Interleukin-6 and its considerable role in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in postmenopausal women

Interleukina 6 i jej istotna rola w patogenezie zaburzeń obrotu kostnego w przebiegu tyreotoksykozy u kobiet po menopauzie

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Abstract

Background: Thyrotoxicosis is more frequent in postmenopausal women than in the general population, effectively accelerating bone turnover. Interleukin-6 has been shown to be involved in the pathogenesis of bone disorders. Thus, the aim of the present study was to assess the role of IL-6 and its soluble receptor in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in oestrogen-deficient women.

Material and methods: The study was carried out in 40 subjects with toxic nodular goitre in three groups: Group 1 — 13 premenopausal females, mean age 36 ± 15 years (PremTxÆPremEu); Group 2 — 12 postmenopausal females, mean age 66 ± 14 years (PostTxÆPostEu); and Group 3 — 15 males, mean age 45 ± 21 years (MTxÆMEu). Overt thyrotoxicosis and euthyreosis after treatment with thyrostatics were confirmed by thyrotropin, free thyroxine and free triiodothyronin concentrations. Serum levels of bone turnover markers: TRACP5b and osteocalcin as well as serum IL-6 and IL-6sR were determined using ELISA kits.

Results: TRACP5b/osteocalcin quotient was significantly elevated in the PostTx females compared to the PremTx women (p < 0.02). There was a positive correlation between serum TRACP5b and osteocalcin in the studied patients (R = 0.45, p < 0.001). Levels of serum IL-6 values were significantly elevated in PostTx: 3.0 (2.14–6.40) and MTx: 2.24 (1.60–5.10), compared to PremTx females: 1.39 (0.96–2.14) (p < 0.01 and p < 0.05 respectively). There were significant positive correlations between IL-6 and IL-6sR concentrations (R = 0.22, p < 0.05) and between IL-6sR and TRACP5b serum levels (R = 0.23, p < 0.05).

Conclusions: The results of our study suggest that interleukin-6 plays a considerable role in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in oestrogen-deficient women.

Key words: IL-6, IL-6sR, hyperthyrosis, osteoporosis, menopause

Introduction

The early years of the menopause are usually characterised by a loss of bone mass that leads to reduced skeletal strength and increased susceptibility to frac-
formation, resulting in a loss of about 10% of mineralised bone per cycle [1].

Oestrogen depletion in the menopause is accompanied by an increased production of osteotrophic factors, such as interleukin-1 (IL-1), IL-6 and tumour necrosis factor [2, 3]. Those cytokines stimulate the differentiation of myeloid precursor cells into osteoclasts, cells that are responsible for bone resorption [4].

Interleukin-6 has been shown to be involved in the pathogenesis of several bone diseases characterised by a negative balance between bone resorption and formation, such as Paget’s disease, renal osteodystrophy and postmenopausal osteoporosis [5–7]. Moreover, in ovariectomised mice, IL-6 appeared to be crucial in bone turnover acceleration and a selective inhibitor of IL-6 activity has been documented to suppress bone mass reduction [8, 9]. Recently, we have seen experimental data suggesting an important role of IL-6 in thyrotoxicosis-related disturbances in bone turnover [10].

Interleukin-6 exerts its full effect through binding to both of its glycoprotein receptors present on target cells: IL-6R and gp130. A complex of IL-6 and a soluble form of IL-6R (IL-6sR) cleaved from the cell membrane may connect to membrane gp130 that leads to biological action [11].

This current study was an attempt to assess the role of IL-6 and its soluble receptor in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in oestrogen-deficient women.

Material and methods

The study was carried out in forty subjects with toxic nodular goiter divided into three groups:

Group 1 — 13 premenopausal females, mean age 36 ± 15 years (PremTx Æ PremEu);
Group 2 — 12 postmenopausal females, mean age 66 ± 14 years (PostTx Æ PostEu);
Group 3 — 15 males, mean age 45 ± 21 years (MTx Æ MEu).

Overt thyrotoxicosis and euthyrosis after treatment with thyrostatics was confirmed by thyrotropin, free thyroxine and free triiodothyronin concentrations. No acute infections were observed in the studied subjects three weeks prior to the study.

Serum was collected before treatment and in stable euthyrosis state and kept frozen at minus 70°C until used. The serum levels of bone turnover markers: osteoclast-derived tartrate-resistant acid phosphatase form 5b (TRACP5b) and osteocalcin were determined by enzyme-linked immunosorbent assay commercial kits produced by Immunodiagnostics Systems Ltd, Boldon, UK: TRACP5b (BoneTRAP Assay; sensitivity 0.5 U/l; intra-assay precision 6.6%; inter-assay precision 7.2%), osteocalcin (N-MID Osteocalcin ELISA; sensitivity 0.5 ng/ml; intra-assay precision 4.2%; inter-assay precision 2.2%) and BAP (OstaseBAP; sensitivity 0.5 ng/ml; intra-assay precision 4.2%; inter-assay precision 2.2%). The serum levels of IL-6 and its soluble receptor (IL-6sR) were estimated using R&D Systems, Minneapolis, MN, USA commercial kits: IL-6 (Quantikine; sensitivity 0.7 pg/ml; intra-assay precision 2.0%; inter-assay precision 3.8%) and IL-6sR (Quantikine; sensitivity 1.5 pg/ml; intra-assay precision 2.3%; inter-assay precision 4.7%).

The statistical significance was estimated by Mann-Whitney test. To evaluate relationships between variables, Spearman’s test was performed using Statistica 9.0 for Windows XP (StatSoft, Tulsa, OK, USA).

Results

Figure 1 shows medians, interquartiles and total ranges of the quotient of serum concentrations of TRACP5b (U/L) and osteocalcin (ng/ml) in examined groups. TRACP5b/osteocalcin quotient was significantly elevated in PostTx females compared to PremTx females (p < 0.02). TRACP5b and osteocalcin concentrations analysed separately were not significantly different between groups. All TRACP5b and osteocalcin results in medians and interquartiles are given in Table I. There was a positive correlation between serum TRACP5b and osteocalcin in the studied patients (R = 0.45, p < 0.001) (Figure 2).

Interleukin 6 serum concentration (in pg/ml) is shown as median and interquartile values in Figure 3. Levels of serum IL-6 values were significantly elevated
in PostTx: 3.0 (2.14–6.40) and MTx: 2.24 (1.60–5.10) compared to PremTx females: 1.39 (0.96–2.14) (p < 0.01 and p < 0.05 respectively). In PremEu, IL-6 serum concentrations were 2.14 (1.20–3.40), in PostEu: 2.4 (1.8–3.8) and in MEu: 3.58 (1.60–6.50). Interleukin-6 soluble receptor levels were not significantly different in the studied groups. In PremTx, IL-6sR level was 45.0 (38.3–52.8), in PremEu 47.9 (40.4–54.9), in PostTx 48.6 (42.8–59.1), in PostEu 48.4 (40.8–55.8), in MTx 46.1 (41.2–49.2) and in MEu 50.9 (41.3–57.5).

There were significant positive correlations between IL-6 and IL-6sR concentrations (R = 0.22, p < 0.05) and between IL-6sR and TRACP5b serum levels (R = 0.23, p < 0.05).

Discussion

In the present study, significantly elevated TRACP5b/osteocalcin quotient in postmenopausal thyrotoxic females in comparison with premenopausal women may reflect bone resorption overbalance as far as TRACP5b predominances over osteocalcin-established bone formation factor. Accelerated bone resorption is accompanied by proportional bone formation increase, as shown by a positive correlation between serum TRACP5b and osteocalcin values in the present study. Taking this into account, these results suggest that bone reconstruction does not keep up with accelerated bone resorption in estrogen deficient females burdened with thyrotoxicosis. Although thyroid hormones are necessary for normal skeletal growth, their excess leads to accelerated bone turnover, diminished bone density and increased risk of fractures [12, 13]. Thyrotoxicosis is accompanied by 12-15% reduction of bone mineral density, predominantly of cortical bone, and in consequence greatly increases the risk for hip fracture, especially in postmenopausal women [14]. Bone formation and resorption markers have been shown to be elevated in patients with hyperthyroidism and significant correlations have been observed between bone metabolism indicators and plasma thyroid hormones levels [15, 16]. In experimental thyrotoxicosis model bone formation markers are additionally increased owing to
triiodothyronine, that has been implicated in the enhanced production of osteocalcin, alkaline phosphatase and I collagen [17]. Osteocalcin is an osteoblast product that constitutes the most abundant noncollagenous protein present in bone and its serum levels closely correlate with histomorphometric parameters of bone formation [18].

The precise mechanism by which thyroid hormones regulate osteoblasts and osteoclasts function at a cellular level remains unclear. Osteoblasts were suggested to mediate the action of triiodothyronine on osteoclastic bone resorption releasing osteotrophic cytokines [19, 20]. The results of the studies carried out on osteoblast cell cultures have shown that triiodothyronine induces interleukin-6 expression [21, 22]. Our previous experimental data suggest that IL-6 plays a crucial role in thyrotoxicosis-related disturbances of bone turnover in mice, determining the imbalance between bone resorption and bone formation caused by excess of thyroid hormones, predominantly by inhibition of bone formation [10]. However, data from in vivo studies on the possible role of IL-6 in the pathogenesis of disturbances of bone formation thyrotoxicosis-related remain equivocal [23, 24].

In the present study levels of serum IL-6 values were significantly elevated in estrogen-deficient thyrotropic females (and thyrotropic men) in comparison to premenopausal women. Moreover significant positive correlations of IL-6/R levels and both: IL-6 and TRACP5b serum levels may suggest that IL-6 exerts its effect via its soluble receptor and that consequence of this interaction is bone resorption augmentation. IL-6 may exert its inhibitory effect on bone formation directly through gp130-STAT 1/3 signaling or indirectly by influencing balance between osteoproetgin (OPG) and receptor activator of nuclear factor B (RANK) and its ligand (RANKL) [25, 26]. The influence of IL-6 on RANK-RANKL/OPG was suggested to be more powerful effect leading to increased bone mass loss [27, 28]. The results of our recent experimental study suggest that IL-6 plays a key role in stimulation of RANKL-RANK/OPG system and this effect is strongly enhanced in conditions of accelerated bone turnover such as thyrotoxicosis especially when accompanied by another factor that enforces bone resorption: estrogen depletion [29].

To sum up, the results of the present study suggest that interleukin-6 plays a considerable role of in the pathogenesis of thyrotoxicosis-related disturbances of bone turnover in estrogen-deficient women.

References