Increased transforming growth factor- β 1 plasma concentration is associated with high plasma 3,3',5'-tri-iodothyronine in elderly patients with nonthyroidal illnesses

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Abstract

Objective: To study transforming growth factor- β 1 (TGF- β 1) plasma concentrations in elderly patients with nonthyroidal illnesses (NTI).

Design: Case-control study.

Methods: We measured plasma concentrations of tri-iodothyronine (T_3), reverse T_3 (rT_3), thyroxine (T_4), free T_3 (fT_3) and free T_4 (fT_4) estimates, TSH, and TGF- β 1 in 48 elderly NTI patients consecutively admitted in our Division of Internal Medicine and Metabolic Diseases, and in 11 healthy age- and sex-matched controls.

Results: The data on thyroid hormones enabled us to identify three groups: Group A, subjects (8 patients) with T_3 and fT_3 levels comparable to those in controls; Group B, subjects (30 patients) with T_3 and fT_3 levels lower than controls but rT_3 levels comparable to those of controls; Group C, subjects (10 patients) with T_3 and fT_3 levels lower than those of controls and higher rT_3 levels. The patients of Group C showed higher plasma levels of TGF- β 1 compared with controls. Moreover, we found a positive correlation between TGF- β 1 and rT_3 ($r_s = 0.38$, $P < 0.01$) in the whole group of NTI patients.

Conclusions: Our data seem to confirm the hypothesis that TGF- β 1 could play a role in the pathogenesis of some modifications of thyroid function observed in patients with nonthyroidal illnesses.

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Introduction

In elderly patients with nonthyroidal illnesses (NTI), decreased plasma tri-iodothyronine (T_3) and free T_3 (fT_3), and increased plasma reverse T_3 (rT_3) levels are frequently observed, while thyrotropin (TSH) usually remains normal. These changes in thyroid hormone metabolism are referred to as the 'sick euthyroid syndrome'. The lower T_3 and higher rT_3 plasma values are determined by a lower T_3 production rate and a decreased rT_3 clearance rate due to diminished iodothyronine 5'-deiodinase activity (1).

Cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), and interferon- γ (IFN- γ), have recently been shown to have an inhibitory influence on the hypothalamic–pituitary–thyroid axis *in vivo* and *in vitro*. In particular these cytokines inhibit [125 I]iodide transport and organification and type I deiodinase in rat thyroid cells (2–5). Several authors have indicated TNF- α , IL-1, IL-6, and acid and basic fibroblast growth factor as putative mediators of the modifications in thyroid function observed in NTI

patients (6–9). Elsewhere we reported on the links between the low T_3 syndrome and erythropoietin and TNF- α plasma concentrations in NTI patients (10).

Transforming growth factor- β 1 (TGF- β 1) is a cytokine that inhibits *in vitro* growth, differentiation and 5'-deiodinase activity in thyroid cells (5, 11). These findings allowed us to hypothesize that TGF- β 1 could play a role in the pathogenesis of the thyroid dysfunction of NTI patients. In order to verify this hypothesis, we evaluated TGF- β 1 plasma concentrations and thyroid function tests in elderly patients with a variety of nonthyroidal illnesses.

Subjects and methods

Our series consisted of 60 elderly informed consent patients with nonthyroidal illnesses consecutively admitted in our division. Patients with thyroidal, hypophyseal or hypothalamic disease ($n = 7$), those on drugs affecting the metabolism or regulation of thyroid and pituitary hormones (i.e. glucocorticoids,

propranolol, amiodarone, estrogens and antiepileptics) ($n=3$), and those treated with cytokines (i.e. IL-2 or IFN- α in malignant tumors) ($n=2$), were excluded from the study. The remaining 48 patients (23 females, 25 males; mean age 73.1 ± 5.4 years) were not on dopamine and/or drugs known to interfere with thyroid hormone assays, and presented with the following diagnoses: 10 congestive heart failure (CHF), 14 diabetes mellitus in a poor metabolic control (NIDDM), 10 chronic liver disease (CLD), 8 chronic renal failure (CRF), and 6 neoplasia. The control group consisted of 11 age- and sex-matched healthy subjects. Nutritional status was evaluated in all subjects by the measurement of the following parameters: body mass index, lymphocytes, serum iron, hemoglobin, plasma albumin, transferrin and cholesterol. Plasma was obtained from all subjects after centrifugation of whole blood samples with EDTA, taken after an overnight fast, and was stored at -70°C until the assay.

Plasma T_3 , thyroxine (T_4), TSH, fT_3 and free T_4 (fT_4) estimates were evaluated with an ELISA kit (Enzy-mun Test, Boehringer Mannheim Immunodiag-nostics, Milan, Italy). Plasma rT_3 was evaluated with an RIA kit (Reverse T_3 , Biodata, Guidonia Montecelio, Italy).

Plasma TGF- $\beta 1$ immunoassay was performed with an ELISA kit (Predicta TGF- $\beta 1$ kit, Genzyme Diagnostics, Cambridge, MA, USA). The interassay variation for the TGF- $\beta 1$ method was 5.2% at 1.44 ng/ml and 4.8% at 3.1 ng/ml. The intraassay variation was 5.2% and 4.8% respectively.

Statistical analysis was performed using the Kruskal–Wallis nonparametric test because data did not involve distributional assumptions. Spearman's correlation coefficient was used for evaluation of the relationship between TGF- $\beta 1$ and thyroid function parameters. Data were expressed as mean $\pm$ s.d. or median with 25th and 75th percentiles when appropriate. Values of $P < 0.05$ were considered significant.

Results

The data on thyroid hormones allowed us to identify the following groups: Group A, subjects (8 patients) with T_3 and fT_3 estimate levels comparable to those in controls; Group B, subjects (30 patients) with T_3 and fT_3 estimate levels lower than controls but rT_3 levels comparable to those of controls; Group C, subjects (10 patients) with T_3 and fT_3 estimate levels lower than those of controls, and higher rT_3 levels (Table 1). Diagnoses of patients' illnesses in the groups studied were: Group A: three NIDDM, one CHF, two CLD, one CRF and one neoplasia; Group B: ten NIDDM, seven CHF, four CLD, six CRF and three neoplasia; Group C: one NIDDM, two CHF, four CLD, one CRF and two neoplasia.

The patients of Group C showed significantly higher TGF- $\beta 1$ levels with respect to controls (Table 1). Data values for plasma TGF- $\beta 1$ in the groups studied are shown in Fig. 1.

No statistically significant difference was found, amongst the groups studied, as regards the nutritional status.

Spearman's correlation analysis performed on the whole group of NTI patients showed a positive correlation between TGF- $\beta 1$ and rT_3 ($r_s = 0.38$, $P < 0.01$) (Fig. 2). This correlation was not found within each group. No correlation was detectable between TGF- $\beta 1$ and T_3 , T_4 , fT_3 and fT_4 estimates, and TSH.

Furthermore, when we divided NTI patients in subgroups according to their diagnoses, in order to investigate whether any of the underlying illnesses could affect plasma TGF- $\beta 1$ levels regardless of the thyroid status, no statistically significant differences were found amongst the subsets of NTI patients for both thyroid function and plasma TGF- $\beta 1$ levels. Moreover, within the diagnosis subgroups, no correlation was found between TGF- $\beta 1$ plasma concentrations and rT_3 , T_3 , T_4 , fT_3 and fT_4 estimates, and TSH.

Table 1 General characteristics, thyroid function and TGF- $\beta 1$ plasma levels in 48 consecutive elderly patients with nonthyroidal illness and in 11 healthy subjects. Data are expressed as median, with 25th and 75th percentiles (the interquartile range) in parentheses (Kruskal–Wallis test). Group A: T_3 , fT_3 and rT_3 levels comparable to those of controls. Group B: low T_3 and fT_3 levels but normal rT_3 compared with controls. Group C: low T_3 and fT_3 , and high rT_3 levels compared with controls.

	Normal range	Controls ($n = 11$)	Group A ($n = 8$)	Group B ($n = 30$)	Group C ($n = 10$)
Age (years $\pm$ s.d.)	—	72.3 ± 6.5	71.9 ± 6.3	73.1 ± 5.3	73.8 ± 5.3
Sex (M/F)	—	6/5	5/3	14/16	6/4
rT_3 (ng/ml)	0.09–0.35	0.19 (0.12–0.21)	0.16 (0.14–0.20)	0.15 (0.13–0.21)	0.48 ^{a,c,f} (0.37–0.65)
T_3 (nmol/l)	1.20–2.70	1.40 (1.30–1.40)	1.50 (1.35–1.55)	1.20 ^{a,c} (1.10–1.20)	1.20 ^{a,c} (1.00–1.20)
T_4 (nmol/l)	58.0–151.0	90.1 (72.1–102.9)	89.4 (77.2–96.5)	84.9 (77.2–95.2)	91.4 (87.5–95.2)
fT_3 estimate (pmol/l)	5.40–9.30	7.40 (6.91–7.68)	6.83 (6.45–7.68)	6.30 ^{a,d} (6.00–6.45)	6.00 ^{a,d,g} (5.53–6.30)
fT_4 estimate (pmol/l)	11.8–24.6	16.1 (12.3–20.1)	15.4 (14.4–15.8)	15.7 (13.2–17.2)	15.8 (14.5–16.6)
TSH ($\mu\text{U/ml}$)	0.23–4.00	1.05 (0.61–2.00)	1.05 (0.86–1.40)	1.00 (0.70–1.90)	0.98 (0.55–1.50)
TGF- $\beta 1$ (ng/ml)	28.0–157.0	42.5 (25.0–80.0)	47.5 (35.0–57.5)	34.0 (22.5–59.0)	112.5 ^{b,e,g} (80.0–157.0)

^a $P < 0.001$, ^b $P < 0.05$ vs controls; ^c $P < 0.001$, ^d $P < 0.01$, ^e $P < 0.05$ vs group A; ^f $P < 0.001$, ^g $P < 0.01$ vs group B.

Figure 1 Plasma TGF- β 1 in 48 consecutive NTI elderly patients and in controls ($n = 11$). Median values are indicated by lateral arrows.

Discussion

Our findings confirm that elderly NTI patients have an altered endocrine thyroid function. In fact, 40 of the 48 subjects examined had reduced T₃ concentrations, and 10 of these had a typical 'low T₃ syndrome', with an increase in rT₃ concentrations.

The main findings in our study were the higher TGF- β 1 levels in patients with low T₃ syndrome and the direct correlation between TGF- β 1 and rT₃ found in the whole group of NTI patients. This could lead us to hypothesize a causative role for TGF- β 1 in mediating some of the alterations in thyroid function observed in NTI patients.

Although TGF- β 1 shows a regulatory action on the growth and differentiation of thyroid cells (12), and a powerful inhibitory action on type I deiodinase *in vitro* (5, 11), little is known about the effect of this cytokine on thyroid function *in vivo*. Moreover, only the NTI patients with low T₃ syndrome (Group C), in which a

reduced 5'-deiodinase activity was generally shown, had higher TGF- β 1 plasma levels. The lower T₃ and higher rT₃ levels found in this group could represent, at least in part, a consequence of an inhibitory effect of TGF- β 1 on type I deiodinase *in vivo*, as also suggested by the direct correlation found between TGF- β 1 and rT₃ in the whole group of NTI patients. On the other hand, the lack of correlations when the groups are considered separately apparently rules out the possibility that the peculiar endocrine status of the patients of each group could affect TGF- β 1 plasma concentrations. The direct correlation found only in the whole group of NTI patients seems to indicate a major influence of chronic diseases on TGF- β 1 plasma levels. Regarding the high concentrations of TGF- β 1 in Group C patients, it cannot be ruled out that this may depend on the greater severity of the disease in these subjects. This could have caused an increase in the plasma concentrations of TGF- β 1, which could in turn have determined the particular endocrine pattern of 'sick euthyroid syndrome' found by us.

Figure 2 Relationship between TGF- β 1 and rT₃ ($r_s = 0.38$, $P < 0.01$) in the whole group of elderly patients with nonthyroidal illnesses ($n = 48$).

Moreover, the recent finding that TGF- β 1 expression is induced by TNF- α and IL-1 β in rat pulmonary arterial endothelial cells (13) suggests that TGF- β 1 expression could be stimulated by these cytokines also in other cell lines. This hypothesis has been confirmed *in vitro* in aged FRTL-5 rat thyroid cells, in which TNF- α induced TGF- β expression at the transcriptional level (14). These findings suggest another interpretation of our data. Since several authors have indicated TNF- α , IL-1, and other cytokines as putative mediators of the modifications in thyroid function observed in NTI patients (6–9), and higher TNF- α levels were found in NTI patients with alterations of thyroid function (8), one could hypothesize that the inhibitory action of TNF- α on thyroid function is not only a direct one, but could also be mediated by other cytokines, such as TGF- β 1.

Nor can it be ruled out that the effect of TGF- β 1 on thyroid hormones may be mediated by other cytokines, such as IL-6, which are known to affect thyroid function (7). Stouthard *et al.* (15), for example, have shown in humans that chronic IL-6 administration triggers a reduction in the plasma T₃ concentrations and increases rT₃ and fT₄. As TGF- β 1 can induce IL-6 expression (16, 17), it appears likely that the effects of TGF- β 1 on thyroid function are, at least in part, mediated by IL-6; anyway, findings obtained under different experimental conditions led us to hypothesize a more complex modulatory effect of TGF- β 1 on IL-6 expression (18).

Our observations, together with the previously reported *in vitro* findings, suggest that some of the alterations in thyroid function parameters in nonthyroidal illnesses may be related to higher TGF- β 1 plasma concentrations.

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