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Transient changes in thyroid functions tests after zoledronic acid infusion

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Abstract. Zoledronic acid (ZA) induces an acute phase response in association with elevation of serum cytokines, which possibly alter the 3 types of iodothyronine deiodinase activity. We therefore studied the possible alteration in thyroid function tests by ZA. We investigated the acute changes in serum thyroid hormones, TSH, cortisol, white blood cells, CRP, interleukin-6 (IL-6) and tumor necrosis factor (TNF-α), before (0) and 1, 2 and 3 days after iv infusion of 5 mg ZA in 24 asymptomatic postmenopausal women with osteoporosis (ZA group) in comparison with a placebo group. In the majority of patients the ZA infusion was associated with acute phase response and fever within 24h after infusion which became attenuated on day three. Concurrently with increase in serum cortisol, CRP, IL-6 and TNF-α, on day 1 and 2, total serum T3 (TT3), free T3 (fT3), total T4 (TT4) and fT4 decreased with a nadir on day 2 in association with an increase in the fT4/fT3 ratio and reverse T3 (rT3) levels. All thyroid function changes returned to the baseline levels on day 3, with cytokines still at higher levels, although lower than those on day 2. Serum TSH remained essentially unchanged throughout the study. The changes in thyroid hormones were at least in part explained by the increased TNF-α, but not by IL-6. ZA induces short term changes in thyroid hormones, characteristic of nonthyroidal illness syndrome (NTIS), in association with an increase in TNF-α and IL-6.

Key words: Aminobisphosphonates, Thyroid, Lymphocytes, Cortisol, Cytokines

BISPHOSPHONATES are widely used drugs in the treatment of osteoporosis, Paget's disease, multiple myeloma, hypercalceamia of malignancy and palliation of metastatic bone disease [1-3]. The annual intravenous administration of a single dose of the aminobisphosphonate zoledronic acid is now an established therapy in postmenopausal osteoporosis [1]. Side effects that resemble transient flu-like symptoms have been reported after oral or iv bisphosphonate therapy [4]. For example, intravenous administration of aminobisphosphonates usually causes an acute inflammatory response with symptoms including fever, myalgias, vomiting, fatigue and general musculoskeletal pain. Recently this phenomenon was analyzed in a large number of postmenopausal women with osteoporosis [5]. A number of studies have suggested that the stimulation of proinflammatory cytokines, such as IL-6 and TNF-α are important mediators of the acute phase response after the administration of bisphosphonates [6]. On the other hand, the elevation of several proinflammatory cytokines may affect thyroid function tests and may be implicated in the pathogenesis of the NTIS [7]. The entity NTIS is characterized by alterations in serum thyroid function tests that mimic thyroid disorders, in the absence of concurrent hypothalamic–pituitary-thyroid disease [8]. It is very common in severe illness or stressful conditions and represents the net effect of several different disturbances [7]. A low serum T3, normal or low T4 and a high rT3 are commonly observed in NTIS [8]. Additionally, a number of drugs may interact with thyroid hormones and cause changes in thyroid tests [8]. In this regard it would be reasonable to hypothesize that ZA administration could induce NTIS due to the acute phase response (APR).

We investigated whether the iv administration of ZA in patients with osteoporosis induces alterations in several thyroid function tests and cortisol levels similar to those in NTIS. In addition, peripheral blood leukocytes, serum CRP, IL-6 and TNF-α were measured as

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markers of inflammation.

Materials and Methods

This was a placebo-controlled prospective study. Forty seven postmenopausal women with osteoporosis diagnosed for the first time at our outpatient endocrine service were studied. The diagnosis was made incidentally, by a routine screen in asymptomatic, apparently healthy women. The osteoporosis was confirmed by lumbar spine or total hip bone mineral density measurements, corresponding to a T-score of < -2.5. Exclusion criteria from the study were: obesity (BMI > 30 kg/m^2), known thyroid gland abnormalities, other chronic illness, renal failure, stressful conditions or use of bone-modifying drugs. All patients had a calculated creatinine clearance greater than 30 mL/min. After the initial tests, subjects were randomized to receive ZA or placebo. The patients and the investigators were blinded to treatment allocation. To minimize variations of the results the patients who were chosen had no history of bone fractures or severe bone pain and were age and BMI matched. In 24 women, fasting blood samples and fasting double voided urine samples were obtained just before (day 0), and 1, 2, 3 days after the iv infusion over 15 min of zoledronic acid 5 mg solution (Aclasta, Novartis, Basel, Switzerland), in the ZA group. The placebo-control protocol was the same, except that placebo infusion of physiological saline was administered instead of ZA, in 23 women. Serum and urine were stored frozen (-20º C) until the day of the assay. An identical diet was given during the study and was designed to maintain a stable nutritional status. All patients gave informed consent to participate in this study, which was approved by the Scientific Committee of the hospital. The samples of an individual subject were run in the same assay to minimize the inter-assay variation. Peripheral blood leucocytes (PWBC), CRP and creatinine were measured using standard laboratory methods. Serum cortisol, TSH (third generation), total T4 (TT4), total T3 (TT3), freeT4 (fT4), freeT3 (fT3) and cortisol measurements were determined by using the auto-analyzer Immulite 2000 or Centaurus (DPC Limited, San Juan Capistrato, CA). Reverse T3 was measured by RIA (Adaltis Italia S.p.A., BO Italy) with normal range 0.025 to 2.0 ng/mL and inter assay coefficient of variation (CV) 6.21% at 0.582 ng/mL. Urine NTx were measured using ELISA (Osteomark, Ostex International, Inc., Seattle, WA) and were expressed as nmoles of bone collagen equivalents (BCE)/mM creatinine. The sensitivity of the assay was 5.0 nmol/LBCE, the intra-assay CVs were 5.1% and 2.7% and the inter-assay CVs were 10.8% and 7.2% at the 35.3 and 66.7 nmol BCE /mM creatinine levels, respectively. Serum IL-6 and TNF-α were measured by an ultra-sensitive solid phase "sandwich" ELISA (Quantikine, R&D Systems Europe, UK). The normal range (NR) for IL-6 is from 0.378 – 10.1 pg/mL with intra assay CV 5.9 % at the levels of 2.73 pg/mL and inter assay CV 16.5 % at 3.575 pg/mL. The NR for TNF-α is usually non-detectable with intra-assay CV 5.2% at the level of 48.1 pg/mL and inter-assay CV 7.8% at 45.8 pg/mL.

Statistical Analysis

All data are represented as the mean ±SD or SE. In the statistical analysis using two way ANOVA for repeated measurements. The effects of interest were a) between subject effects (such as Group), b) within subject effects (such as Days) and c) between the two types of effects (Groups* Days). Differences of the means of age, BMI and basal variables levels between the 2 groups were evaluated by independent samples *t*-test. Linear regression and multiple regression models were used to assess the association between thyroid hormone levels, cortisol and cytokines. Because many variables were closely inter-related, the possibility of multi-co-linearity was excluded by the estimation of the Variances of Inflation Factors (<2.5) with the estimation of tolerances (>0.89). $p < 0.05$ was considered statistically significant.

Results

The results of the various parameters measured in the 2 groups are summarized in Table 1. Study and control groups were statistically equivalent in terms of age and BMI, as a result of matching and all women were euthyroid. Twenty one patients (87%) in ZA group reported musculoskeletal pain and fatigue within 12-48 hours after the ZA infusion and 19 patients used small doses of acetaminofen on days 2 and 3. In 16/24 (66%) patients, those symptoms were associated with mild fever (37.4 -38.6°C). Two patients experienced breathing discomfort because of severe pleural pain which lasted about 20 hours. As was expected, none of the subjects in the placebo group experienced any

Table 1 Profiles of the various parameters measured at the four different points of time in both groups.

	Day 0	Day 1	Day 2	Day 3
ZA group , n=24				
Age (years)	62.50 ± 2			
Mukoskeletal pain (n, %)	21 (85)			
Fever (n, %)	16 (64)			
uNTx (nM BCE/mM creatinine)	58 ± 36	**19 ± 16***	**14 ± 6***	**12 ± 4***
WBC (x10^3/μL)	6.00 ± 1.58	7.05 ± 0.12	6.40 ± 0.98	6.02 ± 0.12
Ratio NE/LY	1.59 ± 0.86	**8.67 ± 0.07***	**3.47 ± 0.03***	1.62 ± 0.78
TSH (mU/L)	1.97 ± 0.65	2.56 ± 0.53	2.10 ± 0.57	2.23 ± 0.50
TT4 (nmol/L)	138.53 ± 8.70	133.7 ± 17.68	**119.18 ± 27.15***	133.73 ± 8.14
fT4 (pmol/L)	17.11 ± 2.70	15.97 ± 2.44	**14.79 ± 2.48***	16.2 ± 2.51
fT3 (pmol/L)	4.78 ± 0.61	**4.13 ± 0.68**	**3.59 ± 0.83***	4.21 ± 0.92
fT4/fT3 (ng/mL)	3.67 ± 0.89	3.76 ± 0.91	**4.17 ± 1.19***	3.98 ± 0.88
rT3 (ng/mL)	0.12 ± 0.01	**0.38 ± 0.03***	**0.49 ± 0.02***	0.28 ± 0.01
TT3 (nmol/L)	1.72 ± 0.31	**1.50 ± 0.26***	**1.33 ± 0.31***	1.65 ± 0.28
Cortisol (nmol/L)	240 ± 78	**405 ± 164***	**321 ± 139***	247 ± 79
CRP (mg/dL)	0.37 ± 0.13	**1.97 ± 2.10***	**4.32 ± 4.1***	**1.5 ± 0.8***
IL-6 (pg/mL)	1.41 ± 0.85	**8.16 ± 4.8***	**5.66 ± 3.68***	**4.99 ± 2.1***
TNF-α (pg/mL)	1.69 ± 2.01	**10.27 ± 7.30***	**11.28 ± 9.1***	**6.70 ± 4.60***
Placebo group, n=23				
Age (years)	64.04 ± 3			
uNTx (nM BCE/mM creatinine)	57 ± 31	55 ± 29*	59 ± 31*	57 ± 30*
WBC (x10^3/μL)	6,12 ± 1,5	5,94 ± 1,48	6,22 ± 1,20	6,01 ± 0,11
Ratio NE/LY	1.60 ± 0.75	1.55 ± 0.84*	1.52 ± 0.68*	1.61 ± 0.54
TSH (mU/L)	2.01 ± 0.55	1.97 ± 0.46	2.22 ± 0.45	2.1 ± 43
TT4 (nmol/L)	138.70 ±11.65	139.79 ± 10.51	138.10 ± 11.40*	140.87 ± 11.12
fT4 (pmol/L)	17.71 ± 1.92	17.99 ± 1.98	18.06 ± 1.87*	17.66 ± 1.73
fT3 (pmol/L)	4.38 ± 0.48	4.51 ± 0.53*	4.47 ± 0.54*	4.42 ± 0.62
fT4/fT3	3.60 ± 0.74	3.39 ± 0.80	3.70 ± 0.84	3.64 ± 0.82
rT3 (ng/mL)	0.14 ± 0.02	0.089 ± 0.016*	0.14 ± 0.04*	0.16 ± 0.06
TT3 (nmol/L)	2.1 ± 0.25	1.99 ± 0.21	2.01 ± 0.19	1.98 ± 0.22
Cortisol (nmol/L)	228 ± 65	232 ± 64*	221 ± 55*	270 ± 59
CRP (mg/dL)	0.33 ± 0.10	0.32 ± 0.12*	0.34 ± 0.11*	0.33 ± 0.11*
IL-6 (pg/mL)	1.12 ± 0.56	1.09 ± 0.45*	1.02 ± 0.46*	1.09 ± 0.4*
TNF-α (pg/mL)	1.59 ± 2.01	1.68 ±2.4*	1.98 ± 2.21*	1.62 ± 2.42*

Values shown are means ± SD. Values in bold are statistically significant from baseline within the same group. Asterisks show differences between 2 groups at a given time.

significant clinical change. White blood cells were slightly increased on day 1, but not to a significant degree, whereas significant changes were found in the ratio of neutrophils / lymphocytes (NE / LY), Table 1. Fig. 1 displays the time course (mean ± SD) in uNTx, serum CRP, thyroid function tests, cortisol, IL-6 and TNF-α levels on days 0, 1, 2 and 3. No significant differences were found in the mean basal serum parameters between the two groups of subjects. Fig. 1A illustrates the alterations in uNTx levels, and verifies the efficacy of ZA administration, as controls injected with placebo displayed no significant changes. A significant reduction in the mean values of uNTx was observed within 24 h after the ZA infusion and remained constantly low until the end of the study. In ZA group a marked increase in serum CRP, IL-6 and TNF-α was observed on days 1 and 2 followed by a rapid decrease thereafter (on day 3), but remaining at higher levels compared with the baseline or with the placebo group, Fig. 1D, 1 E, 1F, Table 1. Only one of the 24 patients receiving ZA had no change in CRP, whereas in all cases, serum IL-6 and TNF-α levels increased significantly. Total T3 and fT3 declined significantly on days 1 and 2 after ZA infusion, (day 0 *vs* day 1, $p = 0.01$, day 0 *vs* day 2,

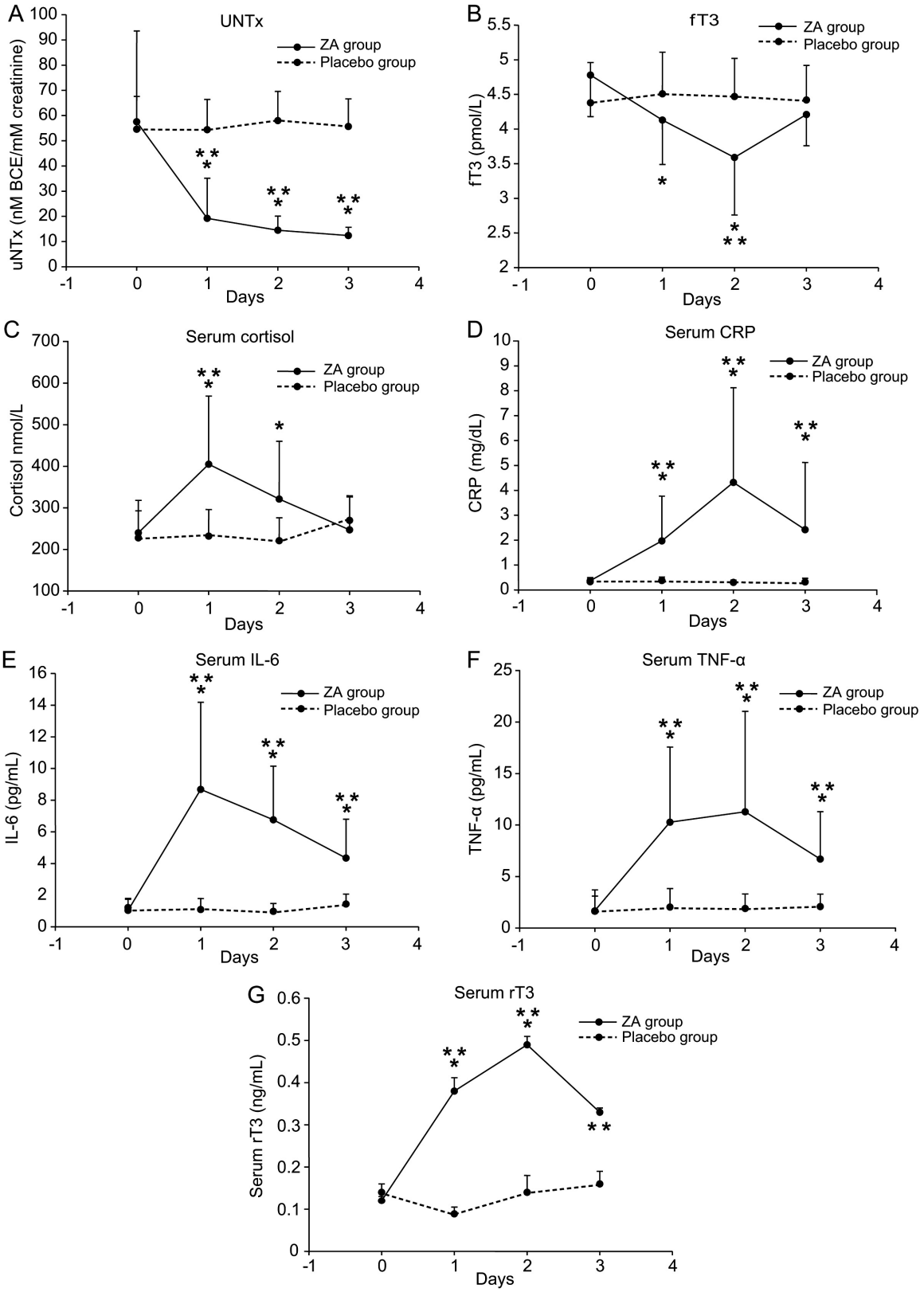

Fig. 1 Changes in serum uNTx, fT3, cortisol, CRP, IL-6, TNF-α and rT3 before (0) and 1, 2 and 3 days after ZA or placebo infusion. Values are the means ± SD. At a given time an asterisk indicates significant difference between two groups and two asterisks significant differences within the same group.

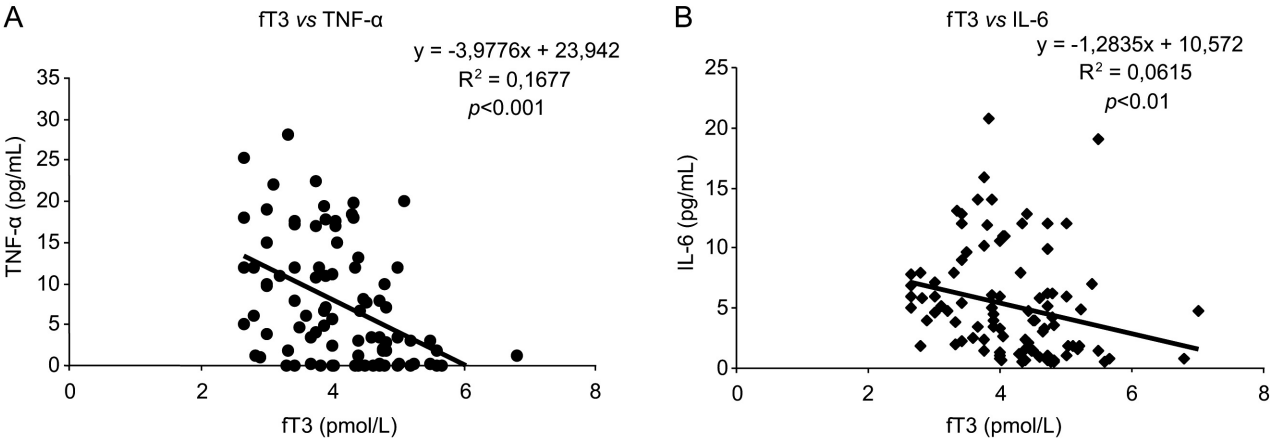

Fig. 2 Linear correlations between fT3 and TNF-α (A) and between fT4/fT3 and TNF-α (B) in ZA group. The values at the different points of time were taken as a combined group, n= 96.

p <0.001) whereas on day 3 levels started to return to the baseline concentrations, p = 0.058, Table 1, Fig. 1B. Individual analysis showed that the transient decrease in serum TT3 and fT3 levels was observed in all patients of the ZA group and in 6/24 (25%) both hormones declined below the lower limit of normal (0.84 ± 0.1 nmol/L and 2.88 ± 0.1 pmol/L respectively). In the placebo group TT3 and fT3 remained within the normal range, with no significant differences from the baseline levels throughout the study. Regarding the effect of ZA the different variables in the two-way ANOVA/repeated measures analysis showed significant differences between the two groups. For example the effect of ZA revealed significant effect regarding the fT3 (F=11.00, p=0.002), fT4 (F=6.46, p=0.01), rT3 (F=92,00 p<0.001), IL-6 (F=78.55, p<0.001) and TNF-α (F=43.24, p<0.001). Free T3 concentrations between the 2 groups at the different sampling times showed that fT3 levels remained significantly lower in the ZA group versus placebo group for the 2 days after ZA infusion, Table 1 (day 1, p=0.028, day 2, p<0.001) whereas on day 3 and fT3 levels were similar in both groups (ZA group *vs* placebo group on day 3, p=0.122). Almost the same pattern of changes were shown in serum TT4 and fT4 levels except that the decrease at a significant level was only on day 2, Table 1. In contrast, rT3 increased significantly the first day and remained significantly high until the end of the study (Fig. 1G). In 3 of 6 subjects with subnormal TT3 and fT3 serum TT4 and fT4 levels also declined below normal (range 64 – 66 nmol/L for TT4 and range 11.7 – 11.9 pmol/L for fT4). In none of them did TT4 declined below 52 nmol/ L [8], but it should be noted that the lowest thyroid hormone levels were observed in the two patients with the breathing discomfort. The time course of rT3, TT3, fT3, fT4/fT3, IL-6, TNF-α and CRP seemed to be closely linked, Fig. 1. In addition, the fT4/fT3 ratio started to increase with a peak value on day 2, in parallel with rT3, (Table 1). The fluctuations in serum TSH were not statistically significant and were almost identical in both groups, Table 1.

Cytokines, CRP and serum cortisol

The peak serum TNF-α and CRP occurred concurrently with the lowest TT3, fT3, TT4 and fT4, and the highest fT3, whereas the peak serum IL-6 was observed 24 h earlier with the beginning of the decline of T3, Figs. 1B, 1E, 1F. The PWBC tended to increase on days 1 and 2 , but did not reach a statistically significant level, (Table 1). In contrast, neutrophils increased significantly, whereas lymphocytes showed a significant decrease on days 1 and 2. Therefore the ratio NE/LY was increased (day 1, p<0.001, day 2, p <0.001) concomitantly with the peak in serum cortisol, (Fig. 1C). Cortisol may be responsible for the significant changes in PWBC. The correlation was stronger between fT3 and TNF-α (r = - 0.408, p<0.001), (Fig. 2A), fT4 and TNF-α (r =- 0.362, p<0.01) and between fT4/fT3 and TNF-α (r = + 0.148, p~0.09) in comparison with the correlations between fT3 and IL-6 (Fig. 2B), and fT4/fT3 and IL-6, whereas fT4 was not correlated with IL-6. In contrast, rT3 showed strong positive correlation with both TNF-α and IL-6 (r = 0.497 and r = 0.492) respectively, but not with cortisol, r = 0.09.

Multiple regression analysis

Free T3, fT4 and rT3 were designated as the dependent variables, whereas IL-6, TNF-α, CRP and cortisol were included as independent variables. We found that fT3 was independently negatively associated with TNF-α with $r^2 = 0.39$ (β -0.137; SE 0.046; 95% CI -0.229 to -0.045, p=0.004), whereas there was no significant association with CRP, $r^2 = 0.12$ (β -0.219; SE 0.126; 95% CI -0.470 to 0.032, p=0.086), IL-6, $r^2 = 0.10$ (β 0.048; SE 0.076; CI -0.104 to 0.199, p=0.053) or cortisol, $r^2 = 0.08$ (β 0.003; SE 0.003; 95% CI –0.002 to 0.009, p=0.218). The independent variable TNF-α explained 39% of the variation of fT3 levels, whereas the variation in fT3 levels by IL-6, CRP and cortisol was explained by 10%, 12% and 8% respectively. Similarly, fT4 was independently negatively associated with TNF-α, $r^2 = 0.14$, (β -0.027; SE 0.010; 95% CI -0.046 to -0.007, p=0.009), whereas there was no significant association between fT4 and CRP, $r^2 = 0.07$ (β -0.173; SE 0.135; 95% CI -0.440 to -0.095, p=0.240), fT4 and IL-6, $r^2 = 0.04$ (β -0.024; SE 0.016; 95% CI -0.57 to -0.008, p=0.148) or fT4 and cortisol, $r^2 = 0.09$ (β 0.019; SE 0.23; 95% CI -0.026 to 0.065, p=0.397). Once again the variation in fT4 levels was explained by 14% from the variable TNF-α and only 7%, 4% and 9% from the variables CRP, IL-6 and cortisol respectively. Almost similar results were observed between cytokines and TT4 and TT3 (data not shown). Considering the rT3 as the dependant variable and in accordance with the previous results of correlations the variability of rT3 levels was explained by 38% from the TNF-α ($r^2 = 0.38$, p<0.001), by 35% from IL-6 ($r^2 = 0.35$, p<0.001) and only by 10% from the cortisol, $r^2 = 0.10$, p=0.30.

Discussion

In this study, under well controlled conditions, we demonstrated that the infusion of ZA in postmenopausal osteoporotic women induces alterations in thyroid function tests, probably *via* the acute-phase response that occurs within 24 h after the infusion.

The acute significant fall in uNTx, a marker of bisphosphonates effectiveness, was associated with a rapid decrease in serum thyroid hormones which in some cases fell below normal values. These changes, which were similar to those associated with NTIS, were an early event in our patients and they appeared at the same time as the significant increase in serum CRP, IL-6, TNF-α and cortisol.

Elevations in cytokine concentrations are routinely cited as potential actors in the pathogenesis of NTIS. In the present study, the changes in serum thyroid hormone levels started after 24 h after the ZA administration, in association with the "flu-like" symptoms, and the lowest serum levels were found 48 h after the ZA infusion. On day three, symptoms start to attenuate with thyroid hormones returning toward the baseline levels, concurrently with the decrease of CRP, IL-6, TNF-α and cortisol. Acute phase response with increased levels of cytokines in the first days after the use of iv aminobisphosphonates have been reported in previous studies, with a median duration of about 3 days [4-5]; this was also the case in our study. Dicuonzo *et al*. observed that ZA administration to cancer patients with bone metastases, induces fever with transient increase in serum IL-6 and TNF-α levels [6].

We can hypothesize that, in our patients, the cytokines acutely altered the iodothyronine deiodinases D1, D2 and D3 expression and activity, reflecting the low conversion of T4 to T3 [9-11]. This is supported by the observations of the increase in rT3 levels and the increase in fT4/fT3 ratio. These alterations tend to return to the baseline levels within 3 days from the end of ZA infusion and accompany resolution of the symptoms, probably due to the rapid decline of ZA in the circulation [12]. Moreover, the combination of reduced serum TT4 and TT3 levels, usually found in more severe NTIS [8], shows, that in the short term, additional mechanisms were activated in our patients [3]. Some patients experienced severe symptoms for almost 24 hours, but in none of them did TT4 decrease to less than 52 nmmol/L (as occurs in fatal diseases) [8]. Despite the slight decrease of fT4 and TT4 the fT4 /fT3 and TT4/TT3 ratios were in favor of TT4 and fT4 showing again declined peripheral conversion of T4 to T3. It could be possible that the depression of both thyroid hormones is simply a reflection of haemodilution or a change in circulating protein levels, but the unchanged hematocrit and serum albumin concentrations (data not shown) did not support this hypothesis. It could be also suggested that the use of acetaminofen diminished the degree of the APR events and thus inhibited the progression to more severe NTIS, although it is known that this drug is a weak inhibitor of the synthesis of prostaglandins [13] and furthermore has no significant effect on serum thyroid hormone levels [14]. We can also hypothesize that acetaminophen

interfers with the thyroid binding globulin as do other common anti-inflammatory agents, but, to our knoweledge, this effect has not been described [15]. Again, this could not be an issue, because the duration of NTIS was the same in the 6 patients who did not receive acetaminophen (data not shown). The rapid clearance of ZA from the circulation, inhibited the effector cells in the periphery that released cytokines and consequently the NTIS was reversible within 3 days.

It has been shown that aminophosphates activate a large number of peripheral γδ T cells, a main source of IL6 and TNF-α production [16]. At present, the only hypothesis we can make is that after ZA infusion and at the time of the lowest thyroid hormone levels, total PBWCs count were slightly changed, whereas blood lymphocytes were significantly decreased, in comparison with the neutrophil count, possibly as a direct action of cortisol and this may explain similar observations in the literature.

However, we can not exclude the possibility that the ZA amino groups are involved in the pathogenesis of NTIS, independently of serum cytokines, either directly acting on thyroid cells or *via* assay interference or *via* peripheral actions, acting as inhibitors of deiodinases. It would be interesting to know whether by the use of a non-amino-bisphosphonate drug, that does not activate an APR [17], the NTIS could be avoided. It is difficult to interpret the normal fluctuations of serum TSH because it would be expected to be low if we suggested that a central hypothyroidism had occurred, but this is not the rule in the NTIS and it is well known that the concentration of TSH may be low, normal or slightly elevated [18].

The high mean cortisol levels 24 and 48 h after the infusion could explain the failure of TSH to increase. On the other hand, the transient increase of cortisol was not sufficient to suppress TSH to very low levels and this also indicates the absence of central hypothyroidism. The simultaneous increase of cortisol and cytokines with the beginning of the decline in serum thyroid hormones may show that cortisol is responsible for the alterations of T4 and T3 acting at peripheral level. However, cortisol could not explain at a significant level the changes we observed and this is in accordance with experimental studies showing that cortisol does not play a dominant role in NTIS [19].

We do not know how early the NTIS appeared in our cases since the first measurements were 24 hr after ZA infusion and the possibility that we may have missed earlier significant changes cannot be excluded. Studies have shown that after acute stress, for example after myocardial infarction, the decrease in T3 appears within 24 h after the heart attack and lasts until recovery [20]. In surgical stress the decline in T3 appears within 30 minutes of the beginning of the surgical procedure, before skin incision and thus is an early event, before the rise of IL-6 and TNF-α [21]. The fact that the elevation in CRP, IL-6 and TNF-α concomitantly with the increase in rT3 levels and fall in serum thyroid hormone levels raises again the issue that cytokines may be one of the causative factors of the changes in our patients. In our cases, the stronger negative correlation between fT3, fT4 and rT3 with TNF-α in comparison with IL-6 supports the hypothesis that TNF-α plays more prominent role in the induction of the acute NTIS after ZA infusion. This is also consistent with the observation that the highest rT3 and the lowest TT3, fT3, TT4 and fT4 levels occurred concurrently with the peak values of serum TNF-α and CRP.

The NTIS has been extensively studied in animals [22]. In humans, most studies are related with critical or non-critical diseases and stressful conditions preceding the appearance of NTIS [23-26, 9]. Our patients were not under stress before ZA infusion, and had only feelings of well being, a condition which is very rare in human studies related to the NTIS. The small sample size may have provoked an overestimation of our results considering a condition like NTIS. However, from the fact that changes in peripheral thyroid hormones were observed in all ZA patients, it is concluded that undoubtedly the ZA infusion affects thyroidal function tests, possibly with no severe clinical consequences, but may be a clinical model for further investigations, with earlier sampling times to see in detail the changes in parameters which were measured. It must be noted that, because the machanism for the NTIS is not universal in all cases, the above changes may be the case in ZA-induced NTIS, but may not be applicable to all cases with a real NTIS.

In conclusion, we observed that ZA infusion induces severe, but short term significant changes in peripheral thyroid hormones which resemble those observed in NTIS. The increase in serum cytokines may at least in part explain these changes.

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