No adverse effects of clodronate on fracture healing in rats

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Clodronate was administered daily 28 days before and after an experimental tibial fracture in 35 male rats, and the effect on fracture healing and post-traumatic bone loss was studied. 5 groups were tested. The clodronate/clodronate group received clodronate in daily doses of 10 mg/ kg body weight for 28 days before being subjected to a standardized fracture of the right tibia, and during the fracture healing period of 28 days. The clodronate/saline group received clodronate before fracture and saline during the healing period. The saline/clodronate group received saline before and clodronate after fracture. The saline/saline group received saline only, while the control group served as unfractured, untreated controls. After 28 days of fracture healing, the tibias were evaluated with dual energy x-ray absorptiometry, and tested mechanically in a 3-point ventral bending test. Bone mineral content and bone mineral density were approximately 30% higher in the groups receiving clodronate during the experiment, compared to the untreated groups. The weight and cross-sectional area of the fracture callus were equal in all groups. Whether clodronate was administered before the fracture, after the fracture or both, did not affect the bone mineral. Ultimate bending moment, energy absorption, stiffness and deflection were not significantly different between the groups. Our findings suggest that clodronate increases bone mineral both when given before and after a tibial shaft fracture, without affecting fracture healing at 28 days.

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Bisphosphonates have been used in the treatment of disorders associated with increased bone resorption, such as Paget's disease and hypercalcemia of malignancy. Several bisphosphonates have also been evaluated in the treatment of postmenopausal osteoporosis, and may increase bone density by 5–10% over 1 year (Ott 1993), and decrease the rate of vertebral compression fractures in postmenopausal osteoporotic women (Storm et al. 1990, Watts et al. 1990, Liberman et al. 1995). Bisphosphonates also prevent loss of cancellous bone from the spine and tibia of estrogen-deficient rats (Wronski et al. 1991), but in some studies in dogs the bisphosphonate etidronate has been associated with an increased incidence of fracture and delayed fracture healing (Flora et al. 1981, Lenehan et al. 1985). The need for further investigations of individual bisphosphonates on the mechanical properties of bone has been emphasized, as the profile of mechanisms is probably different for the various bisphosphonates (Parfitt 1991).

Clodronate does not seem to impair the mineralization of bone in therapeutic doses, and may thus be suitable for long-term use in osteoporosis (Kanis et al. 1993). However, the treatment may affect fracture healing, as clodronate has great effects on bone remodeling (Fleisch 1993). Studies on the effects of clodronate treatment on fracture healing are few and provide conflicting results. An increase in the calcium content in fracture callus during fracture healing in clodronate-treated rats has been shown (Nyman et al. 1993) and the amount of callus may increase during clodronate treatment after fracture (Nyman et al. 1993, Tarvainen et al. 1994). The mechanical strength of the healing fractures may be unaffected when clodronate is administered after fracture induction (Nyman et al. 1993, Hyvonen et al. 1994), but it has also been impaired in nonosteoporotic rats, using therapeutic doses (Tarvainen et al. 1994).

The effect of ongoing bisphosphonate treatment on fracture healing has not previously been investigated, and in this study we assessed the effect of clodronate on tibial fracture healing and bone mineral in rats treated with clodronate 4 weeks before the fracture.

Animals and methods

35 male, 12-week-old Wistar rats with a mean weight of 346 (313–381) g were used. The animals were kept 3 per wire-top plastic cage, with free access to tap water and standard laboratory rodent chow (with 1.1% calcium, 0.8% phosphorus and 1500 IU/kg vitamin D_3) in a 12-hours light/12-hours dark cycle. The experiment conformed to the Norwegian Council of Animal Research Code for the Care and Use of Animals for Experimental Purposes.

Tibial fracture

All animals, except 5, were subjected to a standardized right tibial fracture, using a specially designed fracture forceps (Ekeland et al. 1981). The animals were anesthetized with a combination of fluanisone, fentanyl citrate and midazolam subcutaneously. The fractures were stabilized by intramedullary nailing, using the technique of Nordsletten et al. (1994b). Rotation was checked by comparing the alignment of the foot and thigh. All fractures were stable at the end of the operation. The animals were given one intraperitoneal injection of 75 mg streptomycin preoperatively. The fractures were left without further immobilization. All rats resumed full weightbearing of the fractured limb within 4 days, as assessed by the absence of a visible limp. No signs of infection were detected in any animal.

Clodronate administration

All animals, except the 5 unfractured, received daily injections with either clodronate or saline for 28 days before the fracture. The rats had been allocated to 5 groups: 1) clodronate/clodronate (cl/cl), 2) clodronate/saline (cl/NaCl), 3) saline/clodronate (NaCl/cl), 4. saline/saline (NaCl/NaCl), and 5) unfractured (control).

The cl/cl group (n 7) was injected s.c. with clodronate (Bonefos® "Leiras", Åbo, Finland) in daily doses of 10 mg/kg body weight for 28 days before fracture of the right tibia, and during the whole fracture-healing period of 28 days. The cl/NaCl group (n 7) received clodronate before fracture and an equal amount (0.7 mL) of saline during the healing period. The NaCl/cl group (n 8) was injected with saline before fracture and with clodronate during fracture healing. The NaCl/NaCl group (n 8) received saline only during the whole experimental period, while the control group (n 5) received no drugs.

Evaluation

On day 56 (28 days after fracture), the rats were killed, and the tibias cleaned of all soft tissues, while leaving the callus of the right tibia intact. Alignment of the fractures was evaluated macroscopically, and the intramedullary nails were carefully removed. The bones were weighed to the nearest 0.1 mg (wet weight), and the callus weight was calculated as the weight difference between the fractured and unfractured sides in each animal. The bones were then frozen wet at –20 °C before further testing was carried out. During all later handling, the tibias were kept moist with Ringer-acetate® solution.

Dual energy x-ray absorptiometry (DXA).

The bone mineral content (BMC-g), bone mineral density (BMD-g/cm^2) and the anteroposterior projected area of the tibia (mm^2) were assessed by DXA on a Lunar DPX-l (Lunar, Wisconsin, USA), applying the forearm software in the detail mode (Nordsletten et al. 1994a). The fractured tibia from each rat was scanned once, while immersed in water. The coefficient of variation for BMC was calculated to be 2.5% for duplicate measurements of 30 rat bones.

Mechanical testing

The tibias were loaded until fracture in 3-point ventral cantilever bending, using a loading rate of 0.095 radians/s (5.43°/s), with the fulcrum placed over the callus (Nordsletten and Ekeland 1993). Load/deflection curves were recorded on-line in WorkBenchMac software (Strawberry Tree Incorporated, Sunnyvale, USA), and ultimate bending moment, energy absorption, stiffness and deflection at failure were read out directly or calculated from the computer readings. The term strength in relation to the results was defined according to Burstein et al. (1971), as ultimate bending moment and energy-absorbing capacity. Before fracturing of the operated tibias, the anteroposterior and mediolateral diameters of the callus were measured with a sliding calipers (accuracy of ± 0.05 mm), and the cross-sectional area of the callus was calculated.

Statistics

Results are presented as median (range) and groups were compared using nonparametric testing (Kruskal-Wallis test). The Fisher PLSD test was used for post hoc testing. Level of significance was set at $p < 0.05$.

Results

There were no malaligned fractures. Weight gain during the trial was between 35 g and 54 g in the 5 groups (not significant differences). The callus weight was

Table 1. Number of animals (n), weight gain in each group, callus weight, bone mineral contents (BMC), tibial area, and bone mineral density (BMD) after 28 days of fracture healing in rats injected with clodronate (cl) and/or saline (NaCl) 28 days before and after an experimental tibial fracture, values are median (range)

Group	n	Weight gain, g	Callus weight mg	BMC g	Area cm^2	BMD g/cm^2
cl/cl	7	35 (8–52)	0.14 (0.10–0.37)	0.30 (0.27–0.33) [a]	1.71 (1.62–1.88)	0.17 (0.15–0.20) [b]
cl/NaCl	7	43 (23–51)	0.08 (0.01–0.72)	0.28 (0.27–0.35) [a]	1.75 (1.58–1.93)	0.16 (0.15–0.19) [b]
NaCl/cl	8	54 (28–67)	0.18 (0.05–0.73)	0.29 (0.26–0.32) [a]	1.74 (1.64–1.93)	0.16 (0.15–0.19) [b]
NaCl/NaCl	8	42 (25–75)	0.12 (0–0.50)	0.23 (0.18–0.27)	1.74 (1.32–2.02)	0.13 (0.12–0.15)
Unfractured	5	45 (21–56)		0.22 (0.21–0.24)	1.61 (1.54–1.65)	0.14 (0.13–0.15)

[a] p 0.0003, [b]p 0.0002 versus NaCl/NaCl and unfractured (Kruskal–Wallis test and Fisher PLSD)

Table 2. Transverse callus area and mechanical properties of the tibial shaft 28 days after a tibial fracture in rats receiving clodronate (cl) or saline (NaCl) 28 days before and after the fracture, values are median (range)

Group	n	Transverse callus area mm^2	Bending moment $Nm/° \times 10^{-2}$	Energy absorption $J \times 10^{-2}$	Deflection, °	Stiffness $(Nm \times 10^{-2})$
cl/cl	7	17 (14–25)	3.6 (0–9.9)	0.16 (0–0.75)	0.88 (0–2.63)	0.21 (0–0.54)
cl/NaCl	7	16 (10–24)	6.1 (0–18)	0.19 (0–2.63)	0.94 (0.79–3.58)	0.27 (0–0.57)
NaCl/cl	8	17 (4.1–25)	2.4 (0–14)	0.05 (0–0.32)	0.39 (0–1.97)	0.06 (0–0.95)
NaCl/NaCl	8	15 (5.2–36)	3.6 (0–13)	0.13 (0–0.59)	1.02 (0–5.60)	0.05 (0–0.63)

not significantly different between the groups (Table 1).

The DXA-measured BMC and BMD of the fractured tibias were higher in the groups receiving clodronate than in the NaCl/NaCl group and the unfractured group ($p < 0.05$). The total area of the tibias, as measured with DXA, was not different between the fractured groups, but approximately 8% lower in the unfractured group, reflecting the callus size of the fractured bones (Table 1). The calculated transverse area of the callus was equal between the groups, and there were no statistically significant differences in the mechanical results between the groups with a tibial fracture (Table 2).

Discussion

Clodronate treatment led to a higher BMC and BMD of the fractured tibias 4 weeks after a tibial fracture, compared to the untreated groups. However, we found no significant differences in mechanical strength of the healing tibias, and the callus size was not affected by the clodronate treatment. Whether clodronate was administered 28 days before and/or after the fracture did not influence the results.

High concentrations of clodronate in bone have been measured from 5 minutes and up to 12 months after a single intravenous injection (Mönkkönen 1988), even though the serum half-life of clodronate is short. In a dose-response study, Hyvonen et al. (1994) showed that doses of 3, 10 or 30 mg/kg administered after the fracture did not alter the fracture callus or delay the fracture healing histologically. Doses as high as 50 mg/kg have been shown to increase callus weight and calcium content 2 months after fracture, but the tensile load capacity of the fractured tibias was unaffected (Nyman et al. 1993). In our experiment the drug was given subcutaneously, and the bioavailability of clodronate after subcutaneous administration is compatible with the intravenous route (Mönkkönen 1988). We chose a clodronate dose of 10 mg/kg body weight, corresponding to the doses used in other animal studies (Hyvonen et al. 1994, Lepola et al. 1994, Tarvainen et al. 1994), and relevant to short-term treatment of human hypercalcemia. However, it is higher than the recommended dose for oral treatment of osteoporosis, where a maximum dose of 3200 mg/kg/d corresponds to a subcutaneous dose of 0.5–2 mg/kg/d, as the availability of clodronate after oral administration is 1–4%.

The effects of clodronate on BMC and BMD were substantial in our series, although the animals used were not osteopenic. Both BMC and BMD were approximately 30% higher in the clodronate-treated groups only 4 weeks after the fracture, and whether clodronate was given before or after the fracture obviously had no influence on the bone mineral increase. The increase in BMC and BMD is larger than would be expected in treatment of human metabolic bone

disorders, and may be caused by the very high cell and bone turnover in the fracture area during fracture healing, and the high affinity of clodronate for bone tissue, especially areas with increased bone turnover. The high affinity of clodronate for bone tissue leads to a rapid saturation of clodronate in the bone, and consequently, a slowing down of bone metabolism may be relatively independent of further drug dosages (Mönkkönen 1988). Few previous studies deal with the effects of clodronate on bone mineral contents and density during fracture healing in nonosteopenic animals. However, fast and large increases in bone mineral are known to occur in rats during treatment with other bisphosphonates, as Rosen et al. (1994) found a 32% increase in BMD in the distal femur after only 21 days of pamidronate treatment.

The fracture callus size and weight were not influenced by the clodronate treatment in our experiment, as both callus weight and cross-sectional area were equal between the clodronate groups and the saline-treated group. The fracture healing and bone remodeling processes involve the resorption of bone by osteoclasts, and subsequently the laying down of new bone by osteoblasts (Frost 1989). Furthermore, the resorption of mineralized bone matrix by osteoclasts is preceded by osteoblast collagenase activity (Vaes 1988), and osteoblast and osteoclast activities are thus intimately related. In the early process of fracture healing, the bone mineral turnover is high, and the clodronate effect on osteoclast function at this stage could explain the increased callus size observed by other authors (Nyman et al. 1993, Tarvainen et al. 1994). The reason why our study could not confirm these findings might be the relatively short fracture healing period of 4 weeks, compared to 8–12 weeks in the studies by Nyman and Tarvainen. On the other hand, the fracture healing is a closely regulated process, relatively insensitive to interference by exogenous factors. Hyvonen (1994) found no alterations in the fracture callus histology nor delayed fracture healing in his 22-week experiment, and in Nyman et al.'s (1993) study, all fracture calluses eventually remodeled to lamellar bone, despite the clodronate treatment.

The mechanical strength of the healing bone was not affected by clodronate in our study. Again, there might be a time-related cause for this, as our healing time is shorter than in other studies relating to this matter. Tarvainen et al. (1994) found that the callus strength of osteopenic rats was not influenced by clodronate, but in the normal, nonosteopenic rats a reduced callus strength was found 8 weeks after fracture. This may indicate a different influence of clodronate on the fracture remodeling in normal and osteopenic animals, but our results could not confirm the delay in fracture healing in normal rats. Our results are in line with those of other authors, not being able to detect any effect of clodronate on fracture healing in normal rats (Nyman et al. 1993). A limitation in our study is the relatively small number of animals per treatment group and the wide range of the mechanical data. This limits the ability of the experiment to detect significant differences among the groups.

Our findings have clinical implications, even though comparisons with the clinical situation should be made with care. Clodronate may be a safe drug to use in osteoporosis complicated with fractures, since it does not seem to affect negatively the early stages of fracture healing. Further, loosening of bone screws and implants is a serious problem in fracture fixation in osteoporotic patients, and the holding power of bone screws is closely related to bone mineral (Koranyi et al. 1970). Clodronate's ability to maintain bone mass may be important in preventing the normally occurring posttraumatic bone loss after fracture (Madsen et al. 1995). Thus, by starting clodronate administration immediately after a fracture, it may be possible to maintain an adequate level of bone mineral in osteoporotic patients, and minimize the risk of failure of the osteosynthesis in operated osteoporotic fractures.

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