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Systemic Treatments With the Low-Calcemic $1,25(OH)_2D_3$ Analogs JKF or QW Increase Both the Morphological and Biochemical Responses to Estradiol-17β in Rat Tibiae

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Abstract We demonstrated previously that daily injection for 3 days of the less calcemic vitamin D analogs: JK 1624 F_2-2 (JKF) and QW 1624F_2-2 (QW) followed by estradiol-17β (E_2) in female rats upregulated creatine kinase-specific activity (CK) in skeletal tissues. In this study, we evaluated both histomorphological and biochemical changes due to a regime of 4 days treatment with JKF or QW, followed by injection of E_2 on day 5, repeated for 2.5 months. Ovariectomized female rats (Ovx) were injected 2 weeks after surgery, with JKF or QW at 0.2 ng/g BW followed by injections of E_2 (1 μg/rat) on day 5 of each week for 2.5 months. Rats were sacrificed 24 h after the last injection and bones were analyzed. JKF alone decreased growth plate width, increased % total bone volume (%TBV), with no change in cortical thickness. In contrast, QW restored growth plate width and %TBV with no change in cortical thickness. Combined with E_2, JKF restored %TBV and growth plate width but with no change in cortical thickness, while QW restored significantly all parameters including cortical thickness. Moreover, there was also an increase in the responsiveness of CK to E_2 in epiphyseal cartilage and diaphyseal bone but not in uterus. Thus, vitamin D less calcemic analogs increased responsiveness to E_2 morphologically as well as biochemically. We, therefore, conclude that combined treatment of less calcemic analogs vitamin D and E_2 might be superior for treatment of bone damage caused by ovariectomy in female rats and might be applied for post-menopausal osteoporosis. J. Cell. Biochem. 100: 1406–1414, 2007.

Key words: bone; vitamin D; estrogen; creatine kinase; trabeculi; histomorphometry

Steroid and seco steroid hormones play essential roles in the normal growth and differentiation of bones [Gallagher, 1988; Manolagas and Kousteni, 2001; Eriksen et al., 2002]. Adequate availability of vitamin D_3 and its active metabolite, 1,25 dihydroxyvitamin D_3 (1,25) is essential for skeletal health. By regulating intestinal calcium and phosphate absorption, 1,25 maintains extra cellular calcium levels and bone mineralization, and modulates cell growth and differentiation of both osteoblasts and osteoclasts [Tanaka et al., 1977]. Thus, vitamin D deficiency results in defective bone mineralization. Low vitamin D status is a common phenomenon in the elderly; levels of vitamin D as well as sex hormones are lower in older subjects [Gallagher, 1988; Manolagas and Kousteni, 2001; Eriksen et al., 2002]. However, several vitamin D metabolites and analogs, including the active metabolite 1,25 have shown to have a hypercalcemic effect [Lian et al., 1999; Vieth and Vitamin, 1999]. Since post-menopausal women are vulnerable to osteoporosis, and since bone both vitamin D and gonadal steroids regulate cells, optimal bone growth and prevention of osteoporosis in postmenopausal women, requires adequate concentrations of both

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17β-estradiol and vitamin D_3 [Gallagher, 1988; Manolagas and Kousteni, 2001]. In order to find an effective and restorative agent for bone loss, we looked for vitamin D analogs that will have the ability to prevent bone loss without the adverse calcemic activity.

Using a rat model, we have reported [Somjen et al., 1995, 2000, 2001] that pretreatment with 1,25 or with vitamin D analogs upregulated the responsiveness and sensitivity to 17β-estradiol (E_2) of skeletal cells in vitro or rat skeletal organs, but not in rat uterus in vivo [Kaye et al., 1990; Somjen et al., 1990; 1995; Fournier et al., 1996]. Pretreatment with 1,25 or less calcemic vitamin D analogs such as JK 1624 F_2-2 (JKF), and QW 1624F_2-2 (QW) [Kensler et al., 2000; Posner et al., 2004] upregulated the response of osteoblast-like cells to E_2 as measured by the stimulation of the specific activity of creatine kinase BB (CK) [Somjen et al., 2000, 2001]. Furthermore, these analogs upregulated the responsiveness and sensitivity to E_2 in female rat epiphyses and diaphyses. Daily injection of these analogs, which were found to be non-genotoxic [Posner et al., 2004, 2005] for 3 days did not stimulate significantly the specific activity of CK in rat skeletal long bones, but upregulated the responsiveness and sensitivity of CK to a single injection of E_2 to females [Somjen et al., 2000, 2001].

The present study was designed to analyze the histomorphological and biochemical changes measured following 2.5 months repetitions of a regime of four daily treatments with the analogs JKF or QW, with and without single weekly injection of E_2, in young growing ovariectomized female rats (Ovx), and to compare them with pre-pubertal intact female rats. We measured both CK modulation, which was previously correlated with ovariectomy and histological changes [Kaye et al., 1997], and the changes in histomorphometry and histology. We hope that our results will have a possible future aim for human studies designing a new combined hormone replacement therapy.

MATERIALS AND METHODS

Reagents

Estradiol 17β (E_2) was purchased from Sigma-Aldrich, Ltd, Rehovot, Israel. JKF and QW were synthesized by us (Fig. 1, [Kensler et al., 2000; Posner et al., 2004, 1998; Dixon et al., in press]). All other reagents were of analytical grade.

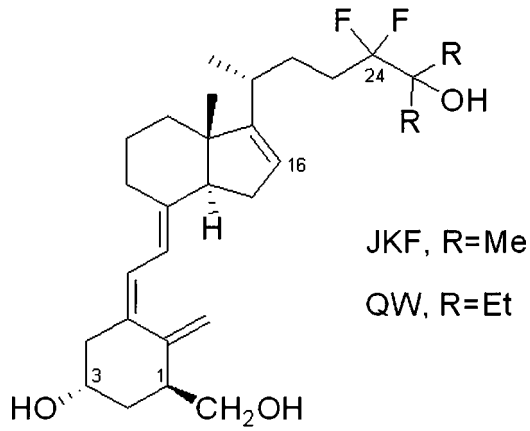

Fig. 1. The structure of the less calcemic analogs of vitamin D; JKF and QW.

Animals

Wistar-derived, locally bred rats were used at initial age of 25 days. The rats were maintained at 23°C, on a 14 h light, 10 h dark schedule and fed pelleted food and water ad libidum. Female rats were divided into seven groups of five animals in each group ($n = 35$). One group remained intact, and the rest ($n = 30$) were bilaterally ovariectomized (Ovx), and the treatments started 2 weeks post surgery for 2.5 months. Rats were injected with vehicle (Ovx), JKF alone, QW alone, E_2 alone, JKF + E_2, or QW + E_2. Rats were sacrificed 24 h after the last injection, by cervical dislocation and organs were removed for both histomorphometry and biochemical measurements. Experiments were carried out according to the regulations of the Committee on Experimental Animals of the Tel-Aviv Sourasky Medical Center and the NIH guidelines.

Hormonal Treatment

Ovariectomized female rats (Ovx) were injected 2 weeks after surgery, with vehicle; 0.1% ethanol in saline (C), E_2 (1 μg/rat), JKF or QW at 0.2 ng/g BW or JKF or QW at 0.2 ng/g BW followed by injections of E_2 (1 μg/rat) on day 5 of each week for 2.5 months or vehicle; 0.1% ethanol in saline (C). The rats were sacrificed 24 h after the last injection.

Histomorphometry

Tibiae were dissected and fixed in 4% formaldehyde in 0.1 M phosphate buffer,

pH 7.2, and decalcified in 10% EDTA in 0.05 M Tris-HCl buffer, pH 7.2. Specimens were embedded in paraffin and 6-μm thick sections, parallel to the long axis of the bone, were cut serially and stained with hematoxylin and eosin. The trabecular bone spicule width was measured at the lower border of the growth plate at random sites in three different fields at five different bones. Measurements were performed using an ocular micrometer, at a magnification of 40 or 100×. The trabecular bone volume (%TBV) was determined in a defined area, directly under the tibial growth plate, using a transmitted light microscope (Zeiss) linked to a CCD video camera and computerized histomorphometric system (Image Pro). The height of the growth plate and the arrangement of cells in the growth plate were also determined.

Creatine Kinase-Specific Activity

Rat organs were collected in cold isotonic extraction buffer [Somjen et al., 2000], homogenized for a few seconds using a Polytron homogenizer (Kinematica A.G., Littau, Switzerland), and enzyme extracts obtained by centrifugation of homogenates at 14,000*g* for 5 min at 4°C in an Eppendorf micro centrifuge. CK activity was measured in a Kontron Model 922 Uvicon Spectrophotometer using a Sigma coupled assay kit (UV-47).

Statistical Analysis

For CK analysis, the significance of differences between experimental and control means was evaluated using Student's *t*-test or ANOVA, in which $n = 5$ number of animals. Statistical analysis for the histological parameters was evaluated using one way analysis of variance and TUKEY non-parametric test, in which $n = 5$ (3×5). In both analyses, the bars represent SEM.

RESULTS

The Effect of the Vitamin D Analogs on CK-Specific Activity in Female Rat Organs

In female rat diaphysis (Di) ovariectomy (Ovx) results in 34% decrease in CK activity, and both analogs JKF and QW increased CK after 2 weeks, while in epiphyseal cartilage (Ep), CK was reduced by 38% by Ovx with no change with the analogs treatment. In the uterus (Ut), Ovx results in 34% decrease in CK activity with no change with the analogs (Fig. 2).

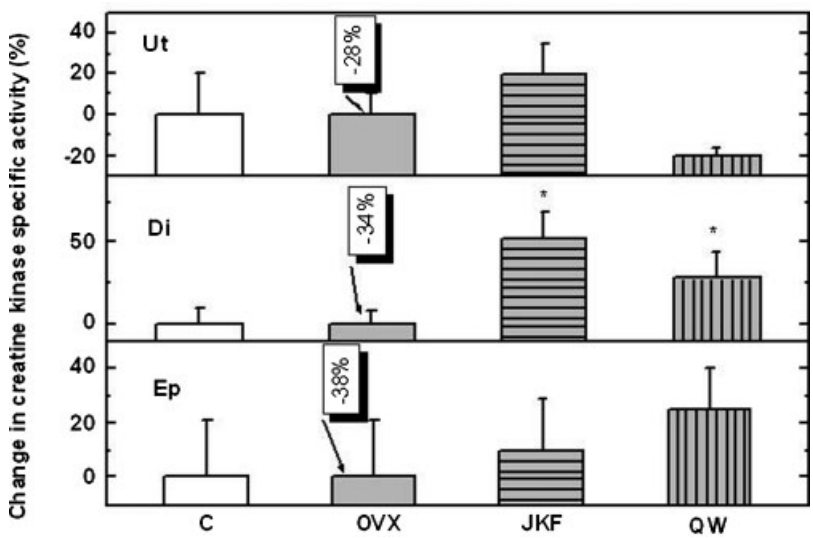

Fig. 2. The effect of JKF (horizontal lined gray bar) and QW (vertical lined gray bar) for 2.5 months on CK in epiphyseal cartilage (Ep), diaphyseal bone (Di), and uterus (Ut) from ovariectomized female rats (Ovx). Rats were injected with the analogs of vitamin D, JKF, or QW (0.2 ng/g BW) or vehicle; 0.1% ethanol in saline (C, white bars for intact and gray bars for Ovx), 4 times/week, followed by injections of vehicle on day 5 of each week, for 2.5 months, as described in Materials and Methods. Results are expressed as % change between the specific activities of CK in hormone-treated and saline-injected control Ovx animals, and also intact control is presented. The basal activity of CK in intact female rats was: in Ep $0.85 + 0.26$ and in Di $0.90 + 0.03$, Ut $0.55 + 0.15$ μmol/min/mg protein, $n = 5$ $^{*}P < 0.05$ of Ovx compared to intact rats and $^{\#}P < 0.05$ of Ovx after vitamin D treatment compared to vehicle treated. The percentage in the boxes above the vehicle-treated Ovx, presented the change in vehicle-treated Ovx compared to vehicle-treated intact female rats.

Stimulation of CK-Specific Activity by Combined Treatment With Vitamin D Analogs Plus Estradiol-17β

In female rat diaphysis (Di), CK was increased $40 \pm 8\%$ by E_2 at 1 μg, and by E_2 after JKF at 0.2 ng/g by $152 \pm 14\%$ and after QW at this concentration by $91 \pm 5\%$. JKF alone increased CK by $30 \pm 10\%$ and QW by $49 \pm 11\%$. In female rat epiphysis (Ep), CK was insignificantly increased $19 \pm 11\%$ by E_2 at 1 μg and by E_2 after JKF at 0.2 ng/g by $75 \pm 15\%$ and after QW at this concentration by $81 \pm 12\%$. JKF or QW alone had no significant effect on CK in this organ. In female rat uterus (Ut), CK was increased $63 \pm 20\%$ by E_2 at 1 μg and by E_2 after JKF at 0.2 ng/g by $10 \pm 11\%$ and after QW at this concentration by $19 \pm 9\%$. JKF or QW alone had no significant effect on CK in this organ (Fig. 3).

Histomorphometric and Histological Changes After Ovariectomy

Examination of control (vehicle-injected) intact animal group at the end of the experiment revealed that at the age of 4 months, the bone had a mature young adult appearance as indicated by the growth plate (Fig. 4a). The typical arrangement of the growth plate was disrupted, with numerous hypertrophic cells and fewer chondroblasts (Fig. 4a). The cortical bone was thick and thick trabeculae were observed at the bone marrow area. Thick trabecular bone with hardly any spicules was observed underneath and adjacent to the lower aspect of the growth plate (Fig. 4c). After 3 months of ovariectomy, control animals (Ovx) demonstrated changes in the growth plate structure. The overall growth plate architecture was dominated by hypertrophic chondrocytes, disrupted with fewer proliferative and chondroblastic cells. The metaphysis underneath the growth plate contained dense trabeculae as compared to the intact control tibiae. Examining %TBV, no statistical difference was found between intact and control Ovx animals. Numerous adipocytes were observed in the bone marrow (Fig. 4b). Ovx cortical bone thickness was significantly reduced compared to cortical bone from intact female rats (Fig. 4d, $P = 0.001$).

Histomorphological Changes After Treatment With Estradiol-17β alone

After 2.5 months treatment of Ovx female rats with E_2, the tibial morphological appearance was restored compared to vehicle-treated Ovx rats (Fig. 5d vs. a), but less matured than bone

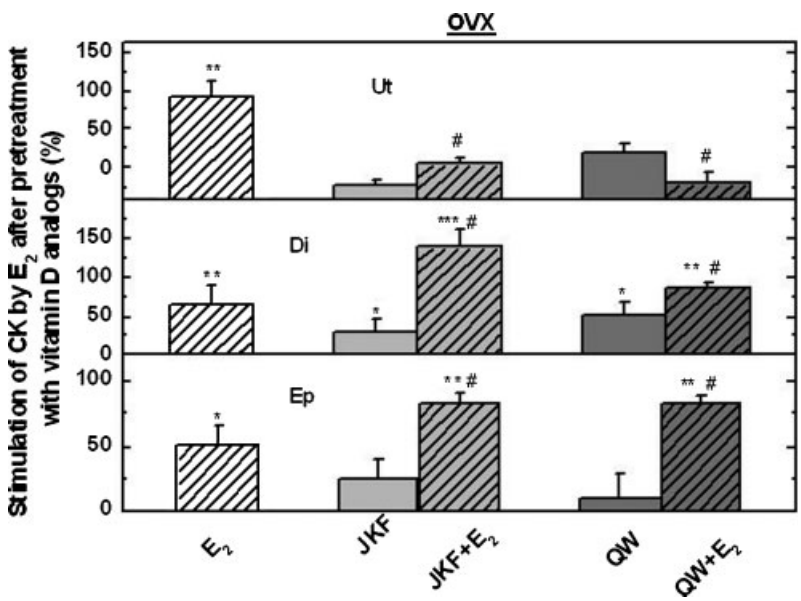

Fig. 3. The effect of JKF and QW at 0.2 ng/g/rat/day for 2.5 months on CK activity in Ep, Di, and Ut compared to the stimulation of E_2 at 10 μg/rat and to the combination of the vitamin D analogs with E_2 at 1 μg/rat. Ovx female rats were injected with E_2 at 10 μg/rat or the analogs of vitamin D, JKF or QW at (0.2 ng/g BW) or saline (C), 4 times/week, followed by injections of E_2 at 1 μg/rat /rat or vehicle on day 5 of each week, for 2.5 months, as described in Materials and Methods. Results are expressed as the ratios between the specific activities of CK in hormone-treated and vehicle-injected control animals. The basal activity of CK in Ep, Di, and Ut were as in Figure 2, n = 5. *, $P < 0.05$, **$P < 0.01$, ***$P < 0.001$; $^{\#}P < 0.05$ in the difference between E_2 after the analogs and E_2 after vehicle.

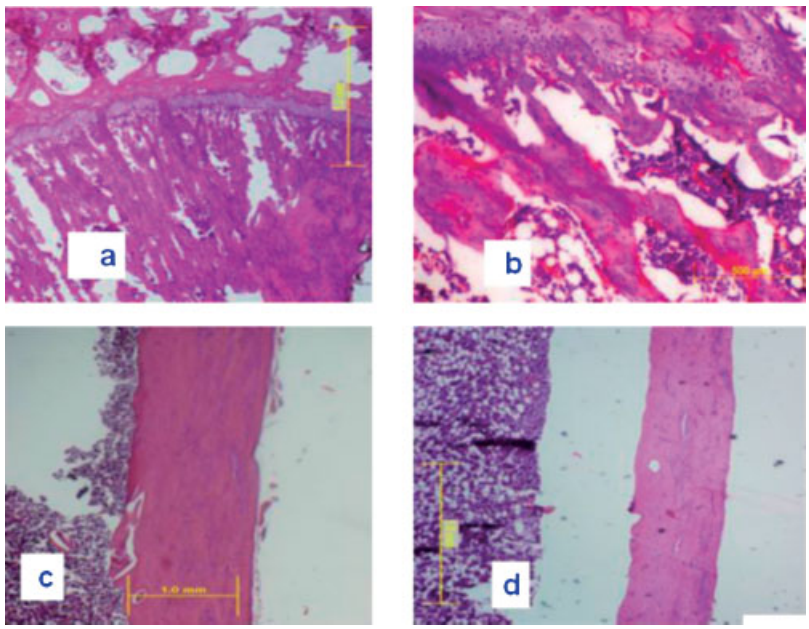

Fig. 4. Sections of female rats tibial growth plate and cortex. Rats were injected with 0.1% ethanol in saline (vehicle). **a**: Control intact tibial growth plate (**b**) Control Ovx tibial growth plate. **c**: Control intact tibial cortex. **d**: Control Ovx tibial cortex. Paraffin-embedded sections were stained with hematoxylin and eosin magnification, 40×.

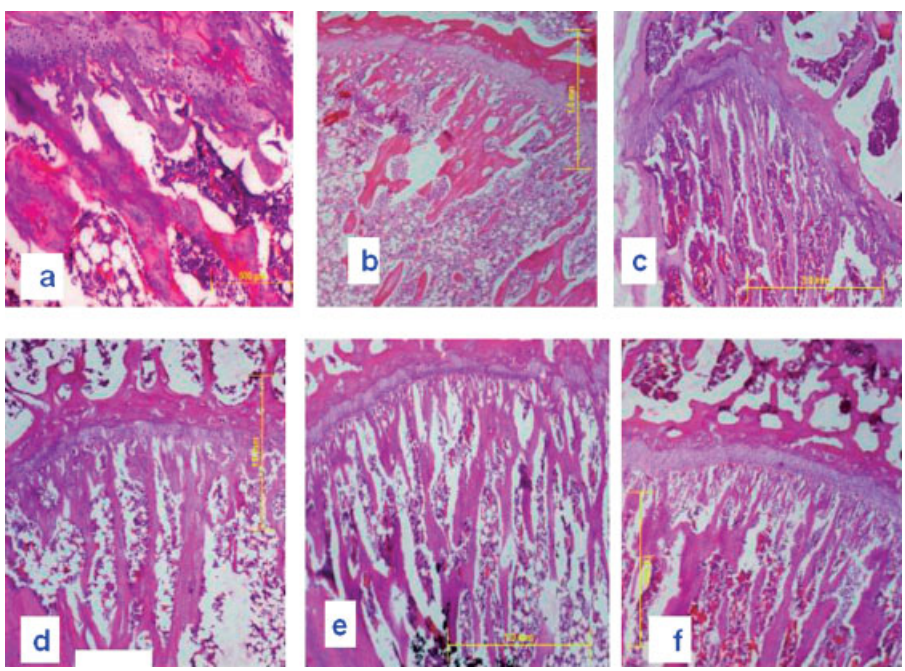

Fig. 5. Sections of female rats' tibial growth plate. Rats were injected with the low-calcemic analogs of vitamin D: JKF or QW at 0.2 ng/g BW followed by injections of E_2 (1 or 10 μg/rat) on day 5 of each week for 2.5 months, as described in Materials and Methods. **a**: Ovx control; (**b**) JKF; (**c**) JKF + E_2 (1 μg/rat); (**d**) E_2 (10 μg); (**e**) QW, and (**f**) QW + E_2. Paraffin-embedded sections were stained with hematoxylin and eosin magnification, 100×.

from the control intact rats (Fig. 4). The bone contained numerous trabeculae. The growth plate was not fused and although in some of the bones, the growth plate was reduced and narrow, it contained mostly proliferative cells. The bony trabecules observed in the primary spongiosa appeared to be thinner as compared to Ovx control animals (Fig. 5d vs. a). There was also reduction in %TBV after this treatment (Table I). The bone marrow contained less adipocytes than control Ovx (Fig. 5d vs. a). Cortical bone thickness was significantly reduced compared to control Ovx (Fig. 6d vs. a).

Histomorphological Changes After Treatment With JKF Alone.

Treatment of 2.5 months with JKF resulted in some recovery of the bone architecture of Ovx animals (Fig. 5b), but not similar to what was observed in the intact bone. Along with a thin epiphyseal cartilage with not many proliferative cells, vast hypertrophic cells, with thick trabeculae, and numerous adipocytes in bone marrow. JKF caused a significant reduction in growth plate width compared to control Ovx (Fig. 5b vs. a) and intact bones (Fig. 4a).

TABLE I. Effects of Less Calcemic Analogs of 1,25(OH)$_2$D$_3$; JKF and QW With and Without Estradiol-17β on Bone Histomorphometry in Proximal Tibial Metaphysis of Intact and OVX Rats

	Site		
Treatment	Cortical thickness (μm)	Growth plate width (μm)	% TBV
Intact + C	1,146 ± 13	261 ± 17	38 ± 4
Ovx + C	754 ± 12[a]	279 ± 19	46 ± 4
Ovx + E_2	581 ± 20[a]	237 ± 12[b,d]	40 ± 2
Ovx + JKF	661 ± 10[a]	236 ± 13[b,e]	55 ± 22
Ovx + QW	586 ± 30[a,d]	283 ± 11	43 ± 2
Ovx + JKF + E_2	490 ± 15[a,b,c]	267 ± 13	48 ± 2
Ovx + QW + E_2	680 ± 10[a]	294 ± 8[b]	45 ± 2

The effects of less calcemic analogs of 1,25(OH)$_2$D$_3$ JKF and QW with and without E_2 on bone histomorphometry in proximal tibial metaphysis of intact and Ovx rats. Rats were injected with the low hypercalcemic analogs of vitamin D, JKF or QW at 0.2 ng/g BW followed by injections of E_2 (1 μg/rat) on day 5 of each week for 2.5 months, as described in Materials and Methods. Significance of differences was evaluated using one way analysis of variant and Tukey test, in which n = 5 (3 × 5).
[a]Significant difference from intact ($P = 0.001$).
[b]Significant difference from Ovx + C ($P = 0.05$).
[c]Significant difference from Ovx treated with QW + E_2 ($P = 0.05$).
[d]Significant difference from Ovx treated with JKF ($P = 0.001$).
[e]Significant difference from Ovx treated with QW ($P = 0.001$).

Significant difference was found in cortical bone thickness and %TBV (Fig. 6b and Table I).

Histomorphological Changes After Combined Treatment With JKF and Estradiol-17β

Addition of E_2 to the treatment with JKF restored some aspects of the bone architecture. The growth plate arrangement was typical young and normal, with prolonged thin trabeculae with hardly any adipocytes, with no change compared to Ovx alone (Fig. 5c). This treatment resulted in restoration of %TBV (Table I). This treatment significantly reduced cortical thickness as compared to control Ovx (Fig. 6c, $P = 0.05$).

Histomorphological Changes After Treatment With QW Alone

Treatment of Ovx rats with QW significantly restored the growth plate width (Fig. 5d $P = 0.05$) compared to the Ovx vehicle-treated bone, better than JKF (Fig. 5e). The growth plate displayed normal appearance with thin trabeculae. There was a slight disruption in the organization of the growth plate and trabeculae compared to control Ovx (Fig. 5e). In the growth plate, proliferative cells were observed and although the mineralized zone appeared to be resorbed, it was not accompanied with bone trabecular formation, and less adipocytes in bone marrow. This treatment resulted in significant reduction in %TBV compared to treatment with JKF alone (Table I, $P = 0.05$). The cortical bone thickness was not affected and remained as the control Ovx bone (Fig. 6e).

Histomorphological Changes After Combined Treatment With QW and Estradiol-17β

Addition of E_2 to the treatment with QW resulted in a complete restoration of the growth plate architecture. There was a significant increase in growth plate width (Fig. 6f, $P = 0.007$) and resulted in a complete repair of the control Ovx bone (Fig. 6f). The typical division of the growth plate zone (proliferative, chonrocytes, and hypertrophic cells) was observed. Moreover, this restoration was observed also in %TBV (Table I). The primary spongiosa contained thin bone trabeculae and almost no adipocytes were observed in the bone marrow (Fig. 6f). Cortical thickness was significantly increased compared to the treatment with E_2 or JKF + E_2 (Fig. 6f).

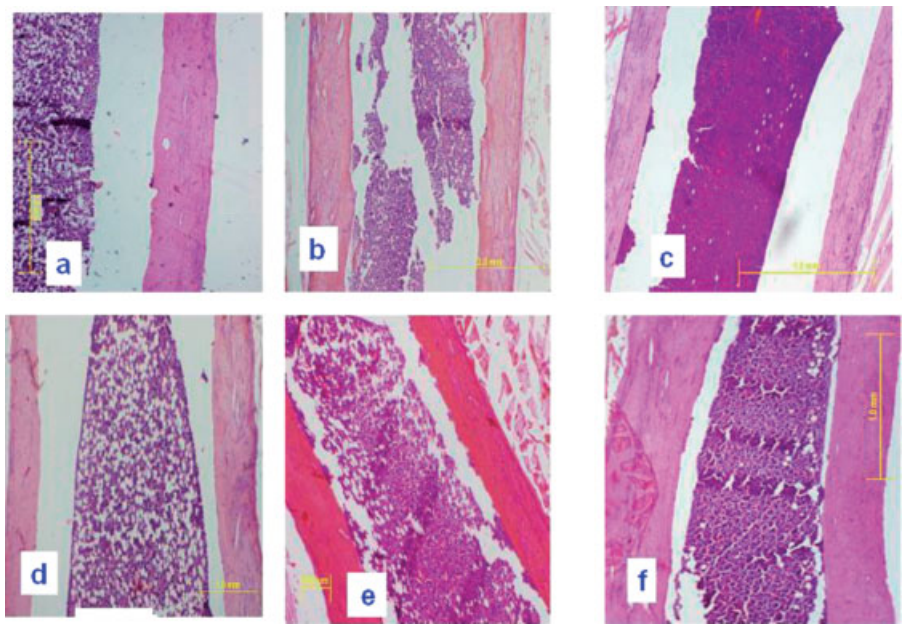

Fig. 6. Sections of female rats' tibial cortex. Rats were injected with the low-calcemic analogs of vitamin D JKF or QW at 0.2 ng/g BW followed by injections of E_2 (1 or 10 μg/rat) on day 5 of each week for 2.5 months, as described in Materials and Methods. **a**: Ovx control; (**b**) JKF; (**c**) JKF + E_2; (**d**) E_2 (5 μg); (**e**) QW, and (**f**) QW + E_2. Paraffin-embedded sections were stained with hematoxylin and eosin magnification, 100×.

DISCUSSION

The problem of hypercalcemia as a result of chronic treatment with 1,25 dihydroxy vitamin D_3, led to the development of vitamin D analogs which were less calcemic but retained anti-proliferative properties. Among these, the hybrid fluorinated analog JKF was tested against a variety of cancer cells including prostate cells [Oades et al., 2002] and skin cancer [Dixon et al., 2005]. QW. a different hybrid fluorinated analog of 1,25 dihydroxy vitamin D_3, has also been tested in different systems.

We utilized a combination of these two analogs with E_2 to demonstrate anabolic effects on rat tibial bone when four daily injections of vitamin D analogs were followed by a single injection of E_2 on day 5, repeated for 2.5 month.

Histological examinations revealed that 2.5 months treatment of Ovx rats with any of the substances resulted in rejuvenation of the bone, among all, QW was the best (Figs. 5 and 6, Table I). Intact cortical bone was significantly thicker than the non-treated Ovx bone ($P = 0.001$). The different treatments showed to affect bone thickness. No significant change was observed between treated and non-treated OVX rat's cortical thickness, except the combine E_2 + JKF treatment, that significantly reduced cortical thickness, as compared to control Ovx rats. Furthermore, the combined QW + E_2 treatment significantly increased cortical thickness as compared to the combined JKF + E_2 treatment ($P = 0.05$).

Short-term treatment (1 or 2 weeks) with JKF or QW alone enhanced maturation of the tibiae and increased cortical and trabecular bone thickness and less cartilage and more bone were observed (data not shown). Generally, a cluttered epiphyseal plate was observed, and the zone of resorption was disarranged with thick trabecular bone spicules. Cortical bone, as reported previously [Berger et al., 1999], was mature and much wider. In females, as shown before [Berger et al., 1999], E_2 injections increased the width of cortical bone close to the epiphysis (data not shown). The prolonged treatment with JKF had a different pattern compared to treatment with QW alone. JKF reduced cortical and trabecular thickness with similar cluttered, disarranged epiphyseal plate. On the other hand, treatment with QW significantly restored the growth plate width % TBV values compared to treatment with JKF alone ($P = 0.05$) and caused the growth plate appeared to be younger.

Both short-term and long-term experiments revealed that gonadal steroids restored the damage caused by treatment with vitamin D analogs alone, to all the parameters measured in the epiphyseal growth plate and the adjacent area underneath as well as %TBV. In long-term experiment, E_2 alone acted similarly to JKF, by reducing cortical thickness and growth plate width. E_2 showed a general reduction in %TBV that was significant in comparison to bones treated with JKF ($P = 0.001$, Table I). Treatments with JKF or E_2 alone caused a significant reduction in growth plate width ($P = 0.05$), as compared to treatment with QW alone. On the other hand, the combined E_2 with JKF treatment resulted in restoration of the bone, as seen from %TBV and growth plate width and morphology. The addition of E_2 to QW further and significantly improved the growth plate width ($P = 0.05$). The combined treatment of $E_2 + QW$ caused a significant increase in growth plate width ($P = 0.007$) and resulted in a complete repair of the control Ovx bone.

The responsiveness of CK to E_2 in Ep and Di (Fig. 3) was significantly enhanced by the combined treatment of JKF or QW with lower dose of E_2 compared to E_2 alone, resulting in significantly higher stimulation of CK-specific activity (Fig. 3). The rapid stimulation of the specific activity of the brain type isozyme of creatine kinase (CK BB) is an almost universal marker of cell stimulation. We have studied its stimulation in skeletal-derived cells and shown that the increase in its activity is closely correlated with the biochemical parameter of cell proliferation, thymidine incorporation into DNA, and with the morphological parameters of bone growth, increase in thickness of cortical bone and of the number of cells in the proliferating zone of the epiphyseal growth plate [Kaye et al., 1997].

Ovariectomy caused an almost complete cessation of bone growth, as was demonstrated by the disruption of the growth plate cell organization, and the complete disappearance of thin bone spicules. The increased amount of adipocytes replacing the bone marrow adjacent to the growth plate could indicate that this treatment affected the overall metabolism of the bone marrow. Such change reflects a more mature stage of the bone as observed in aged animals. In a mouse model of accelerated aging (SAMP6 strain), osteoblastogenesis is decreased with correlation to increased number of adipocytes [Uchiyama et al., 1994]. In newborn humans, the marrow contains few adipocytes and is characterized as "red" or erythropoietic. With advancing age, the number and size of adipocytes increase in a linear manner [Gimble, 1990] resulting in a "yellow" marrow. Fat then occupies 50% of the human marrow cavity [Gimble et al., 1996]. Clinical observations documented an inverse relationship between adipocytes and osteoblasts [Minaire et al., 1984]. In osteoporotic patients, increased bone marrow adipose tissue correlates with decreased trabecular bone volume [Minaire et al., 1984]. Treatment with E_2 reversed the aging process of the bone marrow by reducing the lipid-containing cells in the bone marrow. This treatment also repaired the morphology of the growth plate.

Osteoblasts and adipocytes originate from common mesenchymal precursor in bone marrow [Owen, 1985; Aubin et al., 1985]. Also, the adipocyte is the most abundant stromal cell phenotype in adult human bone marrow [Gimble et al., 1996] possibly due to a cellular stress response pathway activation with aging [Kirkland et al., 2002]. The trabecular bone and adipose tissue content in bone marrow are inversely related in human disuse osteoporosis [Minaire et al., 1984]. Since, ovariectomy causes aging of the bone overall, one of the age signs may also be adipogenesis, which replaces osteogenesis, for example, causing osteoporosis [Martin and Zissimos, 1991]. However, our study showed that the adipogenesis caused by ovariectomy is a reversible process and can be corrected and rejuvenate the bone marrow to its normal accurate chronological age. Moreover, in the bone sites analyzed in the present study, the noncalcemic vitamin D analogs analyzed had a beneficial effect on bone and bone marrow restoration.

As indicated from the parameters measured, and together with the morphological evidence, combined treatment of $QW + E_2$ appeared to be more suitable with best results for the treatment of bone damage caused by ovariectomy in female rats.

This chronic (long term) treatment with the low calcemic vitamin D analogs JKF and QW, increased the responsiveness to E_2 in all parameters measured, both biochemically and morphologically resulting in net growth of bone at approximately normal levels.

REFERENCES

Aubin JE, Turksen K, Heersch JNM. 1985. Osteoblastic cell lineage. In: Noda M, editor. Cellular and Molecular Biology of Bone. New York: Academic Press. pp 1–45.

Berger E, Frisch B, Lifschitz-Mercer B, Weisman Y, Somjen D. 1999. Sequential treatment with vitamin D analogs and gonadal steroids augments anabolic changes in rat bone and cartilage. Abs. 11th Inter. Workshop on Calcified Tissues, Eilat, Israel, February 7-11. p 106.

Dixon KM, Deo SS, Wong G, Slater M, Norman AW, Bishop JE, Posner GH, Ishizuka S, Halliday GM, Reeve VE, Mason RS. 2005. Skin cancer prevention: A possible role of 1,25dihydroxyvitamin D3 and its analogs. J Steroid Biochem Mol Biol 97:137–143.

Eriksen EF, Glerup H, Vitamin D. 2002. deficiency and aging: Implications for general health and osteoporosis. Biogerontology 3:73–77.

Fournier B, Haring S, Kaye AM, Somjen D. 1996. Stimulation of creatine kinase specific activity in human osteoblast and endometrial cells by estrogens and antiestrogens and its modulation by calciotropic hormones. J Endocrinol 150:275–285.

Gallagher JC. 1988. Drug therapy of osteoporosis: Calcium, estrogen and vitamin D. In: Riggs BL, Melton LJ III, editors. Osteoporosis: Etiology, diagnosis and management, vol. 1: New York: Raven Press. 389.

Gimble JM. 1990. The function of adipocytes in the bone marrow stroma. New Biol 2:304–312.

Gimble JM, Robinson CE, Wu X, Kelly KA. 1996. The function of adipocytes in the bone, marrow stroma: An update. Bone 19:421–428.

Kaye AM, Weisman Y, Harell A, Somjen D. 1990. Hormonal stimulation of bone cell proliferation. J Steroid Biochem Mol Biol 37:431–435.

Kaye AM, Kim TY, Kohen F, Somjen D. 1997. Anabolic effects of estrogen and parathyroid hormone on skeletal tissues: The use of creatine kinase B activity as a response marker. Arch Gerontol Geriatr 24:197–209.

Kensler TW, Dolan PM, Gange SJ, Lee L-K, Wang Q, Posner G. 2000. Conceptually new deltanoids (vitamin D analogs) inhibit multistage skin tumorigenesis. Carcinogenesis 21:1341–1345.

Kirkland JL, Tchkonia T, Pirtskhalava T, Han J, Karagiannides I. 2002. Adipogenesis and aging: Does aging make fat go mad? Exp Gerontol 37:757–767.

Lian JB, Stein GS, Stein JL, van Wijnen AJ. 1999. Regulated expression of the bone-specific osteocalcin gene by vitamins and hormones. Vitam Horm 55:443–509.

Manolagas SC, Kousteni S. 2001. Perspective: Nonreproductive sites of action of reproductive hormones. Endocrinology 142:2200–2204.

Martin RB, Zissimos SL. 1991. Relationships between marrow fat and bone turnover in ovariectomized and intact rats. Bone 12:123–131.

Minaire P, Edouard C, Arlot M, Meunier PJ. 1984. Marrow changes in paraplegic patients. Calcif. Tissue Int 36:338–340.

Oades GM, Dredge K, Kirby RS, Colston KW, Vitamin D. 2002. receptor-dependent antitumour effects of 1,25-dihydroxyvitamin D_3 and two synthetic analogues in three in vivo models of prostate cancer. British J Urol Int 90:607–616.

Owen ME. 1985. Linage of osteogenic cells and their relationship to the stromal system. In: Peck WA, editor. Bone and Mineral Research. Amsterdam: Elsevier. 1–25.

Posner GH, Lee L-K, Wang Q, Peleg S, Burke M, Brem H, Dolan PM, Kensler TW. 1998. Noncalcemic, antiproliferative, transcriptiopnally active, 24-fluorinated hybrid analogues of the hormone 1α, 25-dihydroxyvitamin D_3 Synthesis and preliminary biological evaluation. J Med Chem 41:3008–3014.

Posner GH, Jeon HB, Sarjeant A, Riccio ES, Doppalapudi RS, Kapetanovic IM, Saha U, Dolan P, Kensler TW. 2004. Low-calcemic, efficacious, 1alpha, 25-dihydroxyvitamin D3 analog QW-1624F2-2: Calcemic dose-response determination, preclinical genotoxicity testing, and revision of A-ring stereochemistry. Steroids 69:757–762.

Posner GH, Lee SH, Kim HJ, Peleg S, Dolan P, Kensler TW. 2005. Novel A-ring analogs of the hormone 1alpha,25-dihydroxyvitamin D3: Synthesis and preliminary biological evaluation. Bioorg Med Chem 13:2959–2966.

Somjen D, Harell A, Weisman Y. 1990. Reciprocal modulation by sex steroids and calcitropic hormones of skeletal cell proliferation. J Steroid Biochem Mol Biol 37:491–499.

Somjen D, Weisman Y, Kaye AM. 1995. Pretreatment with 1,25 $(OH)_2$ vitamin D or 24,25 $(OH)_2$ vitamin D increases synergistically responsiveness to sex steroids in skeletal-derived cells. J Steroid Biochem Mol Biol 55:211–217.

Somjen D, Waisman A, Weisman Y, Kaye AM. 2000. "Non-hypercalcemic" analogs of 1α, 25-dihydroxy vitamin D augment the induction of creatine kinase B by estrogen and selective estrogen receptor modulators (SERMS) in osteoblast-like cells and rat skeletal organs. J Steroid Biochem Mol Biol 72:79–88.

Somjen D, Waisman A, Lee J-K, Posner GH, Kaye AM. 2001. A non-calcemic analog of 1α, 25 dihydroxy vitamin D_3 (JKF) upregulates the induction of creatine kinase B by 17β estradiol in osteoblast-like ROS 17/2.8 cells and in rat diaphysis. J Steroid Biochem Mol Biol 77:205–212.

Tanaka H, Seino Y, Vitamin D. 1977. metabolites and bone. In: Feldman D, Glorieux FH, Pike JW, editors. Vitamin D. San Diego: Academic Press. 305.

Uchiyama Y, Miyama K, Kataginri T, Yamguchi A, Takmori H, Nakashima K, Sato T, Suda T. 1994. Adipose conversion is accelerated in bone marrow cells of congenitally osteoporotic SAMP6 mice. J bone Miner Res 9(Suppl I):B365.

Vieth R, Vitamin D. 1999. supplementation, 25-hydroxyvitamin D concentrations, and safety. Am J Clin Nutr 69: 842–856.