

Evaluation of certain antiresorptive drugs in growing dogs affected with osteopenic bone diseases (rickets, NSH)

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ABSTRACT

Growing dogs (92) affected with osteopenic bone diseases like nutritional secondary hyperparathyroidism and rickets, were subjected to treatment with different antiresorptive drugs. In all the animals, calcium (250 mg/kg)-vitamin D₃ (3 lakh iu/wk) and zinc (5 mg/wk) were administered. In group 1 animals, no other treatment was given. In groups 2–5, in addition to Ca-vit.D-Zn therapy, Nandrolone deconoate (12.5 mg/wk im), Raloxifene (15 mg/kg PO), Alendronate (5 mg/day PO) and TGF β (20 ng/wk im), respectively, were administered. The owners were advised to continue the treatment till satisfactory improvement was seen for at least 1 month duration. All the animals, which came for follow up examination and treatment, were observed for different clinical and biochemical observations at weekly intervals and radiographic observations at every 15th day. In group 1, in general the response was seen after 2nd/3rd week of initiation of therapy. In majority of cases, good response was seen after 6–8 weeks of treatment. Animals of groups 3 and 4 showed relatively early signs of improvement as compared to group 1. In animals treated with anabolic steroid (group 2) and TGF β (group 5), the clinical response was quicker than all other groups. Our results indicated that treatment with different antiresorptive drugs had positive response in growing dogs affected with osteopenic bone diseases. Nevertheless, anabolic steroid, probably was the most effective drug, and Raloxifene was the least effective in growing animals with osteopenia.

Key words: Antiresorptive drugs, Bone disease, Growing dogs, Nutritional secondary hyperparathyroidism, Osteopenia, Rickets

The incidence of osteopenia and growth abnormalities in young dogs have increased and is reported to be as high as 30% (Kushwaha 2003, Kumar 2007). The commonly reported skeletal diseases in growing dogs are rickets, nutritional secondary hyperparathyroidism and hypertrophic osteodystrophy (Kushwaha 2003).

Osteopenic bone conditions are commonly managed with balanced Ca, P preparations along with vit. D supplementation. Antiresorptive drugs like Raloxifene and Alendronate that are used in humans for treatment of post-menopausal osteoporosis have not been evaluated in animals. Nandrolone, an anabolic steroid, is also being used for treatment of established osteoporosis in human patients, however, very little is known about how anabolic steroids affect on bone metabolism in animals. Calcitonin, a thyroid hormone, increased the bone mass by transiently inhibiting osteoclast activity (Stephan *et al.* 2003), and was used to

treat disease conditions like osteoporosis and rickets (Ladelnet 1999). Transforming growth factor (TGF), a dimeric protein of 25kDa molecular weight (Roberts and Sporn 1993), regulated normal cell growth and development, and participated in cartilage growth metabolism, particularly in the control of chondrocytes differentiation and hypertrophy (Loveridge *et al.* 1993). Keeping these facts in view, in the present study different classes of antiresorptive drugs were evaluated for the management of osteopenic bone conditions in growing animals.

MATERIALS AND METHODS

The study was conducted on dogs affected with osteopenic bone diseases (rickets and NSH) reported for treatment at Polyclinics, IVRI, from September 2004 to April 2005. After recording detailed history, clinical and radiographic signs, osteopenia condition was diagnosed (as rickets or NSH). Ninety-two such cases of osteopenic bone diseases were subjected to treatment with a particular antiresorptive drug. Care was taken to avoid variation among different treatment groups with respect to disease condition, age and sex of the animal. The owner's willingness, economic status of the owner and the availability of the drug were, however,

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considered before selecting a particular drug. In all the animals, calcium (250 mg/kg)-vitamin D₃ (3 lakh IU/wk) and zinc (5 mg/wk) were administered orally. In group 1 animals (n=40), no other treatment was given. In group 2 (n=17), 3 (n=12), 4 (n=13) and 5 (n=6), in addition to Ca-vit D-Zn therapy, Nandrolone deconoate (12.5 mg/wk im), Raloxifene (15 mg/day PO), Alendronate (5 mg/day PO) and TGF β (20 ng/wk im), respectively, were administered. The animal owners were advised to continue the treatment till satisfactory improvement was seen or for at least 1 month duration.

Clinically all the animals were observed for lameness, bowing/bending of bones/limbs, broadening of metaphysis, pain on palpation and posture of the animal, and were graded on a scale of 0 to 3 (0– no, 1– slight, 2–moderate and 3– severe). These parameters were evaluated in all the animals at the time of reporting and in the animals reported for follow up examination on days 7, 15, 30, 45 and 60. In a few cases, the response was obtained from the animal owners through telephonic conversation. The response of the animal was graded as slight, moderate, good and very good based on overall improvement in different clinical parameters.

Radiographs of the most affected limb(s) of diseased animals were made in medio-lateral and antero-posterior planes. These radiographs were compared with normal radiographs of the same breed of dogs at the same age group for different bony changes. Radiographic evaluation was done at 15 days' interval during the treatment period to evaluate the efficacy of treatment.

Venous blood (5 ml) was collected before the start of treatment and at weekly intervals during the treatment period. Plasma was separated and used for estimation of calcium, inorganic phosphorus, total protein and alkaline phosphatase using standard methods. The data obtained for different biochemical parameters were statistically analysed using paired 't' test.

The efficacy of the treatment was evaluated on the basis of improvement in clinical, radiographic and biochemical changes at different intervals of treatment.

RESULTS AND DISCUSSION

Out of 92 cases with osteopenic bone conditions reported during the period, 35 cases were diagnosed as rickets and 57 cases as NSH based on clinical and radiographic observations. Several researchers have also recorded high incidence of NSH and rickets in growing dogs (Capen and Rosol 1993, Kushwaha 2003), as also observed in the present study.

Clinical observations

In group 1, cases (40) of skeletal diseases were treated by administration of calcium, vitamin D₃ and zinc. These cases mainly included rickets (12) and NSH (28). Only 17 cases

were available for clinical evaluation. In general, in group 1, the response was seen after 2nd/3rd week of initiation of therapy. In most of the cases, good response was seen after 6 to 8 weeks of treatment. This suggests that the administration of calcium and vit-D₃ has hastened the recovery of dogs affected with bone diseases. In most of the cases plasma calcium levels indicated hypocalcemia, suggesting probable calcium deficiency in the diet. Further, phosphorus levels in the plasma were increased leading to imbalance in the Ca-P ratio. Administration of Ca+vit D₃+Zn might have helped in early recovery of cases affected with bone diseases, and hence, it is suggested that calcium-vitamin D₃ supplementation should be advocated for growing dogs affected with bone diseases.

Majority of the dogs treated with Raloxifene and Alendronate also showed signs of improvement at different intervals. Further, the signs of improvement appeared earlier in these animals as compared to those treated with only Ca+vit D₃+Zn. This indicates that these antiresorptive drugs have positive effect on dogs affected with bone diseases. Raloxifene, the antiresorptive agent is thought to work by inducing conformational changes in the estrogen receptor, resulting in differential expression of specific estrogen regulated genes in different tissues. (Mitlak and Cohen 1997). Whereas biophosphonate compound acts as a potent, specific inhibitor of osteoclast mediated bone resorption. Alendronate effectively decreased the bone turnover in a dose-related manner and increased lumbar bone mineral density @ 2.5 mg/day (Shiraki *et al.* 1998). Biphosphonates are said to be capable of suppressing parathyroid hormone (PTH)-mediated bone resorption and are useful for the prevention and treatment of postmenopausal osteoporosis (Rossini *et al.* 2001). However, the mechanism of action of these drugs in growing animals is not known. It is probable that these drugs inhibit osteoclast induced bone resorption in growing animals affected with bone diseases and may prove to be good adjuncts in such cases.

The dogs treated with Nandrolone and TGF β showed good response clinically as indicated by early improvement in the clinical signs of the disease. The response was better than that of control group and also to that of raloxifene and alendronate treated animals. TGF β stimulated collagen synthesis and AP activity and helped in maturation and differentiation of cultured bone cells (Morike *et al.* 1993). However, the mechanism by which TGF β helped to improve the condition of growing dogs affected with bone diseases is not clear. It is probable that TGF β may help in differentiation of bone cells and help to improve deposition of minerals in the bone by stimulation of collagen synthesis/alkaline phosphatase activity. Anabolic steroids are currently used in the treatment of established osteoporosis (Schot *et al.* 1993), and it has been demonstrated that they increase the bone density by stimulating bone formation. In rats and mice these agents increase longitudinal and periosteal bone growth and

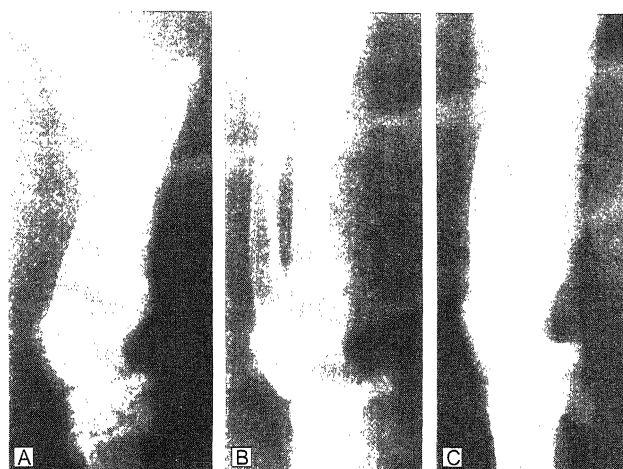


Fig. 1. Radiograph of a pup with rachitic signs before treatment (A); 25 days after treatment with Ca+vit D₃+Zn shows improvement in rachitic changes (B); and on day 70 shows good mineralization of long bone cortices (C).

bone mass, and also decrease trabecular bone resorption. In elderly dogs, Nandrolone stimulated endosteal bone formation (Schot *et al.* 1993). In the present study also, it showed some positive response in growing dogs affected with bone disease. It is likely that the drug acts by inhibiting the bone resorption in different disease conditions, however, the exact mechanism of its action in growing dogs with osteopenic bone conditions needs to be further investigated.

Radiographic observations

In the present study, the dogs affected with rickets showed different radiographic signs like enlarged physes of long bones (most commonly affected bone was ulna and radius), increased width of metaphyses with 'cupping' at its borders, generalized thinning of long bones etc, as also recorded by earlier workers. The pups affected with NSH showed near normal physes of long bones in most cases with varying degrees of thinning. In certain cases pathological fractures were also recorded. Similar changes were reported by Schwarz (1991) in growing dogs affected with NSH.

In animals of control group treated with Ca+vit D₃+Zn, the radiographic changes at different treatment intervals included gradual increase in density at the physes leading to normalcy of physes (in rickets cases) and varied levels of increase in cortical thickness and density (Fig. 1). The radiographic changes differed among various animals. The radiographic changes supported the clinical findings and proved that supplementation of Ca+vit D₃+Zn helped in early mineralization of skeleton and regression of bony changes.

The follow up radiographs of dogs treated with Alendronate and Raloxifene (Fig. 2) and TGF- β also showed signs of mineralization of skeleton and regression of bony changes at the physes. However, there was no marked



Fig. 2. Radiograph of a pup shows signs of NSH with thin long bone cortices and saucer shaped preferential mineralization at the metaphyseal ends of long bones before treatment (A); and 30 days after treatment with Ca+vit D₃+Zn and raloxifene shows increased thickness of cortices and dissolution of increased bone density at the metaphysis (B).

difference when compared to that of control animals, which was in contrast to the clinical findings. This may be due to the fact that the follow up radiographic examination could be done only for a short duration (about 1 month) and it is possible that longer time is required for skeletal change to be appreciable radiographically. Further, radiographs of only a few cases in these groups could be examined during the treatment period, more number of cases and follow up for prolonged period might have helped to come to a definite conclusion.

Radiographs of growing dogs treated with Nandrolone showed clear radiographic signs of mineralization like increase in density and thickness of long bone cortices, as early as on 15th day of treatment (Fig. 3). The increase in

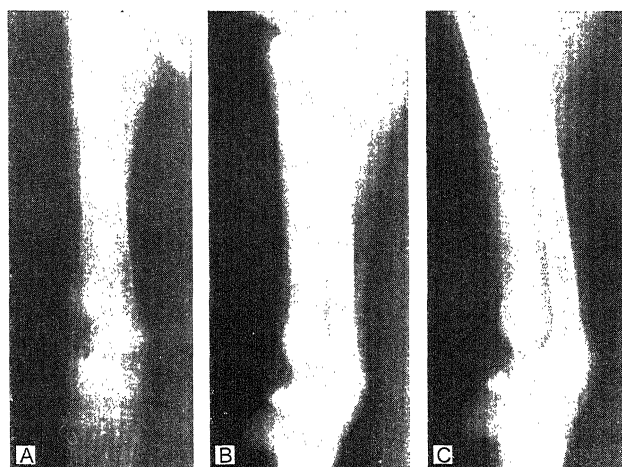


Fig. 3. Radiograph of a pup shows signs of NSH with very thin cortices before treatment (A); 15 days (B) and 45 days (C) after treatment with Ca+vit D₃+Zn and nandrolone shows mineralization and thickened long bone cortices.

cortical thickness in this group of animals is better than in all other groups, which supports the clinical findings. Anabolic steroids prevented the bone resorption and facilitate periosteal and endosteal new bone formation (Schot *et al.* 1993). Our results indicated that anabolic steroid was also effective in the treatment of osteopenic bone diseases in growing dogs.

Biochemical observations

Mean plasma Ca levels recorded in animals of different groups were less than the normal levels reported in growing animals, suggesting hypocalcemia. In animals of control group, plasma Ca levels increased gradually up to 45 days of observation (Fig. 4), suggesting positive response to therapy. In animals treated with raloxifene and alendronate also there was increasing trend. However, several earlier studies in animals showed decreased serum Ca levels during treatment with antiresorptive drugs indicating inhibition of bone resorption (Shiraki *et al.* 1998, Rubin *et al.* 2003). The growing dogs in the present study were hypocalcemic, and the treatment of these cases also included administration of Ca and vit D₃. Increasing trend in the plasma Ca probably is the positive indication of correction of mineral imbalance. On the other hand the animals treated with anabolic steroid (group 2) showed decreased plasma Ca levels on treatment days 7 and 15. This probably indicates strong inhibitory effect of the anabolic steroid on bone resorption or enhanced deposition of calcium in the bone, as also seen in radiographic study. In TGF β treated group, no marked difference was recorded in the plasma Ca values and it fluctuated at different intervals.

In animals of all groups, plasma P levels recorded on day 0 were above the normal range. Hyperphosphataemia in these cases might be due to dietary imbalance, increased resorption of minerals from the bone (under PTH influence) or due to reduced excretion of phosphorus from the kidneys. In animals of control group, plasma P levels decreased gradually and significantly at different treatment intervals, suggesting positive response in terms of correction of mineral imbalance. Similar trend was seen in biophosphonate compound and anabolic steroid treated groups (Fig. 5). As a result of

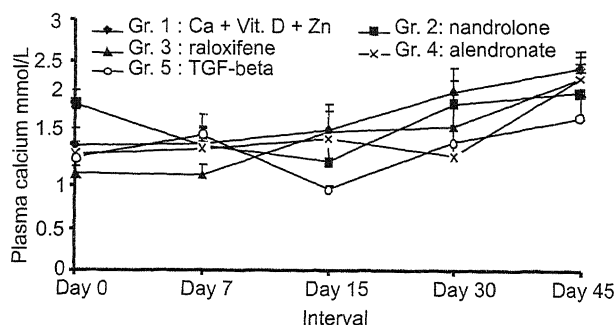
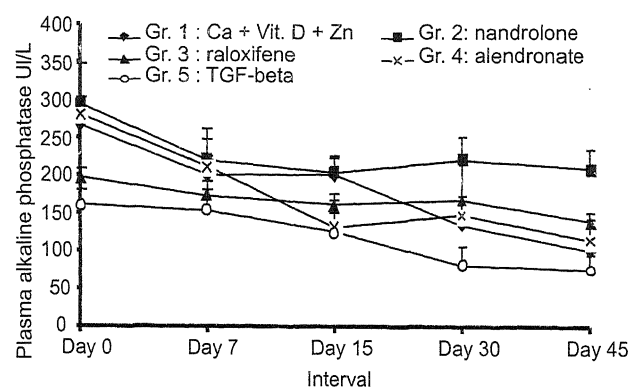
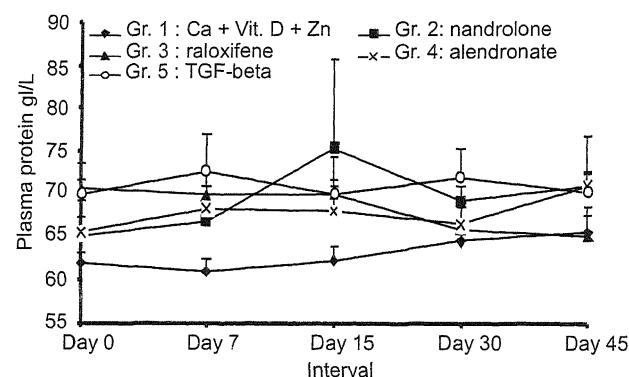
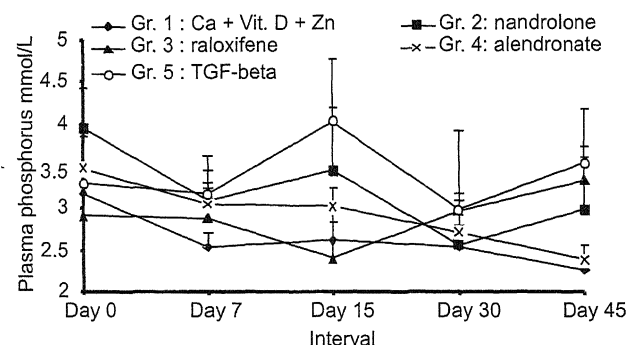


Fig. 4. Plasma calcium levels (mmol/l) in animals of different groups treated with antiresorptive drugs.

inhibition of bone resorption, biophosphonate compound has generally been shown to induce mild, transient and asymptomatic decreases in serum Ca and phosphate (Shiraki *et al.* 1998). The reduction in serum P may reflect not only the positive bone mineral balance, but also a decrease in renal phosphate re-absorption. In Raloxifene treated animals, plasma P levels though slightly reduced on day 15, on day 45 the mean value remained above the base value recorded on day 0. In this group of animals plasma Ca also showed an increasing trend. These effects of Raloxifene on plasma minerals can not be explained, as there are no animal studies



Figs. 5–7. 5. (top) Plasma phosphorus levels (mmol/l) in animals of different groups treated with antiresorptive drugs. 6. (middle) Plasma protein levels (g/l) in animals of different groups treated with antiresorptive drugs. 7. (bottom) Plasma alkaline phosphatase activity (U/l) in animals of different groups treated with antiresorptive drugs.

available to support or contradict these findings. In TGF β treated group, there was fluctuation in plasma P levels at different intervals.

Plasma protein values slightly increased at different treatment intervals in animals of control group as well as those treated with biophosphonate compound, anabolic steroid and TGF β (Fig. 6), suggesting positive response to these treatments. Whereas in antiresorptive drug treated group, there was decreasing trend in plasma protein levels. Plasma alkaline phosphatase activities in animals of different groups were in higher range on day 0 (Fig. 7). Increased AP activity is considered as a marker of bone resorption. Different antiresorptive drugs decreased the levels of AP. Administration of biophosphonate compound in patients of primary hyperparathyroidism led to significant reduction in serum total AP level at 6 months and remained decreased at 12 months (Hassani *et al.* 2001). Similarly, in Japanese patients with osteoporosis, biophosphonate compound treatment resulted in significant decrease in serum alkaline phosphatase activity (Shiraki *et al.* 1998). In the present study, animals treated with different antiresorptive drugs showed significant decrease in the AP activity, from day 7 onwards; and by day 30, the decrease was more than 50% in animals treated with Ca-vit.D₃-Zn, biophosphonate compound, anabolic steroid and TGF β . In animals treated with Raloxifene also, the AP activity decreased gradually; however, the decrease was to a lesser extent indicating that probably it was less effective as antiresorptive agent as compared to other drugs used in the present study, as also seen in clinical and radiographic observations.

From the results of this study, it can be concluded that calcium-vit.D₃-zinc therapy would help in early recovery of dogs affected with bone diseases like rickets and NSH. Antiresorptive drugs, Alendronate and Raloxifene do not show marked improvement over Ca-vit. D₃-Zn therapy, however, they do not either show deleterious effect on the skeleton in growing dogs. TGF β showed early clinical signs of improvement in dogs affected with skeletal diseases, but its lack of effect on the mineralization of bone requires further testing in more number of clinical cases. Anabolic steroid seemed to have positive effect on the mineralization of growing dogs affected with bone diseases and hence may be useful in clinical situations.

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