done within 48 hours of death. All bones were similarly oriented in a material testing machine (Instron Ltd Wycombe, Buckinghamshire, United Kingdom), and the area to be tested was defined as the medial aspect of the fracture callus. A custom jig ensured consistent alignment of the bone axis with the axis of the testing machine.

The bones were placed on two
TABLE 2

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Morphometric Measurement of Tibial Fractures in the Rat (Mean±SD)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group 1 (n=13)</td>
</tr>
<tr>
<td>Callus length (mm)</td>
<td>8.92±2.38</td>
</tr>
<tr>
<td>Callus diameter (mm)</td>
<td>0.91±0.21</td>
</tr>
<tr>
<td>Trabecular bone in callus (%)</td>
<td>92.04±2.80</td>
</tr>
<tr>
<td>Trabecular bone area of callus (mm²)</td>
<td>7.34±1.54</td>
</tr>
</tbody>
</table>

*P<.05, versus group 1.
†P<.05, versus group 2.
‡P<.05, versus group 3.
§P<.05, versus group 4.

There were no postoperative infections. In the remaining 63 rats, the intra-medullary fixation maintained axial alignment of the fracture without interfering with the formation of external fracture callus.

Mechanistic Measurements of Fracture Callus

Specimens tested mechanically all failed at the original fracture site (Table 3). Mechanical testing indicated parathyroid hormone administration resulted in an increase in ultimate load in ovariectomized rats, whereas no significant differences were seen between the groups injected with saline or 17β-estradiol (group 1; n=5, group 2; n=4, and group 3; n=3, 17β-estradiol). Other significant differences were in the increase in the absorbed energy and the ultimate stress of the group of saline-treated normal rats and high doses of parathyroid hormone-treated ovariectomized rats (group 1; n=5, group 2; n=4, and group 3; n=3, 17β-estradiol).

Scanning electron microscopy showed a tendency for parathyroid hormone-treated rats to have more trabecular bone in the callus resulting in the increase of callus diameter and cross-sectional area of the fracture than that of sham, ovariectomized only, or ovariectomized plus estrogen-treated groups. However, they also were more porotic than the sham operated group (Figure).

DISCUSSION

This study confirmed earlier observations that fracture healing is impaired in the ovariectomized rat. An inhibition of mineralized bone formation and reduction of trabecular bone formation in later stages of fracture healing have been noted in this model.

Although in this study there were no significant differences in external cal-
Table 3

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=13)</th>
<th>Group 2 (n=14)</th>
<th>Group 3 (n=12)</th>
<th>Group 4 (n=13)</th>
<th>Group 5 (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultimate load</td>
<td>79.73±6.06</td>
<td>34.93±9.57*</td>
<td>36.24±12.39*</td>
<td>55.08±7.27***</td>
<td>70.55±8.02†††‡‡‡</td>
</tr>
<tr>
<td>Deflection (mm)</td>
<td>0.21±0.08</td>
<td>0.35±0.15*</td>
<td>0.31±0.17</td>
<td>0.25±0.09</td>
<td>0.27±0.14</td>
</tr>
<tr>
<td>Absorbed energy (N/mm)</td>
<td>10.60±3.47†</td>
<td>4.41±1.46*</td>
<td>4.59±2.34*</td>
<td>4.72±2.00*</td>
<td>8.32±1.29†††‡‡‡</td>
</tr>
<tr>
<td>Ultimate stiffness (N/mm)</td>
<td>51.15±12.49</td>
<td>23.45±6.84*</td>
<td>27.69±9.43*</td>
<td>34.17±12.90*</td>
<td>42.67±19.52†††‡‡‡</td>
</tr>
<tr>
<td>Ultimate stress (N/mm²)</td>
<td>37.94±5.32</td>
<td>16.10±2.90*</td>
<td>15.70±2.34*</td>
<td>19.32±4.02*</td>
<td>31.18±2.52†††‡‡‡</td>
</tr>
</tbody>
</table>

*P < 0.05, versus group 1.
†P < 0.05, versus group 2.
‡P < 0.05, versus group 3.
§P < 0.05, versus group 4.

Figure: Scanning electron microscopy findings of fracture callus cross-section in sham-operated (A) and parathyroid hormone-treated ovariectomized rats (B). Parathyroid hormone-treated ovariectomized rats (group 5) had more trabecular bone in their callus than that of the sham-operated group and they were more porous than sham-operated rats.

In this study, we investigated the effect of intermittent parathyroid hormone treatment on immature rats. We found that intermittent parathyroid hormone treatment increased the trabecular bone formation and possibly a small increase in endosteal bone formation at early times postovariectomy or are a characteristic pattern of fracture repair found in ovariectomized rats.

The primary question addressed in this study was whether parathyroid hormone improves fracture repair in the ovariectomized rat model. Our results demonstrated intermittent parathyroid hormone administration increased the morphometric and mechanical parameters of fracture callus. Parathyroid hormone is commonly believed to trigger the catabolism of bone by stimulating osteoclastic resorption. On the other hand, intermittent administration of parathyroid hormone has been found to stimulate bone formation in many human and animal studies. In human studies, reported side effects have been confined to transient reddening around the injection site and transient elevation of plasma calcium above the upper limit of normal approximately 6 hours after injection.

The mechanism behind the anabolic effect of intermittent parathyroid hormone treatment is not fully understood, but most studies in rodents using parathyroid hormone analogues suggest c-AMP dependent pathways play the predominant role in mediating the osteoinductive actions of parathyroid hormone. Parathyroid hormone acts directly on the osteoblast by binding to membrane receptors. The resulting increased presence of secondary messengers as c-AMP, phosphoinositol metabolites, and cytosolic Ca²⁺ may lead to cellular proliferation and differentiation.

Recently, it has been suggested that the anabolic action of parathyroid hormone is substituted or partly mediated via an increased synthesis of local growth factors in the bone tissues. Prefilchta et al. revealed the anabolic effect of parathyroid hormone on bone mass is accompanied by progressive increases in bone matrix-associated insulin-like growth factor-I (IGF-I) and transforming growth factor-b, and parathyroid hormone has no effect on circulating IGF-I. It may suggest the increase of bone matrix IGF-I is due to the local effect of parathyroid hormone on bone tissue directly rather than to an increase of circulating IGF-I. Recent studies also demonstrated regulatory roles for those local growth factors in the initiation and the development of the fracture callus. On the basis of those findings, we questioned whether parathyroid hormone also enhances fracture healing.

The major effect of parathyroid hor-
mone on fracture healing was an increase in the bony tissue of the fracture callus, as reflected by morphometric parameters. However, as we did not make serial observations of the entire fracture healing process, we are uncertain whether the increase in bony callus is due to an increase in cartilaginous cell number or an increase in intracellular or extracellular matrix. Although we cannot clarify whether our findings resulted from increases in one of two distinct mechanisms (intramembranous bone formation and endochondral replacement), a seemingly increased cross-sectional area associated with relative narrowing of the central marrow cavity of the fracture surface noted in scanning electron microscopic study in parathyroid hormone-treated rats suggests both possibilities.

Histologic findings and scanning electron microscopy also indicated parathyroid hormone-treated rats have more trabecular bone in their callus resulting in an increase of callus diameter, but remained more porotic than the sham-operated group. This fact explains the lack of significant differences in ultimate load and ultimate stress value between sham-operated and parathyroid hormone-treated groups despite differences in their external callus diameter or cross-sectional area. It means a qualitative difference rather than a quantitative difference in callus tissue between groups, and that parathyroid hormone treatment of the ovariectomized rats induces increased amounts of bone tissue in their callus, but which also has the altered mechanical properties induced by ovariectomy.

From studies concerning the effects of estrogen in ovariectomized rats, signs of both enhanced and delayed bone repair in the rats have been reported. Danielsen et al noted estrogen treatment of both intact and ovariectomized rats tended to reduce the rate of periosteal bone formation. Turner et al found transient increases in the rate of bone formation, an early effect of ovariectomy, were reversed by the administration of 17β-estradiol. Our results showed administration of 17β-estradiol, an inhibitor of bone resorption, does not significantly influence any of the parameters in question, which means estrogen offers no advantage in terms of fracture healing in ovariectomized rats.

CONCLUSION

The development of systemic methods for the enhancement of fracture healing is attractive, but the introduction of a systemic agent that targets the fracture healing process requires a high degree of specificity and more extensive investigation. To date, no exogenously administered systemic factors or treatments have been shown to accelerate fracture healing in a reproducible manner. Based on our preclinical study, it is suggested that intermittent parathyroid hormone therapy in the estrogen-deficient osteogenic state benefits fracture healing, and parathyroid hormone is likely to receive increasing study on fracture repair as well as osteoporosis. Further study is needed in large animal models, and attention should be focused on the effects of different doses or duration of the drugs used, and the relationship with local growth factors.

REFERENCES


