THE EFFECT OF HALF-YEAR ADMINISTRATION OF CLODRONATE ON THE FRACTURE HEALING IN RAT

*Jämsä, T; *Koivukangas, A (E-Leiras Oy); *Tuukkanen, J; **Kippo, K; **Hannuniemi, R; **Pasanen, I; ***Väänänen, K; +*Jalovaara, P
+*University of Oulu, Oulu, Finland. P.O.Box 5000, 90014 University of Oulu, Finland, +358-8-3152011, Fax: +358-8-3155318, pekka.jalovaara@oulu.fi

Introduction. Bisphosphonates inhibit bone resorption by inactivating osteoclasts, and they are widely used in the treatment of bone disorders associated with increased bone resorption. However, the skeletal half-life of bisphosphonates is long. It is therefore important to know what effects they have on healing fractures when administered in the long term. Also clinicians have been concerned if fractures heal in individuals exposed to long-term bisphosphate treatment.

The aim of the present study was to investigate how half-year treatment with clodronate affects fracture healing of the long bones in the growing rat.

Methods. A total of 199 three-month-old female rats were used. The animals received three different treatments: 1) physiological saline, 2) 2 mg/kg disodium clodronate or 3) 10 mg/kg disodium clodronate. All doses were given subcutaneously twice a week. After 24 weeks of clodronate administration, the rats were anaesthetized and the prenailed right tibiofibular bone was manually fractured. The clodronate and vehicle treatments were continued until the animals were sacrificed at 4 or 8 weeks after the fracture.

The visibility of the fracture line was observed from radiographs (Fig. 1) and scored as totally visible, partly visible, or absent. The mean bone mineral density (BMD) and the cross-sectional bone area (CSA) of the callus were scanned with a peripheral quantitative computer tomography (pQCT) system, Stratec XCT 960A (software version 5.20, Norland Stratec Medizintechnik GmbH, Birkenfeld, Germany) (1). The mechanical strength of the callus was evaluated by axial tension test in the 4-week healing group, and by compression test in the 8-week group (1). The undecalcified histological sections were stained with the Masson-Goldner and toluidine blue methods and analyzed using a digital image analysis system (MCID/M4 with software version 3.0 rev. 1.1, Imaging Research Inc., Canada). The area of callus, excluding the intramedullary space, the area of cartilaginous tissue in callus, the area of fibrotic tissue in callus, and the area of mineralized callus were measured. The proportional areas of cartilaginous, fibrotic and mineralized callus out of total callus were measured.

The data were analyzed using two-way ANOVA to identify differences between the treatments and the time-treatment interactions. The fracture lines in x-ray analysis and histological evaluation were analyzed using Fisher’s exact test. The biomechanical data were analyzed with one-way ANOVA.

Results. The body weight of the animals increased continuously over the study period, with no significant differences between the treatments. The tibial fractures in clodronate-treated rats healed equally well as those in vehicle-treated rats. There were no significant differences in radiological healing at 4 or 8 weeks as indicated by the visibility of the fracture line. The cross-sectional area of the callus, measured by pQCT, was greater in the fractures of clodronate-treated rats (p<0.001), with no difference between the dose levels, but there was no significant difference between treatments in bone mineral density. The percentage area of mineralized, fibrotic and cartilaginous tissues in the tibial callus did not differ between the treatments, and there was no significant difference in the periosteal mineral apposition rate between the treatments at 4 or 8 weeks. No significant difference between the treatments was found in the mechanical failure load of the callus at 4 or 8 weeks.

Discussion. This was a unique experiment, where the fracture healing process was investigated in growing rats given long-term clodronate treatment before experimental fracturing, and no similar studies have been made earlier with either clodronate or any other bisphosphonate. Therefore, the comparison with previous studies is not possible.

Long-term clodronate treatment does not seem to prolong the fracture healing process significantly, although it has some effects on callus size. Our results suggest that the healing of fractures during or after clodronate treatment is not inhibited. It is therefore evident that the clinicians’ concern for the effects of clodronate on fracture healing is not justified, i.e. clodronate can safely be administrated to patients with increased fracture risk.

Fig. 1. Radiograph of a tibia 8 weeks after the fracture showing a solid union and hypertrophic callus.

References.

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**Leiras Oy, Turku, Finland.
***University of Turku, Turku, Finland.