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Efficacy and safety of DT56a (Femarelle) compared to hormone therapy in Greek postmenopausal women

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ABSTRACT

Background: Hormone therapy is the treatment of choice for the alleviation of menopausal symptoms; concerns, however, about its concomitant long term health risks have limited its use. DT56a (Femarelle) is a unique enzymatic isolate of soybeans. The purpose of our study was to evaluate the efficacy and safety of DT56a, compared to hormone therapy (HT), in symptomatic postmenopausal women.

Subjects and Methods: 89 postmenopausal women were studied prospectively. Women with climacteric symptoms were randomly assigned to receive either DT56a (n=27) or oral low dose continuous combined HT (n=26). Symptomatic women not wishing to receive any treatment served as controls (n=36). Menopausal symptoms as assessed through the Kupperman index, serum lipids and lipoproteins, calcium, as well as bone mineral density, endometrial thickness, and mammography were assessed at baseline and at 12 months.

Results: Patients receiving HT and DT56a showed a significant and independent decrease in menopausal symptoms (mean difference in Kupperman score, DT56a group: -3.98, HT group -5.601, no treatment group +1.76, p-value < 0.001). Lumbar spine bone mineral density T score was significantly lower in women receiving no treatment, as opposed to the two treatment arms which showed no significant change (No treatment, baseline: -0.60, final: -0.85, p=0.001; HT, baseline:-84, final -0.99, p=0.79; DT56a, baseline -0.51, final:-0.76, p=0.75). No differences in femoral bone density, endometrial thickness or mammography classification were detected in any of the treatment arms. Likewise, serum lipids or lipoproteins did not differ between the three groups.

Conclusions: DT56a decreased menopausal symptoms significantly and in the same degree as HT.

Key words: phytoestrogens, DT56a, isoflavones, Hormone Therapy, menopause, hot flushes, bone mineral density

INTRODUCTION

Menopause, a natural event in a woman's life is characterized by loss of reproductive function and decline in estrogen levels (1). Menopause is associated with multiple symptoms such as hot flushes, night sweats, sleep disturbances, urinary incontinence, decreased libido and sexual dysfunction, poor memory, anxiety and depression that may affect the quality of life (2). Hot flushes have been documented to occur in over 75% of midlife women and constitute the most characteristic and distressing symptom of the climacteric (3, 4, 5). Furthermore, one third of women over the age of 60 and two thirds over the age of 75 complain about urogenital symptoms which bear a serious impact on their social and sexual life (6). Moreover, menopause accelerates bone loss leading to osteoporosis. Postmenopausal osteoporosis and osteoporotic

fractures comprise a serious threat to the aging population, presenting ever increasing incidence rates (7). The lifetime probability of hip fractures in women at the age of 50 exceeds 20% in developed countries, while the lifetime probability of any osteoporotic fracture is over 40% in postmenopausal women (8).

The most effective treatment for the management of climacteric symptoms and the prevention of osteoporosis is hormone therapy (HT) (9, 10). Concerns, however, about the long term health risks of HT have limited its use. The Women's Health Initiative (WHI) trial reported that the overall health risks of long term estrogen therapy, including increased risk of venothrombotic disease, breast cancer, stroke, and coronary artery disease, exceed its benefits (11). Alternative treatment options are therefore sought for the alleviation of climacteric symptoms lacking a stimulating effect on the breast or the clotting system (12, 13, 14, 15, and 16).

Although evidence is not consistent (17, 18), phytoestrogens are reported to have a modest effect on climacteric symptoms and bone mineral density (19, 20, 21), DT56a Tofupill/Femarelle) is a unique enzymatic isolate of soybeans. In fact, it represents a composition of substances derived from soy through an enzymatic process resulting in a more potent extract with an efficacy of 8:1 compared to the raw material (22). Studies have also indicated that it acts as a phyto SERM (23). DT56a has been shown to increase bone mineral density in postmenopausal women (24) and to relieve vasomotor symptoms with no effect on sex hormone levels or endometrial thickness (25).

The purpose of our study was to evaluate the efficacy of DT56a, compared to HT, in the management of menopausal symptoms, as well as its effect on bone mineral density, lipid profile and endometrial thickness in symptomatic Greek postmenopausal women.

METHODS

Subjects

100 postmenopausal women recruited prospectively by the Menopause Clinic of the 2nd and 3rd Department of Obstetrics and Gynecology of the Athens University, in Aretaieion and Atticon Hospital respectively. 89 women completed the study period and were included in the analysis in an intention to treat design. 11 women dropped out of the study (9 lost to follow up and 2 withdrew their consent). The decision to treat was based on the presence of climacteric symptoms. Women with climacteric symptoms were randomly assigned to receive either Femarelle (644 mg/day, DT56a, Se-cure Pharmaceuticals, Yavne, Israel) or HT (17β-estradiol 1mg plus norethisterone acetate 0.5mg, Activelle, Novo-Nordisk, Copenhagen, Denmark). 36 symptomatic women not wishing to receive any treatment served as controls. This sample size was sufficient to evaluate statistical differences with an anticipated effect size of 0.8 and a statistical power of 80% ($\alpha = 0.05$, two tailed).

Before commencing therapy, patients underwent a gynecological and biochemical examination including a bimanual cervical examination, PAP smear, transvaginal sonography, breast

examination and mammography, thyroid – liver – renal function as well as blood coagulation tests and bone densitometry.

Criteria for inclusion in the study were the absence of menses for at least 12 months, FSH>25mIU/ml, serum estradiol <50 pg/ml,endometrial thickness less than or equal to 5mm, the absence of gynecological malignancy, ischemic heart disease,thromboembolism, diabetes mellitus, pharmacologically treated dyslipidemia, and non-treated thyroid dysfunction. Women who were past users of HT, tibolone or raloxifene were not included in the study unless they had been off therapy for at least 6 months. All subjects gave informed consent and the study protocol received approval by the Ethics Committee of Attikon and Aretaieion Hospital.

Anthropometric measurements

A detailed medical history including age, years since menopause, education, exercise, alcohol, tobacco consumption, and parity was recorded for each subject. Weight was measured in light clothing and on an electronic scale, and height was measured using a stadiometer in the upright position. Body mass index (BMI) was calculated using the equation: BMI = weight (kg)/ height (m2). Two blood pressure measures were obtained from each subject by oscillometry using the automated Omron 705IT device (Omron). The measures were subsequently averaged for use in the data analysis stage.

Biochemical measurements

Patients were instructed to abstain from food and smoking for 12 hours. Subsequently, fasting venous blood samples were drawn at 8:30 – 9:30 a.m. and serum was stored at 680O C until analyzed. The following parameters were assessed at baseline and at 12 months: serum total cholesterol (TC), LDL cholesterol (LDLc), HDL cholesterol (HDLc), triglycerides (TG), Apolipoprotein A1 (ApoA1), Apolipoprotein B (ApoB) and serum Ca. Serum total cholesterol, HDL cholesterol and triglycerides were assessed enzymatically by an autoanalyzer (COBAS – MIRA, Roche Diagnostics Limited, Lewes, East Sussex, UK). Apolipoprotein A1 and apolipoprotein B were determined by an immunoturbimetric assay (ABX Diagnostics BP7290 – 34187 Montpellier, France). AIP (Atherogenic Index of Plasma) was computed by the following equation: log [TG (mmol/l) / HDLc (mmol/l)].

Bone mineral density measurements

Bone mineral density (BMD) was assessed through Dual Energy X-ray Absorptiometry (DXA), applied to sites of biological relevance, including the hip and spine. BMD was measured using a Norland Excell Plus XR-36 densitometer (Norland Medical Systems, Fort Atkinson, WI, USA). Within subject coefficient of variation was 1.1%. In postmenopausal women and men aged 50 years or more, T-score should be reserved for diagnostic use. The T-score is defined as the number of standard deviations below average for a young adult at peak bone density, adjusted for gender and ethnicity (26, 27).

Psychometric and gynecological measurements

Menopausal symptoms were assessed through the Kupperman index, filled by subjects at baseline and at 12 months. The Kupperman index (28) considers common climacteric symptoms; vasomotor symptoms such as hot flushes and sleep disturbances, psychological symptoms such as anxiety and depression, aggressiveness, loss of concentration, sense of weakness or fatigue, myalgia, headaches and palpitation, feeling of dizziness, urogenital atrophy, decreased libido and sexual dysfunction. For each of the above mentioned symptoms the score is 0, 1, 2 or 3 for no, mild, moderate or severe intensity, respectively.

Mammography was also performed at baseline and at 12 months on dedicated mammographic equipment (Senographe 600T or 700T, GE Medical Systems). All mammograms were obtained by using a high resolution screen6 film combination and under maximum compression pre-adjusted by the GE technologists at 20 daN.

Mammography consisted of a standard bilateral craniocaudal and mediolateral oblique position. Mammographic density was evaluated using the modified Wolfe criteria (29). Patients underwent baseline TVUS (transvaginal ultrasound) in the month before randomization and after 12 months, with the patient in lithoid position and the bladder empty. Endometrial thickness (ET) was also assessed. The anteroposterior measurement of ET was obtained from the longitudinal axis view at the widest point of the endometrial–myometrial interface, while recording the maximum width of the double layer (excluding anechoic endometrial fluid collection). The ultrasonographic examination was performed with an Ultramark 69 HDI ultrasound machine using a 6.5 MHZ transvaginal transduser (advanced technology laboratories, Bothell, WA, USA).

Statistical Analysis

Data analyses were performed using SPSS version 17.0 for Windows (SPSS Inc, Chicago, IL, USA). Fischer's exact test was used to examine correlations between categorical factors and treatment arms, and Analysis of Variance (ANOVA) to examine differences in measurements of quantitative factors between treatment arms at baseline. The focus was on changes between initial and final visit, any occurrence of which was of interest to the study; any change of classification according to categorical factors was investigated using Fischer's exact test (statistical significance would imply correlation among initial and final classification, i.e. patients sustaining their classification). Changes in quantitative measurements were computed as final minus baseline values and examined through paired T-test, within treatment arms, and through Analysis of Co-Variance (ANOVA), between treatment arms; the latter was examined in order to investigate variation of change after adjustment for baseline values and for years of menopause.

RESULTS

Table 1 illustrates baseline demographic, lifestyle and anthropometric characteristics as summarized and compared among treatment arms. Our population comprised young, recently

postmenopausal women with no significant differences in baseline parameters. Regarding changes throughout the duration of the study, classification according to DEXA measurements and mammography was sustained (baseline classification was highly correlated to final classification, Fischer's exact test p-values <0.05); changes among treatment arms could therefore not be noticed. As for baseline and final values in quantitative measurements of interest, results are summed up in Tables 10, 2 and 3. Patients receiving no treatment had a higher mean baseline BMI than those receiving HT (Bonferroni corrected p value 0.001).Lumbar T-score decreased in women not receiving any treatment (paired t-test p-value 0.001). Women receiving HT presented a significant decrease in the mean Kupperman score (paired t test p value 0.032), and a significant decrease in the mean ApoB levels (paired t-test p value 0.036).

Women receiving DT56a exhibited a significant decrease in the mean Kupperman score (paired t-test p-value 0.001). As far as Kupperman subscale scores are concerned, women in both treatment arms showed a significant reduction in hot flushes / night sweats (HT baseline 2.25 ± 1.24 final 0.25±0.56, p<0.001; DT56a baseline 2.17±0.94 final 0.75±0.69, p<0.001) and in sleep disturbances (HT baseline 1.75 ± 0.93 final 0.34±0.47, p=0.01; DT56a baseline 1.96±1.08 final 0.91±0.82, p=0.02). None of the remaining symptoms showed individually a significant difference in any of the two treatment arms.

Table 4 presents results for estimated mean change in values of measurements of interest, among treatment arms, adjusted for baseline values and for years since menopause. Both HT and DT56a significantly decreased the Kupperman score, compared to no treatment (no treatment vs. HT Bonferroni corrected p-value <0.001 and no treatment vs. phyto-SERMs Bonferroni corrected p-value 0.013). No differences in mean change of endometrial thickness, BMD T-scores, or the lipid profile were recorded between the three study groups.

DISCUSSION

The present study showed that DT56a decreased menopausal symptoms significantly and in the same degree as HT. It also maintained BMD, in contrast to 11 women receiving no treatment who exhibited a decrease in lumbar spine BMD.

DT56a use was not associated with changes in the lipid profile, mammography or endometrial thickness.

DT56a is a unique enzymatic isolate of soybeans. Experimental studies have shown that DT56a can function as an estrogen receptor modulator. The induction of the creatine kinase (CK) activity has been used as a response marker of estrogenic activity, since estrogens, both in vivo and in vitro, stimulate CK activity in cells containing active estrogen receptors (ER) (31, 32). DT56a induces CK activity both in skeletal (23) and vascular tissues (33), while it does not activate CK activity in the uterus. The selective estrogen receptor modulator (SERM) raloxifene blocked the stimulation of CK by both DT56a and E2, pointing towards a common receptor mechanism of action (23, 30). DT56a also stimulated CK and DNA synthesis in cultured bone cells (33), while DT56a, unlike E2, retained the CK and the DNA synthesis stimulation in

hyperglycemic condition (34), suggesting that DT56a could be an effective bone restoring agent in diabetic postmenopausal women. In the breast tissue, DT56a displayed its antagonistic action by causing no stimulation of MCF-7 human breast cancer cultured cell lines, contrary to the use of E2 (35). Finally, DT56a did not affect platelet reactivity adversely as measured by PFA (Platelet Function Analyzer) closure times in symptomatic thrombophilic postmenopausal women or normal controls, suggesting the absence of thrombogenic activity (36).

The abundance of soy based dishes in the Asian diet provides a possible explanation for the difference recorded in the experience of hot flushes between Western and Asian population, since only 10%-20% of the Asian population suffer from menopausal symptoms, compared to the 80%-90% prevalence in the United States (37). Soy is a main source of phytoestrogens which have both estrogenic and 12 anti-estrogenic properties. Phytoestrogens can be classified into two main categories: isoflavones and lignans. Soy is a common source of isoflavones. In line with our study, the intake of DT56a by 84 postmenopausal women, either in the standard dose of 644 mg/ day or even in the low dose of 322 mg/day, resulted in a significant reduction of menopausal symptoms by 78% in both groups (25). The improvement was still evident following 12 months of treatment, while there was no significant change in the endometrial thickness. Furthermore, a meta-analysis (19) based on randomized, controlled, parallel group studies comparing isoflavone therapy (using either soy products or red clover products) to a non-isoflavone, non-estrogenic comparator showed that isoflavone supplementation was associated with a significant reduction in hot flushes (effect size −0.28, 95% confidence intervals −0.39 to −0.18, $P < 0.0001$). The percentage reduction in hot flushes was significantly related to the number of baseline flushes per day and the dose of isoflavone studied ($\beta = -0.49$ and -0.26, respectively, both $P < 0.0001$). These results suggest that isoflavone supplementation may lead to a modest reduction in the number of daily hot flushes in postmenopausal women and that the benefit may be more apparent in women experiencing a high number of hot flushes per day.

On the other hand, other studies have not demonstrated an efficacy of phytoestrogens in relieving menopausal symptoms (38). A recent meta-analysis reviewing 11 trials on soy isoflavones extracts did not show efficacy in the treatment of hot flushes (39). A double blind clinical trial involving 177 breast cancer patients who received either soy tablets or placebo showed no difference in hot flush frequency between the two groups after 4 weeks of treatment. Interestingly, at the end of the trial more women chose to continue placebo than the soy product (40).

According to a recent Cochrane meta-analysis which summarized the findings of 30 13 clinical trials investigating the effect of various forms of phytoestrogens on hot flushes, there was no evidence of effectiveness in the alleviation of menopausal symptoms (41).

As in the case of menopausal symptoms, the phytoestrogen rich diet of the Asian women possibly accounts for the low rate of osteoporosis in these populations. The role of phytoestrogens in the prevention of osteoporosis is, however, controversial, since some studies have shown beneficial effects (20, 21) and others have not (42). In experimental studies, the

effectiveness of DT56a was comparable with estrogens in ovariectomized (OVX) rats, since the administration of DT56a could inhibit the decrease in trabecular bone volume and in trabecular and cortical thickness induced by ovariectomy (43). DT56a, like estrogens, restored bone structure measurements to the values obtained in the intact rats. In the uterus, DT56a did not activate estrogen receptors while estrogens did elevate CK activity (43). A clinical study evaluating the effect of DT56a in the bone mineral density of postmenopausal women showed that the standard dose of 644 mg/ day had a positive effect (an increase of 3.6% in spine and of 2% in the hip), whereas the low dose of 322 mg/ day resulted in a decrease of 0.6% in both sites (21). Endometrial thickness did not change after 12 months of treatment.

Limitations in our study include the small size of study groups; as a result, possible differences regarding bone mineral density, the lipid profile or mammography classification may have not surfaced due to the lack of power. It should be noted, however, that our women had normal values of BMD and no osteopenia or osteoporosis, presenting, thus, a low risk population. In these populations an effect on BMD requires significantly higher number of subjects. Furthermore, women not receiving any treatment had a higher BMI compared to the 14 other two groups. All comparisons, however, were made after adjustment for baseline values, in order to account for this difference. On the other hand, our study is the first to demonstrate an efficacy of DT56a in relieving menopausal symptoms in direct comparison to HT.

In conclusion, DT56a decreased menopausal symptoms significantly and in the same degree as HT. Women receiving DT56a or HT did not exhibit any change in bone mineral density, as compared to women not receiving treatment who exhibited a decrease in lumbar spine BMD. No change either in endometrial thickness or mammography classification was recorded after 12 months of treatment. DT56a may serve as an alternative to hormone replacement therapy for the relief of climacteric symptoms in postmenopausal women.

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