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Evaluation of the effect of tamoxifen citrate on model of osteoporosis in dog: biomechanical and histopathological studies

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Summary

The effect of tamoxifen citrate on bone mass in immobilization osteoporosis was studied in 10 dogs. Osteoporosis was induced by fiberglass cast immobilization of the right hind-limb for 28 days, while the left hind-limb served as a non-immobilized control. Five dogs received tamoxifen citrate (1.5 mg/kg per os) once daily for 28 days; five dogs received no treatment. All dogs were euthanized on day 28 and tibiae were harvested. Bone biomechanical properties and microscopic structures of tibiae from casted and uncasted limbs were studied. Significant differences in the percent of decreased values of examined mechanical properties were found between untreated and tamoxifen-treated dogs. No remarkable histopathological changes indicative of osteoporosis were detected in the tibiae of casted limb of tamoxifen-treated dogs. These findings indicated that short term tamoxifen therapy may have promising effects on prevention of osteoporosis in dog.

Key words: Antiestrogen, Tamoxifen, Osteoporosis, Biomechanics, Dog

Introduction

Diminished bone mass, osteoporosis, is a well-recognized consequence of immobilization. Immobilization (disuse) osteoporosis may result from prolonged cast or splint fixation, stress protection secondary to plate fixation of fractures, incapacitation due to chronic illness or spinal cord injury, or weightlessness associated with orbital space flight (Jimenez *et al*., 1997; Hajela *et al*., 2001). Immobilization osteoporosis is the result of an imbalance between bone resorption and bone formation (Weinreb *et al*., 1989; Black *et al*., 1994).

Tamoxifen citrate is a non-steroidal antiestrogen compound used in the treatment of human breast cancer and is under intense investigation for its potential as a chemopreventive agent in women at risk for breast cancer (Jordan, 1988; Turner *et al*., 1988). As an antiestrogen, its potential for an adverse effect on bone metabolism (e. g., osteoporosis) have generated concern (Vogel *et al*., 2002; Fontana and Delmas, 2003). However, results of retrospective studies of tamoxifen-treated women with breast cancer have failed to demonstrate a deleterious effect on bone mass (Hershman *et al*., 2002; Fontana and Delmas, 2003). In fact, experimental evidence suggests that tamoxifen and related compounds may have a bone mass sparing effect in rats after ovariectomy (Black *et al*., 1994; Frolik *et al*., 1996; Visentin *et al*., 2000) or immobilization (Wakley *et al*., 1988). Since tamoxifen metabolism in dogs less closely resembles tamoxifen metabolism in rats (Waters *et al*., 1991), the present study was conducted to investigate the potential bone mass sparing effect of tamoxifen citrate in canine immobilization osteoporosis.

Materials and Methods

Ten adult apparently healthy mongrel dogs, mean weight 20 kg, were studied. The dogs were housed in individual cages and had access to water and food ad libitum. Right hind-limb osteoporosis was induced

by immobilization in a fiberglass cast from mid-femur to digits for 28 days, while the left hind-limb served as a non-immobilized control. Evaluation prior to cast fixation included physical examination, anterior-posterior and lateral radiographs of both hind-limbs from the stifle to the digits, complete blood count, and serum biochemical profile. The dogs were matched for body weight and then randomly assigned to one of two groups: tamoxifen-treated (n = 5), and untreated (n = 5). Tamoxifen-treated dogs received tamoxifen citrate (10 mg tablet, Iran Hormone Co., Tehran, Iran) at a dose of 1.5 mg/kg of body weight per os every 24 hrs for the duration of the experiment. The dogs were examined daily. All cast applications were performed under light anaesthesia with mixture of acetylpromazine (Hoogsrraten, Belgium) (0.1 mg/kg, intravenously) and ketamine HCl (Alfasan, Woerden, Holland) (5 mg/kg, intravenously). Complete blood counts and serum biochemical profiles were repeated on day 28. All dogs were euthanized with an overdose of sodium thiopental solution on day 28. Immediately after euthanasia, both tibiae were removed, cleaned of soft tissue, wrapped in saline-soaked tampon and subjected to mechanical testing. The mechanical properties were measured by a manual custom-made three-point bending machine (designed by Rezazadeh *et al*., College of Engineering, University of Urmia) using 500 gr load cells and displacement accuracy of 0.01 mm. The Young's modulus of elasticity, ultimate strength, failure load, and maximum bending moment were determined. Immediately after bone failure, samples were cut and placed in 10% neutral buffered formalin for subsequent histopathological studies.

Data derived from mechanical testing were expressed as the mean (±SD) for each group. Differences in fractional changes in mechanical properties between treated and untreated dogs were analysed with an unpaired Student's t-test. Differences were considered significant if $p<0.05$.

Results

All dogs remained healthy throughout the study. Tamoxifen treatment was well tolerated. Mean total leukocyte count, haematocrit, and serum biochemical profile data, including serum calcium, phosphorus, and alkaline phosphatase in the dogs of the studied groups remained within the normal ranges throughout the experimental period. Cast sores were not observed.

Table 1: Statistical comparison of biomechanical parameters of tibia in untreated and tamoxifen-treated groups

		Untreated	Tamoxifen-treated
Young's modulus of elasticity (MPa)	Uncasted limb	758.2 ±53.58	818.42 ±54.36
	Casted limb	464.6 ±39.27	732.68 ±52.17
	%Decrease	38.72 ±9.5	10.47 ±11.7*
Ultimate strength (MPa)	Uncasted limb	198.24 ±35.21	208.87 ±39.65
	Casted limb	70.12 ±9.36	186.52 ±40.26
	%Decrease	64.62 ±9.5	10.7 ±4.5*
Failure load (N)	Uncasted limb	709.4 ±78.56	652.7 ±71.54
	Casted limb	250.3 ±54.62	582.12 ±48.67
	%Decrease	64.71 ±9.5	10.81 ±9.3*
Maximum bending moment ($N.m/m^2$)	Uncasted limb	301.17 ±58.54	308.21 ±69.68
	Casted limb	107.24 ±51.91	232.22 ±47.78
	%Decrease	64.39 ±7.8	24.65 ±5.9*

*Differ from value for untreated dogs ($P<0.05$)

Mechanical properties measured for the two groups are shown in Table 1. Significant differences in the examined mechanical properties were found between percent of decreased values of untreated and tamoxifen-treated dogs. Statistically, the percent of reduction in biochemical values of tamoxifen-treated group was significantly less than untreated one, which revealed that bone resorption was significantly decreased in the former group.

The effects of limb casting on bone architecture of right tibiae in untreated dogs were loss of cortical bone thickness and trabecular bone volume, which resulted in increasing cortical porosity. Increased quantity of osteoid and osteoid surfaces, normal osteoid seam width, increased

resorption surfaces, and peritrabecular fibrosis and increased osteoclast number were obviously observed. While in the left hind-limb cortical bone was thick, and normal components of compact bone with healthy periosteum and endosteum were distinguished. In tamoxifen-treated group normal microscopic morphology of the bone was observed in both hind-limbs. Normal structure of cortical and trabecular bone, periosteum and endosteum were preserved in immobilized limbs as well as the uncasted ones, and there was no evidence of osteoporosis in the tissue samples.

Discussion

Immobilization causes net bone loss as a result of an imbalance between bone resorption and bone formation (Weinreb *et al*., 1989; Fisher *et al*., 1998; Hajela *et al*., 2001). Increased bone resorption is evidenced by increased numbers of osteoclasts and percent resorption surface (Thompson and Rodan, 1988; Wakley *et al*., 1988). Although an increased sensitivity to normal concentrations of circulating factors (e. g., parathyroid hormone) has been proposed (Turner and Bell, 1986), this concept has been challenged (Resch *et al*., 1998; Hajela *et al*., 2001; Delmas, 2002), and the underlying stimulus for enhanced bone resorption remains unknown. Several factors have been implicated in the decreased bone formation associated with immobilization. Weight-bearing may stimulate osteoblasts directly (Pead *et al*., 1988), whereas mechanical unloading may eliminate osteoblast stimulation (Somjen *et al*., 1980; Delmas, 2002) or impair osteoblast function or recruitment (Uhthoff and Jaworski, 1978; Avioli, 1999). Alternatively, hormonal changes and alterations in blood flow have been incriminated (Schoutens *et al*., 1988; Delmas, 2002).

Immobilization osteoporosis has been studied experimentally after unilateral motor denervation (Turner and Bell, 1986; Wakley *et al*., 1988), patellar or calcanean tenotomy (Thompson and Rodan, 1988), and cast fixation (Uhthoff and Jaworski, 1978; Uhthoff *et al*., 1985; Waters *et al*., 1991). Long-term (16 weeks) unilateral fore-limb cast immobilization resulted in a 45% decrease in bone mass in the distal radial metaphysis of adult dogs (Uhthoff *et al*., 1985). In the present study, 28-day unilateral hind-limb cast immobilization in untreated dogs resulted in great decrease in bone mechanical parameters. Thus, profound osteopenia may be achieved in dogs after a relatively brief period of immobilization, making this a useful model in the investigation of therapeutic strategies to prevent immobilization-induced bone loss. In this study, treatment with tamoxifen citrate provided a partial bone mass sparing effect in immobilized limbs. The bone mass sparing effect of tamoxifen in dogs with immobilization osteoporosis in this study is consistent with the results of a previous investigation using a sciatic neurectomy model in male rats (Wakley *et al*., 1988). In that study, tamoxifen treatment resulted in a 60% sparing effect on the trabecular bone loss associated with immobilization. Osteoclast number in immobilized trabecular bone of untreated rats increased 65% over sham-operated controls; tamoxifen-treated rats showed no increase in osteoclast number. These results, and results of bone culture studies (Roodman *et al*., 1985; Resch *et al*., 1998; Hershman *et al*., 2002), suggest that tamoxifen exerts its bone mass sparing effect by inhibition of osteoclast-mediated bone resorption.

In this study, the evidences of increased bone resorption and accelerated turn over in the immobilized tibiae of the control dogs, reflecting the histological criteria of active osteoporosis (Kissane, 1990). Nevertheless, the histopathological findings of both hind-limbs of the tamoxifen-treated dogs revealed identical normal microscopic bone morphology, which indicated the bone mass sparing effect of tamoxifen on prevention of immobilization osteoporosis.

Turner *et al*., (1988) suggested that tamoxifen may have an effect on the bone microscopic structure by decreases in both the number and activity of osteoclasts, and net loss of trabecular bone. Although the precise mechanism for antiestrogen effects on bone is unknown, histomorphometric data from Turner *et al*., and Moon *et al*.,

showed that tamoxifen and estrogen have similar actions in the diaphysis and metaphysis of the tibia, suggesting an agonist or partial agonist activity for tamoxifen on bone (Black *et al*., 1994).

The beneficial effect of tamoxifen in an ovarian hormone-independent osteopenia such as immobilization is not surprising because the biological effects of tamoxifen are diverse and not limited to estrogen receptor-mediated events (Norval *et al*., 1988; Jordan, 1998; Marttunen *et al*., 1998; Resch *et al*., 1998; Hershman *et al*., 2002). Tamoxifen has been shown to inhibit prostaglandin synthesis in vitro (Vogel *et al*., 2002), and it was previously reported that prostaglandin E, a potent bone resorbing agent, increases in bone after immobilization (Waters *et al*., 1991). Tamoxifen is able to modulate protein kinase C, an enzyme involved in signal transduction processes that control cell growth and division (O'Brian *et al*., 1985; Tritton and Hickman, 1990; Hershman *et al*., 2002). Tamoxifen has also been shown to regulate the expression of genes that control polyamine biosynthesis (Thomas *et al*., 1989), and polyamine inhibitors have been shown to inhibit parathyroid hormone-mediated bone resorption (Lucas *et al*., 1989). Induction of transforming growth factor-beta by tamoxifen has also been reported (Knabbe *et al*., 1987). Transforming growth factor-beta is believed to play an important role in intercellular communication within bone (Martin and Suda, 1989).

In conclusion, short-term consumption of tamoxifen citrate in dog attenuated the decrease in bone mass induced by disuse. The results of this study may be of benefits in dealing with bone defects and complicated fractures (e. g., delayed union). However, extrapolation of these data to dogs with fracture-associated disuse osteoporosis must await further studies on the effects of tamoxifen citrate on fracture healing.

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