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Original Article

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Interaction between nandrolone decanoate and calcitonin in bone formation markers (osteocalcin and bone specific alkaline phosphatase) and IGF-I in rats

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Abstract

Bone tissue has been shown to contain numerous cell-to-cell signaling peptides called growth factors. These growth factors are thought to have important regulating effects for bone remodeling, due to their potent effects on bone cell metabolism. Our investigation was intended to assess the effect of nandrolone decanoate and calcitonin treatment on biochemical markers of bone formation (bone alkaline phosphatase – osteocalcin) and insulin-like growth factor-I in rats. We studied 48 adult male rats. The animals were divided into four groups. Group (A) served as control. Animals in Group (B) were injected with 4 mg/kg/day nandrolone decanoate. Animals in Group (C) were injected with 400mU/rat/day calcitonin and Group (D) received combined therapy for seven days. Nandrolone decanoate and calcitonin have a mild but significant effect on insulin-like growth factor-I without affecting osteocalcin levels, while calcitonin alone decreases the BALP levels. The coadministration of two agents caused notable elevation on insulin-like growth factor-I, followed by a significant increase of osteocalcin and bone alkaline phosphatase.

Keywords: Anabolic Steroids, Calcitonin, Insulin-Like Growth Factor-I (IGF-I)

Introduction

Anabolic steroids are synthetic androgens (testosterone derivatives) that possess the ability to retain nitrogen. This effect of androgens was first demonstrated in castrated dogs injected with androgen extracts from the urine of normal men[1]. The anabolic action of androgens is mediated by the same protein receptor that mediates the action of sex hormones in target tissues[2].

Nandrolone decanoate (ND), an anabolic steroid with an acceptable side effect profile, has been shown to stimulate human and murine osteoblastic cell proliferation *in vitro* and to induce expression of the osteoblast line differentiation markers, presumably by an androgen receptor-mediated mechanism[3].

Calcitonin (CT) is a peptide hormone produced mainly by the parafollicular cells of the thyroid gland in mammals and acts to reduce plasma calcium by inhibiting the output of calcium from skeletal tissues[4]. There is some evidence that calcitonin stimulates formation of bone by osteoblasts in addition to inhibiting bone resorption[5].

This study intends to investigate the possible potency of nandrolone decanoate, calcitonin and the cooperative effect of this combination in bone turnover parameters (bone specific alkaline phosphatase (BALP- osteocalcin) and insulin-like growth factor-I (IGF-I), in serum.

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Materials and methods

Forty-eight (48) adult (8 weeks old) male Wistar rats (mean weight 200 ± 20 gr) were obtained from the Athens Pasteur Institute. The animals were then divided into four groups (n=12). Group A was sham-treated and the other groups received drug therapy. The sham-treated group was injected subcutaneously with 0.25 ml arachis oil every day. Group B was injected subcutaneously with 4mg/kg/day ND (Deca-Durabolin, Organon int.B.R.Oss, The Netherlands). Group C was injected subcutaneously with 400mU/rat/day

salmon CT (Miacalcic, Sandoz, Basel, Switzerland) and 0.25ml arachis oil and group D received combined treatment (ND 4mg/kg/day and 400mU/rat/day CT) for 7 days.

Body weight of rats was measured on the first and the seventh day of the experimental procedure and the differences in body weight were evaluated.

The rats were sacrificed 20-22 hours after the final injection. Blood samples were collected from the left atrium after thoracotomy, were centrifuged at 3000g for 15 minutes and serum was collected and stored at – 32 ˚C until assayed.

Serum biochemistry

Serum bone specific alkaline phosphatase (BALP) was measured on Hitachi 717 analyzer using a wheat germ lectin precipitation method (Boehringer Mannheim, Germany). Insulin-like growth factor (IGF-I) was extracted from plasma by chemical extraction with chlorhydric acid ethanol. IGF-I levels were measured by RIA, using a rat radioimmunoassay kit (Nichols Institute, San Juan Capistrano, CA). Serum osteocalcin was measured by RIA using a kit specific for rat osteocalcin (Biomedical Technologies, Stoughton, MA). Serum phosphate, albumin and calcium were measured by autoanalyzer techniques.

Statistical analysis

The results are expressed as the mean ± SE. Comparisons between groups were made using one –way analysis of variance with post hoc Bonferroni-Dunett test.

Results

Estimation of IGF-I levels in serum

Levels of IGF-I in blood samples showed a mild but significant increase after administration of ND or calcitonin alone, compared with the control group. Administration of either ND or calcitonin alone caused an increase in IGF-I from 0.55 ± 0.03 μg/lt in the control group to 0.64 ± 0.025 μg/lt and 0.65 ± 0.033 μg/lt respectively, $p<0.05$. Coadministration of ND with calcitonin caused an increase from 0.55 ± 0.03 μg/lt in controls to 0.77 ± 0.024 μg/lt, $p<0.05$. This increase was notably greater than the one caused by ND or calcitonin alone, $p<0.05$ (Fig. 1).

Estimation of BALP levels in serum

Administration of ND did not affect levels of BALP compared with the control group, 520.45 ± 32 iu/ml vs. 511.6 ± 18.6 iu/ml respectively, p=n.s. Administration of calcitonin reduced BALP to 413.5 ± 16 iu/ml, $p<0.05$, while coadministration of ND with calcitonin increased BALP to 668.6 ± 26.4 iu/ml, $p<0.05$ (Fig. 2).

Estimation of osteocalcin levels in serum

Administration of ND or calcitonin alone had no effect on the levels of osteocalcin, 56.9 ± 3.1 mg/lt and 56.65 ± 3.4 mg/lt, p=n.s. respectively vs. 56.58 ± 3.3 mg/lt in the control group. On the other hand, the combination of ND with calcitonin raised levels of osteocalcin to 70.6 ± 0.9 mg/lt, $p<0.05$ (Fig. 3).

Changes in animal weight during the first week

Administration of either ND or ND-CT caused a significant increase in animal weight from 9 ± 5 g/7d in the control group to 22 ± 4 g/7d and 20 ± 2 g/7d respectively, $p<0.05$. Administration of CT alone did not affect animal weight compared with the control group, 8 ± 3 g/7d vs. 9 ± 5 g/7d respectively, p=n.s. (Fig. 4).

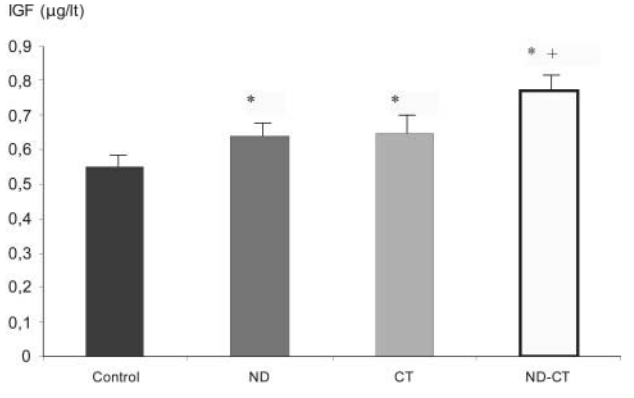

Figure 1. Levels of IGF-I in the blood. Control: non-treated animals, ND: animals treated with nandrolone decanoate, CT: animals treated with calcitonin, ND-CT: animals treated with nandrolone decanoate and calcitonin. IGF: Insulin-like growth factor-I. * $p<0.05$ vs. control, + $p<0.05$ vs. ND and CT groups.

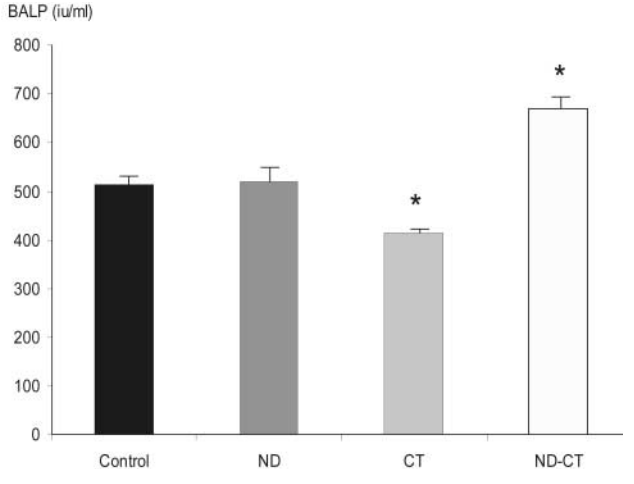

Figure 2. Levels of BALP in the blood. Control: non-treated animals, ND: animals treated with nandrolone decanoate, CT: animals treated with calcitonin, ND-CT: animals treated with nandrolone decanoate and calcitonin. BALP: bone alkaline phosphatase. *$p<0.05$ vs. control and ND groups.

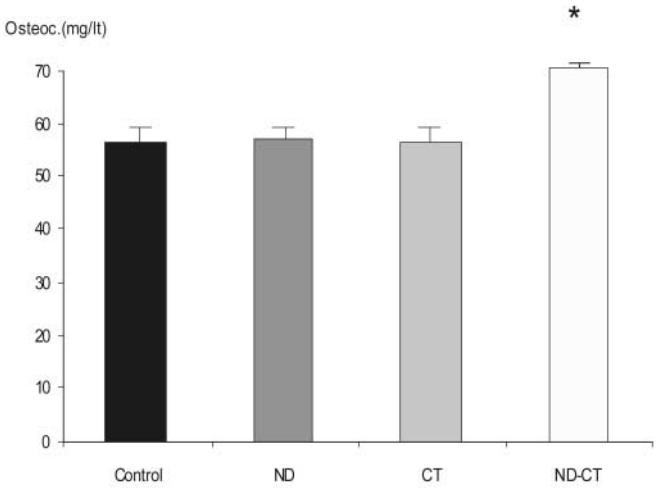

Figure 3. Levels of osteocalcin in blood. Control: non-treated animals, ND: animals treated with nandrolone decanoate, CT: animals treated with calcitonin, ND-CT: animals treated with nandrolone decanoate and calcitonin. Osteoc: Osteocalcin. *p<0.05 vs. other groups.

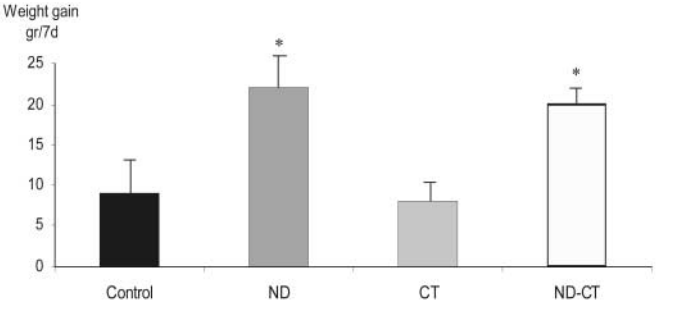

Figure 4. Weight gain during the first week. Control: non-treated animals, ND: animals treated with nandrolone decanoate, CT: animals treated with calcitonin, ND-CT: animals treated with nandrolone decanoate and calcitonin. *p<0.05 vs. control and CT groups.

Estimation of calcium, phosphate and albumin levels in serum

Coadministration of ND with calcitonin or CT alone caused a decrease in calcium levels compared with the control group. Administration of ND did not have any effect on calcium levels compared with the control group.

Changes in phosphate levels were not observed in any group.

Administration of calcitonin alone had no effect on the serum albumin levels compared with the control group. On the other hand, coadministration of ND with CT or ND alone caused an increase in albumin levels compared with the control group (Table 1).

Discussion

In our investigation ND increased IGF-I content without affecting BALP and osteocalcin levels in serum. IGF-I concentration was elevated after ND administration due to its synthesis in many target tissues such as liver[6] and bone[7]. The increase in the levels of serum albumin, a protein synthesized by the liver, after anabolic steroids administration suggested that ND may have an effect on both bone and liver IGF-I.

In contrast, in our study, ND did not affect serum osteocalcin and BALP levels. In this case, it would be proposed that BALP and osteocalcin synthesis reflects different aspects of osteoblastic activity and therefore this effect of ND on their serum levels may indicate that androgens could act at a specific stage of osteoblastic maturation[8].

Finally ND had a positive effect on body weight gain due to both sodium and water retention by the kidney and increase of the metabolic functions[9].

Calcitonin administration resulted in a decrease in BALP concentration in serum. Middle-term treatments, more than five days and/or higher doses have revealed a diminished response or a decrease of BALP in serum, while low dose and short-term treatment are associated with the anabolic action of CT and increased BALP levels[10].

It is well known that CT can decrease growth hormone (GH) concentrations and heparin IGF-I production[11] and otherwise can induce gene expression of IGF-I in osteoblasts[12]. These opposite effects of CT on IGF-I synthesis in different target tissues are of much interest, but are very difficult to explain.

On the other hand we cannot disregard the alternative that CT treatment can decrease the levels of calcium in serum, increasing parathormone (PTH) secretion[13,14]. PTH has an inhibitory effect on differentiated osteoblasts by inhibiting BALP activity, although PTH could stimulate osteoblasts replication by producing growth factors from the matrix, including IGF-I, *in vitro*[14]. Therefore the increase in IGF-I may be attributed to CT-induced proliferation of osteoblasts while the reduction of BALP may be the result of CT on differentation.

	CONTROL	CT	ND	CT-ND
Albumin g/lt	28.8 ± 0.7	28.2 ± 1	33 ± 0.2*	34.5 ± 1*
Phosphate mg/dl	4.1 ± 0.2	4.3 ± 0.15	4.1 ± 0.6	4 ± 0.2
Calcium mg/dl	10.4 ± 0.7	8.1 ± 0.3**	11 ± 1	8 ± 0.7**

Table 1. Biochemical variables following administration of ND and CT. *p<0.05 vs. control and CT, **p<0.05 vs. control and ND.

Another hypothesis could be that IGF-I levels in serum are the result of CT activity in tissues other than bone, such as the pituitary gland, brain and testis[15,16].

Finally, the above data revealed that ND and CT in combined infusion augments the levels of IGF-I, osteocalcin and BALP in serum. An explanation could be that CT and ND have an additive effect on IGF-I production. Furthermore, previous studies have reported that CT causes an upgrade in the secretion of androgens *in vitro*[17]. However *in vivo* studies have not yet confirmed these observations. The significant elevation of BALP and osteocalcin in serum due to their production in bone may be induced by the marked stimulatory effect of IGF-I[18].

We can conclude that parallel administration of CT and ND can modify bone metabolism parameters in serum with two possible mechanisms: a) CT and ND can directly interfere in bone metabolism functions b) CT and ND may trigger systemic production of growth factors such as IGF-I. Subsequently IGF-I may exert a positive effect on osteocalcin and BALP formation in bone, resulting in further modulation of bone metabolism.

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