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Oral Isotretinoin Therapy in Severe Acne Induces Transient Suppression of Biochemical Markers of Bone Turnover and Calcium Homeostasis

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Although dietary vitamin A is required for normal growth and development, long-term or high-dose administration of vitamin A derivatives (retinoids) may produce a variety of skeletal side-effects in man. In this study we investigated the early effects of oral isotretinoin therapy on bone turnover and calcium homeostasis in eleven consecutive patients with nodulocystic acne. The effects on bone metabolism were correlated to radiological and bone mineral density measurements following drug therapy for six months.

Markers of bone turnover, i.e. serum osteocalcin, the carboxyterminal propeptide of type I collagen, bone specific alkaline phosphatase, the carboxyterminal telopeptide of type I collagen, and urine levels of calcium and hydroxyproline decreased significantly within five days of treatment ($p < 0.05$). There was also a statistically significant decrease in serum calcium, with a minimum on day five, and a marked increase in serum parathyroid hormone ($p < 0.05$). With continued treatment, however, the abnormal levels of these markers returned to baseline values within 14 days. No significant roentgenological changes or effects on bone mineral density were found in response to the drug.

The observed inhibitory effects of isotretinoin on bone turnover, despite elevated parathyroid hormone levels, indicates that the drug exerts a direct effect on bone tissue. ***Key words: vitamin A; retinoic acid; isotretinoin; bone mineral density.***

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Vitamin A is known to affect bone formation and structure. Thus, a chronic deficiency state may lead to impaired growth of bone, whereas hypervitaminosis A can induce osteoporosis or even spontaneous fractures (1). Similarly, synthetic analogues of vitamin A, retinoids, may produce a variety of skeletal manifestations such as bone spurs, ossification of ligaments, and periosteal thickening (2–4). In severe cases, slender long bones and osteoporosis have been reported (5, 6). Recent studies using dual X-ray absorptiometry has shown significantly reduced bone mineral density (BMD) values in patients with long-term etretinate treatment (6, 7). We have previously demonstrated that human osteoblasts express nuclear receptors for the active vitamin A metabolites all-*trans* retinoic acid (RA) and 9-*cis* RA (8). Both these compounds induce bone resorption *in vitro* (9).

Synthetic retinoids are commonly used for systemic treatment of keratinizing skin disorders and severe acne. Etretinate and its main metabolite, acitretin, are both effective in the treatment of ichthyotic conditions and psoriasis. Like the aromatic retinoids, isotretinoin (13-*cis*-RA) increases the incidence and severity of vertebral hyperostoses (10) although the bone density seems to decrease as judged from radiological data (11). Surprisingly, studies on isotretioin has not revealed any effect of this retinoid on markers of bone turnover or BMD after long-term treatment of mixed patient populations (6, 12, 13).

To better understand the paradoxical effects of retinoids on bone tissue, inducing ectopic bone formation despite reduced BMD, we have assessed the acute and long-term effects of isotretioin on bone turnover in patients with severe nodulocystic acne. The biochemical response was related to BMD measurements and X-rays obtained after 6 months' therapy.

PATIENTS AND METHODS

Subjects and treatment

Eleven Caucasian patients (9 males) aged 15–43 years (mean 26 years) with nodulocystic acne were studied. The patients were referred to the Department of Dermatology because of therapeutic failure of topical remedies and tetracyclines. Isotretinoin (Roaccutane®) therapy was initiated at a mean dose of 0.71 mg/kg body weight (range: 0.56 to 0.88 mg/kg) and then adjusted to 0.88 mg/kg/day (range 0.66–1.00) as maintenance dosage after 1–3 months. The drug was administered once daily with a meal. The patients were otherwise healthy and received no concomitant medication which might affect the bone metabolism. The study was approved by the local Ethical Committee at Uppsala University Hospital.

Bone densitometry

Bone mineral density of the lumbar spine (L2–L4) and whole body were measured by dual energy X-ray absorptionmetry (DPX-L, Lunar Radiation Corporation, Madison, WI, USA). The BMD was expressed in grams per cm^2. The measured error in precision of DXA, as tested on a spine phantom in our laboratory, is less than 1%.

Radiography and evaluation

Before entry into the study, spine radiographs were obtained in all patients. A standard technique was used including four projections of the cervical spine: anterio-posterior (AP), lateral, and 45° oblique views. At the thoracic and lumbar level, two projections were used: anterio-posterior, including angled anterio-posterior, and lateral. In total, 12 images per examination were performed. Nine patients were reexamined after drug therapy for 6 months. Radiographs were evaluated by a skeletal radiologist with regard to the presence of new bone formation, periosteal thickening and calcification of ligaments and tendons. At evaluation of radiographs, pre- and posttreatment pictures were mixed without knowledge to the radiologist.

Biochemical markers of bone turnover

Fasting morning blood and urine samples were obtained prior to therapy and on days 1, 5, and 14 after initiation of treatment. A detailed description of the analyses has previously been published (14). Briefly,

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Table I. *Effects of isotretinoin treatment on biochemical markers of bone turnover and calcium homeostasis during the first two weeks of treatment*[a]

Biochemical marker			Baseline	Day 1	Day 5	Day 14
S-Osteocalcin	(μg/l)	(n=10)	14.5±2.4	14.3±2.5	12.8±2.4*	13.5±2.4
S-PICP	(μg/l)	(n=10)	202.3±35.9	184.3±30.5	153.7±19.1*	182.8±27.0
S-bALP	(μkat/l)	(n=9)	1.76±0.81	1.66±0.75	1.61±0.74	1.52±0.65
S-ICTP	(μg/l)	(n=10)	8.8±2.5	7.8±2.1*	7.9±2.0	8.5±2.1
U-Ca	(mol/mol Crea)	(n=10)	0.3±0.1	0.2±0.0	0.2±0.0	0.3±0.1
U-hydroxyproline	(mmol/mol Crea)	(n=9)	17.9±4.5	19.4±3.4	14.5±3.8	22.9±11.6
S-Ca	(mmol/l)	(n=10)	2.49±0.02	2.44±0.03*	2.43±0.03*	2.45±0.02
S-PTH	(ng/l)	(n=10)	17.4±1.6	23.9±3.0*	23.9±2.0*	19.0±1.8
S-ALP	(μkat/l)	(n=10)	3.68±0.74	3.64±0.68	3.73±0.70	3.68±0.63
S-Alb	(g/l)	(n=10)	43.3±0.9	43.6±0.7	44.2±0.8	43.2±0.7
U-Ca	(mmol/24h)	(n=10)	3.39±0.75	2.92±0.70	2.28±0.57	3.07±0.70

[a] Values are mean±SEM.
* Statistically significant difference ($p<0.05$) vs baseline.
PICP: the carboxyterminal propeptide of type I collagen, bALP: bone specific alkaline phosphatase, ICTP: the carboxyterminal telopeptide of type I collagen, PTH: parathyroid hormone.

serum and urine calcium were determined by atomic absorption, and bone specific alkaline phosphatase (bALP) activity was measured by spectrophotometry according to routine procedures at the Department of Clinical Chemistry, University Hospital, Uppsala. The serum calcium values were adjusted to the concomitant serum albumin values.

The other samples were centrifuged at 4°C and stored at −70°C until analysis, with all samples from each patient being analyzed in one batch. Serum osteocalcin was measured by radioimmunoassay (CIS Bio International, Gif-Sur-Yvette, France), parathyroid hormone (PTH) by a sandwich radioimmunometric method (Nichols Institute, San Juan Capistrano, USA), and urinary hydroxyproline by amino acid analyzer. Serum concentrations of the carboxyterminal propeptide of type I collagen (PICP) and the carboxyterminal telopeptide of type I collagen (ICTP) were measured by radioimmunoassay using commercially available kits (Orion Diagnostica, Espoo, Finland).

Statistics

Statview II version 1.04 (Abacus Concepts Inc, Berkeley, CA, USA), was used for statistical calculations. These included analysis of variance (ANOVA) of repeated samples to assess the effect of isotretinoin therapy on biochemical markers of bone turnover and BMD. *P* values less than 0.05 were considered statistically significant.

RESULTS

Due to missed appointments, two patients were unable to produce a complete series of samples for biochemical markers, and three patients were unable to attain both BMD investigations. Thus in each of these cases, data from one sample occasion was lost for statistical analysis.

Biochemical markers of bone turnover

Table I shows that the serum concentrations of three markers of osteoblastic activity, i.e. osteocalcin, bALP and PICP, all decreased early during retinoid therapy. The lowest values were observed on day five. Mean values for these markers returned to pretreatment levels by day 14.

The markers of bone resorption were also affected with slightly reduced creatinine-corrected, fasting urinary calcium (U-Ca/tU Crea) levels during the initial five days (NS), and significantly ($p<0.05$) decreased serum ICTP concentrations on day one. There were no consistent changes in the urinary hydroxyproline levels.

Serum calcium and PTH

The serum calcium levels were significantly ($p<0.05$) reduced during the first five days of isotretinoin therapy. This was associated with a significant ($p<0.05$) increase in serum PTH levels. Both calcium and PTH values returned to baseline after 14 days of treatment (see Table I).

Bone mineral density

Bone mineral density, as measured by DXA of the lumbar (L2–L4) spine, showed no significant change after six months with isotretinoin as compared to pretreatment values (Table II).

X-ray examinations

Spine radiographs obtained before treatment revealed minimal or moderate degenerative changes such as disc height reductions and/or presence of osteophytes in three of the patients. In addition, preexisting asymptomatic skeletal anomalies including block vertebrae, transitional vertebrae and persisting apophyses, were observed in these patients.

In 7 of 9 patients, the follow-up radiographs did not differ from pretreatment pictures. In one case, there was a slight increase in the size of preexisting osteophytes on three cervical and one lumbar vertebra. In another patient a small osteo-

Table II. *Comparison of bone mineral density at baseline and after six months of isotretinoin treatment*[a]

		Baseline	6 months
BMD L2-L4 (g/cm^2)	(n=8)	1.258±0.045	1.258±0.045
BMD total body (g/cm^2)	(n=8)	1.225±0.037	1.235±0.038

[a] Values are mean±SEM.
BMD: bone mineral density.

phyte, not observed before treatment, was noticed at the end of treatment in an otherwise normal spine.

DISCUSSION

Signs of skeletal toxicity from chronic retinoid exposure is important to recognize, but the extent and severity of the problem in clinical practice is still unsolved. Previous studies have shown contradictory results which, to some extent, may be due to heterogenous study populations and widely differing retinoid dosages. In this study, the potential bone effects of an ordinary therapeutic dose of isotretinoin was investigated in a group of patients with severe acne.

Our study shows that initiation of isotretinoin therapy is followed by an acute inhibition of biochemical markers of bone turnover and calcium regulation. The effect was reversed within 14 days despite continuation of drug administration. Moreover, no significant effects on BMD values or radiographs were found after retinoid treatment for 6 months.

Studies *in vitro* and in laboratory animals have demonstrated that all-*trans* RA and 9-*cis* RA may induce bone resorption (9), and that fractures resembling osteoporotic lesions can be induced by high doses of synthetic retinoids (15, 16).

Skeletal alterations in man treated with naturally occurring acidic retinoids (e.g. all-*trans* RA) are generally similar to those seen in hypervitaminosis A (17). The most frequently reported changes are cortical hyperostosis and ligamentous calcifications. Long-term or high doses of etretinate to patients with skin disease have also been linked to severe osteoporosis (5, 6). Even low-dose oral etretinate to psoriatic patients has been accompanied by reduced bone density with concomitant vertebral spur formation and calcification of ligaments (7). Furthermore, administration of the main metabolite of etretinate, acitretin, has been associated with bone-spur formation (18) though a recent study indicates that either retinoid has little or no effect on bone (19).

The seemingly conflicting findings, i.e. increased bone formation with concomitant reduction of BMD, could possibly be explained by tissue-specific retinoid effects on cartilage versus bone. Thus, RA is known to stimulate maturation of chondrocytes into a hypertrophic phenotype (20) preceding enchondral bone formation (21). On the other hand, RA has a putative inhibitory effect on remodelling of preformed bone which would lead to decreased bone density.

BMD measurements were made at the lumbar spine level, since confounding extraosseous calcifications are more common at the cervical and thoracic level. Also, spinal BMD is mainly composed of trabecular bone, which is believed to be more sensitive to metabolic disturbances than cortical bone (4).

Our study showed no effect of isotretinoin on bone density when measured after six months of treatment. This is in accordance with previous reports (6, 12) although radiological studies have shown that the drug can induce skeletal changes similar to etretinate (22). Two of our patients had minimal signs of new bone formation in the spine. This finding could not be unequivocally attributed to the retinoid treatment, and did not affect the dual X-ray absorptiometry values of the spine. Prolonged follow-up studies after isotretinoin therapy may, however, be necessary to detect small changes of clinical significance for the patient (22). Hypercalcemia has previously been reported from near-toxic doses of isotretinoin in neuroblastoma patients (23). However, studies in other patients treated with isotretinoin for 1–4 months showed no significant effect of the drug on markers of bone mineralization (12, 13).

It is difficult to explain why isotretinoin has a temporary effect on markers of bone turnover in acne patients. This biochemical response, which has not been reported previously, may suggest that the drug exerts a direct – though transient – effect on bone tissue. Another explanation would be that isotretinoin affects bone turnover indirectly e.g. due to reversible interaction of the drug with the endogenous vitamin A metabolism. Since it is known that supplementation with vitamin A (retinyl palmitate) may transiently affect circulating levels of vitamin A metabolites such as 13-*cis* RA, it seems possible that pharmacological doses of this compound may interfere with the metabolism of vitamin A. Although isotretinoin therapy in acne patients does not affect circulating levels of vitamin A in the long run (26), one cannot exclude acute effects of the drug on systemic or local vitamin A levels e.g. like that in skin tissue (26). An alternative explanation for the biochemical pattern observed might be that the drug interacts with retinoid and vitamin D signalling pathways as suggested by earlier studies on bone resorption (9). If isotretinoin reduces bone turnover by interaction with vitamin D-induced bone resorption one would expect serum calcium levels to decrease and PTH levels to increase. It is less likely that isotretinoin acts primarily on PTH secretion or intestinal calcium absorption since this would result in increased bone turnover and concomitant decrease in serum calcium and subsequent rise in PTH levels.

Whichever mechanism is involved, compensatory processes seem to correct the drug-induced biochemical effects in course of time. This is in accordance with previous studies showing normal levels of bone turnover markers as measured one month after initiation of retinoid treatment (12, 13). At this point in time our data suggest that the levels of bone markers would already have returned to baseline values.

Taken together, our data indicates that isotretinoin has a specific effect on bone turnover and calcium homeostasis.

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