

Effects of Risedronate, Alendronate, and Raloksifene on Fracture Healing in Rats

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ABSTRACT

Bisphosphonates are a unique class of drugs that inhibit bone resorption, however recent studies also suggest their stimulatory effect on osteoblast formation. There are still controversies about the effects of bone resorption inhibitors during fracture healing. A prospective longitudinal randomized controlled study was designed in rat tibia to test the effects of various types of bisphosphonates on fracture healing. 48 skeletally mature female Wistar rats with a mean weight of 340 (316-351) g were used. Rats were allocated into four study groups, 12 animals in each group. Right tibial diaphysis was then fractured and fracture was stabilized with long leg cast. No other treatment was given to the control group, other groups received; risedronate 0.2 mg/kg/day, raloxifene 1.0 mg/kg/day, alendronate 0.2 mg/kg/day separately. Treatment began immediately after an experimental tibial fracture. Animals were sacrificed week four of the experiment. Fractured tibia was evaluated radiologically and mechanically and histologically. Radiographic study showed that bony callus was present in all the fractures and bisphosphonates led not to a larger callus as in the other studies. Mechanical testing revealed that ultimate load of alendronate group was significantly higher than other groups. Histologically, process of fracture healing time was shorter with significantly higher osteoblast in alendronate treated group (P <0.05). The result suggest that systemic alendronate treatment induces stronger callus formation in rats.

Key words: Bisphosphonate; Fracture healing; Mechanical testing; Histopathology

Introduction

Antiresorptive and anabolic drugs are two currently available therapeutic options for the treatment of osteoporosis.

Antiresorptive agents inhibit bone resorption and bone formation to stabilize bone structure and increase bone mass [1,2]. Osteoporosis most frequently affects postmenopausal women, placing them at a significant risk for fractures.

Osteoporotic fractures in women consist of vertebral fractures, wrist and forearm fractures, hip fractures, rib fractures, humeral fractures, pelvic fractures, clavicular, and tibial and fibular fractures, scapular, and sternum fractures [3]. Bisphosphonates, and selective estrogen receptor modulators currently, are used widely in the treatment of osteoporosis in postmenopausal women [2,4]. Animal studies using bisphosphonates such as alendronate, zoledronic acid, incandronate and pamidronate generally indicate an increase in callus size and structural biomechanical changes [5-12]. Risedronate improves bone biomechanical properties through alterations of trabecular structure, especially its shape and connectivity and it is effective in the treatment and prevention of postmenopausal osteoporosis in women and corticosteroid-induced osteoporosis in men and women [13-15].

Bisphosphonates have also been used in the treatment of diseases involving extreme osteoclast mediated bone resorption such as Paget's disease, tumor-induced hypercalcemic and metastatic bone diseases [16,17]. Alendronate in mature dogs did not show any adverse effects on fracture healing, mineralization and mechanical properties [18]. Recent studies with another bisphosphonate, incadronate, showed an enlarged callus that was strong, but incadronate delayed callus remodelling in the fractured femora of rats [19,20]. The size of the callus was either not influenced or was increased but never decreased [21-23]. The influence of bisphosphonates on fracture repair may depend on the mode and dosage of the administration [19]. Possible effects of selective estrogen receptor modulators in fracture healing are not well understood. They suppress bone resorption activity in ovariectomized rats secondarily suppress bone formation activity resulting in lower bone remodeling [23]. The purpose of the study was to investigate the effects of risedronate, alendronate, and raloxifene on early stages of fracture healing and mechanical properties of callus.

Material and Methods

Animals

The experimental protocols were approved by the local animal ethical committee. Forty-eight female, 12-week-old Wistar rats with a mean weight of 340 (316-351) g were used. The animals were housed in a cage (floor area 900 cm² and height 20 cm) with free access to tap water and standard laboratory rodent diet (with 1.1 % calcium, 0.8 % phosphorus and 1500IU/kg vitamin D3) in a 12 h/12 h light-dark cycle.

Experimental protocol

The animals were anesthetized with combination intramuscular injections of ketamine HCL (50 mg/kg, Ketalar; Parke-Davis, Morris Plains, NJ) and xylazine (10mg/kg, Rompun; Bayer, Istanbul, Turkey). All animals were subjected

to a standardized right tibial fracture, using a specially designed fracture forceps [24]. The fractures were stabilized by long leg cast. The fractures were left without further immobilization. All rats resumed full weightbearing of the fractured limb within 1 week as confirmed by the absence of a visible limp.

Experimental groups

The animals were randomly allocated into four groups: one control group and three treatment groups with the same body weight, 12 rats per group. Treatment began immediately after an experimental tibial fracture.

Group 1 (control);

Group 2 (Risedronat); 0.2 mg/kg/day,

Group 3 (Raloxifene); 1.0mg/kg/day,

Group 4 (Alendronate); 0.2 mg/kg/day,

The drugs were administered orally on a daily basis with 16 gauge stainless-steel gavage needle. The compounds were prepared, in sterile 0.9% saline. All dosing solutions were stored refrigerated at approximately 50C. Solutions were warmed to room temperature before administration.

Four weeks after fracture, the rats were sacrificed, and tibias cleaned of all soft tissues, while leaving the callus of the right tibia intact.

Radiography

The anteroposterior soft radiographs of all fractured tibias were taken (30 Kvp, 2 mA) with a Siemens X-ray machine (Model number: 4803404, Germany). Callus maturity was evaluated, described by Goldberg [25].

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences version 15.0 (SPSS for Windows 15.0, Inc., Chicago, IL, USA).

Biomechanical Testing

Both the fractured right tibias were tested by the three-point bending method using a mechanical testing machine (Zwick/Roell, 1446, Germany). The tibia was placed, facing its anterior surface down, on the two lower support bars (12 mm apart) with loading bar positioned at the fracture site, or middle tibia. Load was applied until breakage ultimate load was determined by a connected computer. All specimens were consequently loaded to failure point at a static rate of 20 mm/min and force versus displacement data was recorded. Biomechanical data were studied using the One-Way ANOVA and post hoc Bonferroni test. A P value of less than 0.05 was considered statistically significant for mechanical results.

Histopathology

After mechanical testing, fractured tibias were repositioned, the specimens were fixed in 10% formaldehyde, decalcified with 10% formic acid, and embedded in paraffin, stained

with hematoxylin-eosin. 5 micron thick cross-sections were cut. All histological specimens were examined under light microscope by the blinded pathologist. Histological evaluation was performed according to the grading system of the fracture healing. A point value was assigned to each phase of healing in a continuum, such that 10 points would represent the most mature repair and 1 point the most immature [26]. One-Way ANOVA and post hoc Bonferroni test was used to evaluate the histologic results. A P value of less than 0.05 was considered statistically significant for histologic results.

Results

Of the total 48 rats, one was excluded because of infection in raloxifen group. After fracture, the rats resumed normal activity within a week, and drugs did not cause any side effects. Soft X-ray observation showed external callus formation. Fracture line disappeared in all groups. Callus width was same in all groups. In the alendronate group, at 4 weeks histologic observations of callus showed that there was more woven bone than other groups ($P < 0.05$). [The histologic score for risedronate, alendronate, raloxifene, and control groups were 5 (3-6), 7 (6-8), 4 (3-6), 4.5 (3-6) respectively.] In the three point bending test, all the fractured tibias failed along the original fracture line. Ultimate load of fractured tibias in alendronate ($14,16 \pm 1,02$) group was higher than raloxifene ($12,1 \pm 0,93$), risedronate ($12,13 \pm 0,91$) and control ($11,84 \pm 0,88$) groups. The differences in ultimate load were statistically significant ($P < 0.05$). There was no statistically significant difference between the other groups ($P > 0.05$).

Discussion

Fracture healing was always been a main medical problem and it has been the aim of physicians to shorten the healing time and to prevent nonunion. The effort to develop drugs to promote bone formation have not been successful yet. Invention of bone-forming growth factors, such as the transforming growth factors, fibroblast growth factors, bone morphogenetic protein and others gives hope that soon we shall have use of their anabolic properties [27]. Madsen et al. have confirmed that tibial diaphyseal fracture model is sufficient to be used to investigate the effects of bisphosphonates on the processes of fracture repair [28]. The fracture healing process includes various stages such as endochondral ossification, woven bone production, and callus remodelling to lamellar bone, and fracture callus is heterogeneous with respect to the tissue composition especially in the early stage. These situations make histological evaluation of fracture callus very hard [29]. During fracture healing, osteoclasts play an important role in endochondral ossification and remodelling of woven bone to lamellar bone [30-32]. Bisphosphonates inhibit osteoclast activity and their continuous long-term use inhibits osteoclast differentiation [33-35]. The inhibitory effect may be directly on the osteoclast, partly mediated by other cells, especially osteoblast [36]. Currently, Alendronate, estrogen, Risedronate, and Raloxifene are available therapies used to treat postmenopausal osteoporosis [4,37,38]. The present study showed that process of fracture healing progressed not only in the control group, but also in other groups as evidenced by histological observations. Callus formation evaluated by radiographs showed that all fractures healed with external

osseous callus. It confirms that these drugs do not inhibit mineralization of fibrocartilage. Risedronate, alendronate, and raloxifene treatment led not to a larger callus as evidenced by radiographs. In a previous study alendronate treatment increased the size of the callus compared with other groups [39]. In our study size of the callus was not affected by alendronate. In the early process of fracture healing, the bone mineral turnover is high, and the alendronate effect on osteoclast function at this stage could explain the increased callus size observed by other authors [39]. The reason why our study could not confirm these findings might be the relatively short fracture healing period of 4 weeks. The animals used were not osteopenic. Bending tests are often used to determine mechanical properties because they are faster and convenient [40]. It is possible to locate the loading bar at the fracture site to test the part of the bone by using the three-point bending test [24,41]. The three-point bending test was also used in the present study. There was difference in ultimate load between the alendronate group and other groups. Mechanical strength of fractured bone might be unaffected under treatment with alendronate, tiludronate and clodronate but it affected use with etidronate [18,21,28,42]. Taken together, the previously mentioned studies further confirm that whether bisphosphonates interfere with fracture repair and mechanical strength of fractured bone varies based on their chemical structure, dosage, potency and duration. Our mechanical study indicated that the ultimate load of alendronate group was higher than the other treatment groups and control. Continuous treatment with risedronate, alendronate, and raloxifene appears not to delay the fracture healing at any time point after fracture. In contrast, short-term continuous treatment with alendronate shortened the healing time in the early stages of fracture repair processes as evidenced by histopathology. There was less fibrocartilage and more woven bone than the other groups. The recruitment of periosteal cells to the fracture site, differentiation of these cells to chondrocytes and osteoblasts, and the process of mineralization of fibrocartilage were normal under new bisphosphonates treatment (14,35,36). [22,42,43]. The production of the mineralization matrix in callus by endochondral bone formation and in growth plate was increased by clodronate and incadronate treatment [42,44]. A study by Giuliani et al. have showed that bisphosphonates could directly stimulate formation of osteoblast precursors and mineralized nodules in both murine and human marrow cultures in vitro [45]. Raloxifene and risedronate had similar effects on fracture healing and were also similar to control.

In conclusion, alendronate induced the formation of strong fracture calluses in rats and short term continuous treatment shortened the healing time in the early stages of fracture repair processes. Risedronate and raloxifene had no effect on progression of fracture repair. Risedronate, alendronate, and raloxifene may be safe drugs to use in osteoporosis complicated with fractures, since they do not seem to affect negatively the early stages of fracture healing.

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