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Pharmacological Research 54 (2006) 298–302

Pharmacological research

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Effects of hormone replacement therapy on plasma pro-inflammatory and anti-inflammatory cytokines and some bone turnover markers in postmenopausal women

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Accepted 13 June 2006

Abstract

Objective: The present study was undertaken to evaluate plasma TNFα, IL-1β, IL-10; and urinary hydroxyproline (Hyp) and calcium (Ca) as bone resorption markers in postmenopausal women compared with premenopausal ones; and to assess the effects of HRT upon these cytokines and bone turnover markers.

Patients and methods: The study involved 50 healthy postmenopausal women, and 25 healthy premenopausal women (control group). Postmenopausal women were randomly divided into two subgroups: women receiving *cycle HRT schedule* (0.625 mg conjugated estrogen from days 1 to 28 + 5 mg medroxyprogesterone acetate from days 18 to 28) for 2 months ($n = 25$); and second subgroup consisted of women receiving *continue HRT schedule* (0.625 mg conjugated estrogen + 2.5 mg medroxyprogesterone acetate from days 1 to 28) for 2 months ($n = 25$). Plasma TNFα, IL-1β and IL-10 concentrations were measured with ELISA kits. Fasting urinary Hyp was measured by Ehrlich's spectrophotometric reaction. Ca was determined by oxalate precipitation and the redox titration procedure. Statistical significance was analysed by Kruskal–Wallis plus post hoc Mann–Whitney *U*-tests for multiple comparisons, Wilcoxon signed ranks test for paired data, and Pearson correlation test.

Results: Compared with premenopausal individuals, postmenopausal women have increased plasma TNFα, IL-1β, IL-10 ($p < 0.0001$, $p < 0.0001$, and $p < 0.001$, respectively); and increased urinary Hyp and Ca concentrations ($p < 0.05$). HRT (both cycle and continue schedules) lead to a significant decrease in TNFα, IL-1β and urinary Hyp concentrations, and has no effect uppon IL-10 levels. HRT reverses increased urinary Hyp and Ca excretion to the premenopausal level. There is a significant positive correlation between pre- and post-HRT IL-1β levels in both cycle and continue subgroups ($r = 0.437$, $p < 0.05$; and $r = 0.656$, $p < 0.01$, respectively), and between pre-HRT IL-1β and urinary Ca ($r = 0.509$, $p < 0.01$; and $r = 0.415$, $p < 0.05$). There is a significant negative correlation between post-HRT IL-10 and TNFα levels in continue HRT receiving group ($r = -0.446$, $p < 0.05$). Urinary Hyp in cycle and continue HRT received subgroups are correlated with post-treatment values ($r = 0.455$, $p < 0.05$; and $r = 0.776$, $p < 0.01$).

Conclusions: Plasma TNFα, IL-1β, IL-10; and urinary Hyp and Ca increase with menopause. We suggest that the increase of IL-10 is secondary to the elevation of TNFα and IL-1β and that the increase of IL-10 is a compensatory mechanism, by which this anti-inflammatory cytokine counteracts to pro-inflammatory TNFα and IL-1β, and thus balances their osteoclast activating and oxidative stress-related effects. Two months duration HRT (cycle and continue schedule) lead to the significant decrease in plasma TNFα, IL-1β and urinary Hyp concentrations. HRT reverses increased Hyp and Ca excretion to the premenopausal level. So, HRT, decreasing Th1 cytokines (TNFα, IL-1β) probably improve the aberation of Th1/Th2 balance that is implicated in various pathological conditions. However, because of the relatively small number of participants and short duration of the therapy, further studies are necessary to establish a risk/benefit ratio for HRT to view effects on cytokine pattern and bone metabolism.

Keywords: Cytokines; Hormone replacement therapy (HRT); Bone turnover; Menopause

1. Introduction

At menopause ovarian estradiol and progesteron declines, and risk for osteoporosis and cardiovascular disease increases. Postmenopausal osteoporosis is a heterogenous disorder, characterized by a progressive loss of bone tissue. This progressive

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doi:10.1016/j.phrs.2006.06.006

bone loss is related with altered equilibrium between bone formation and resorption, and is due to increased osteoclast and decreased osteoblast formation.

Pro-inflammatory TNFα and IL-1β are among the numerous cytokines implicated in the regulation of osteoblasts and osteoclasts [1]. These cytokines promote bone resorption *in vitro* [2,3] and cause bone loss and hypercalcemia when infused *in vivo* [4–6]. TNFα and IL-1β activate mature osteoclasts indirectly via a primary effect on osteoblasts [7,8] and inhibit osteoclast apoptosis. In addition, they markedly enhance osteoclast formation by stimulating osteoclast precursor proliferation both directly [9] and by stimulating the pro-osteoclastogenic activity of stromal cells. TNFα and IL-1β are also powerful inducers of other cytokines that regulate the differentiation of osteoclast precursor cells into mature osteoclasts, such as IL-6 [10].

IL-10 is a pleiotropic cytokine that causes the inhibition of pro-inflammatory cytokine synthesis [11,12]. Endogenously synthesized IL-10 limits the immunecomplex-induced secretion of pro-inflammatory cytokines TNFα and IL-1β [13]. IL-10 is also capable of down-regulating the release of IL-6 and prostaglandin E_2 (PGE_2)—well known as pro-osteoporotic substances [11,12,14,15].

It is now recognized that estrogen prevents bone loss through multiple effects on bone marrow and bone cells, which results in decreased osteoclast apoptosis, and decreased capacity of mature osteoclasts to resorb bone [16]. Estrogen modulates osteoclast apoptosis and osteoclast activity both directly [17] and indirectly via regulation of growth factors and prostaglandins [18,19]. Hannon et al. reported that 6 months HRT with transdermal estradiol decrease bone resorption markers such as Hyp, Ca, and deoxypyridinoline [20].

Recently, numerous reports have been published demonstrating that natural or surgical menopause increases blood bone marrow and monocyte levels of IL-1 and TNFα [21,22]. Many studies have also documented the ability of estrogen and HRT to suppress [23,24] or increase [25] the production of these cytokines by bone and bone marrow cells. It was reported that IL-10 concentrations did not change [26], or decrease with menopause [27], and that HRT have no effect uppon IL-10 concentrations [28].

The controversial data about the effect of HRT on TNFα, IL-1β, and IL-10, prompted us to investigate plasma TNFα, IL-1β and IL-10 in postmenopausal women before and after different regimens of estrogen/progesteron administration and to compare them to the levels measured in young premenopausal healthy women. We investigated also whether there may be relationship between above mentioned cytokines and bone turnover markers urinary Hyp and Ca.

2. Material and methods

Fifty healthy postmenopausal women weighing within 15% of their ideal body weight were selected according to following inclusion criteria: (1) natural menopause occured at least 12 months before the onset of the study; (2) body mass index lower than $30\,kg\,m^{-2}$; (3) sustained FSH rise (higher than $40\,IU\,mL^{-1}$). Exclusion criteria were: (1) no history of breast or genital tract malignancy, hepatic, renal, vascular or endocrinological disease and acute infection; (2) no prior exposure to any medication known to influence bone and/or mineral metabolism (e.g. corticosteroids, hormone replacemet, GnRH analogs, anticonvulsants, heparin, aluminium containing antiacids, thyroid medication). Also, excluded were those women suffering from any form of osteoporosis (by fracture history and spinal X-ray), as well as those reporting tobacco and alcohol use.

Twenty-five premenopausal healthy women in productive era constituted the control group. They were multiparas, not on oral contraception and any medication influencing bone metabolism, and were in the follicular phase of the menstrual cycle at the sampling. Also, they had no estrogen deficiency symptoms and were free of hepatic, renal, vascular, or endocrinological disease.

The age of postmenopausal women was 48.6 ± 3.5 years; the age at menopause was 47 ± 2.5 years; and time since menopause was 1.6 ± 1 year. The age of premenopausal women (control group) was 30 ± 2.5 years. Postmenopausal women were randomly divided into two subgroups. While 25 postmenopausal women were treated for 2 months with *cycle HRT schedule* (0.625 mg conjugated estrogen from days 1 to 28, and 5 mg medroxyprogesterone acetate from days 18 to 28), the remaining 25 women were treated for 2 months with *continue HRT schedule* (0.625 mg conjugated estrogen and 2.5 mg medroxyprogesterone acetate from days 1 to 28). Postmenopausal women were in their follicular phase of the artificial HRT cycle at the time of sampling. Venous blood samples were collected at 10^{00} in EDTA.K_3 tubes from the antecubital vein after overnight fasting and centrifuged immediately at 1000 g (10 min) to remove the plasma. After removing the plasma, samples were kept at $-70\,^{\circ}C$ pending analysis. Plasma TNFα and IL-10 were measured by ELISA (Biosource International, Camarello, CA, USA) and IL-1β by ELISA (Diaclone Research, Besançon, France). The sensitivity was: 1.7 pg/mL for TNFα; less than 5 pg/mL for IL-1β; and 1 pg/mL for IL-10. Intra-assay coefficients of variation (CV) were less than 10% for all. For urinary analyses, an overnight and second morning urine samples were collected. Urine measurements included Hyp, creatinine and Ca. To avoid diet-induced interference in measuring Hyp, all subjects were maintained on a gelatin free diet the day before urine collection. Hyp was measured by Ehrlich's spectrophotometric reaction [29] and the results were expressed as the molar ratio to creatinine measured by the Jaffe procedure [30]. Urinary fasting Ca was determined by oxalate precipitation and the redox titration procedure [31] and the results were expressed as the molar ratio to creatinine. The intra-assay coefficient of variation was 4.2% for Hyp, and 3.7% for Ca. Data were given as means ± S.D. Because the data were not normally disturbed, we used the Kruskal–Wallis test for nonparametric data plus post hoc Mann–Whitney *U*-tests to compare differences between groups. Wilcoxon signed ranks test for paired data was used for comparison of baseline and post-treatment values, and Pearson correlation test was used to test the correlation between groups.

Informed consent was given by each woman, and the study was approved by the Istanbul Medical Faculty's institutional review board.