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Effects of genistein on hot flushes in early postmenopausal women: a randomized, double-blind EPT- and placebo-controlled study.

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ABSTRACT

Objective: We evaluated and compared the effects of the phytoestrogen genistein, estrogen-progestogen therapy (EPT), and placebo on hot flushes and endometrial thickness in postmenopausal women.

Design: Ninety healthy, postmenopausal women, 47 to 57 years of age, were randomly assigned to receive for 1 year continuous EPT (n = 30; 1 mg 17β-estradiol combined with 0.5 mg norethisterone acetate), the phytoestrogen genistein (n = 30; 54 mg/day), or placebo (n = 30). Endometrial safety was evaluated by intravaginal ultrasounds at baseline, 6 and 12 months.

Results: By comparison with placebo, daily flushes reduced significantly by a mean of 22% (95% CI: −38 to −6.2; $P < 0.01$) after 3 months, by a mean of 29% (95% CI: −45 to −13; $P < 0.001$) after 6 months, and by a mean of 24% (95% CI: −43 to −5; $P < 0.01$) after 12 months of genistein treatment. Flush score decreased by a mean of 53% (95% CI: −79 to −26; $P < 0.001$) after 3 months, by a mean of 56% (95% CI: −83 to −28; $P < 0.001$) after 6 months, and by a mean of 54% (95% CI: −74 to −33; $P < 0.001$) after 12 months of EPT, as compared with placebo. No side effect was observed on the uterus of the participants.

Conclusions: The present study confirms that genistein might have positive effects on hot flushes without a negative impact on endometrial thickness and suggests a future role of this phytoestrogen as a strategically therapeutic alternative in the management of postmenopausal symptoms.

Key Words: Genistein – Menopause – Hot flushes – Endometrium – Estrogen receptors.

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Menopause is associated with an increased incidence of coronary heart disease and vasomotor disturbances.[1,2]

Vasomotor symptoms have a multifactorial etiopathogenesis, but result primarily from the loss of estrogen as ovarian function ceases.[3] Although hot flushes typically last for 0.5 to 5 years after natural menopause, they may persist for as long as 15 years in a small percentage of postmenopausal women.[3]

Menopause-related hot flushes are associated with profuse sweating, decreased skin resistance, modest tachycardia and cutaneous vasodilatation. With the exception of the last effect, these events are the consequence of an abrupt increase in sympathetic outflow.[4]

Estrogen-progestogen therapy (EPT) can be directed to symptom relief or to prevention or treatment of chronic diseases,[5] but it is also known that this therapy might cause several side effects.[6-8]

As a matter of fact, menopause is a natural event, and understandably women would like to take a natural therapy rather than a drug for managing their menopause symptoms as well as preventing the long-term sequelae of estrogen deficiency.

Postmenopausal women in Europe and North America report an incidence of hot flushes as high as 70% to 80%, whereas Asian women experience them as a rate of 20% to 25%.[9]

It has been reported that vegetarians and residents of Japan have fewer vasomotor disturbances mainly as a consequence of the high soy content of their diets.[10] Albertazzi et al[11] conducted a prospective, double-blind, randomized, placebo-controlled trial comparing the effects of a 3-month dietary soy supplementation (40 g/day) versus placebo in postmenopausal women. The results showed a significant reduction in hot flushes in the group taking soy.[11]

In contrast, another study reported only a modest benefit for hot flushes in postmenopausal women with dietary soy supplementation when compared with placebo or hormone therapy (HT).[12] Furthermore, soy beverage did not alleviate hot flushes in women with breast cancer.[13]

Recently, a great deal of attention has been focused on soy isoflavones such as genistein and daidzein, which are found in abundance in soybeans and their derivative foods, such as tofu, miso, and others.[14] However, there have been conflicting data on the efficacy of such products for the treatment of hot flushes.[15-18]

Genistein has been experimentally shown to be the most efficacious in humans and in animal models,[19] and this isoflavone is now being extracted and produced as a dietary supplement.

The aim of our study was to investigate whether pure genistein (54 mg/day for 1 year) reduces the number of hot flushes in postmenopausal women without endangering the endometrium.

METHODS

Participants

We evaluated the climacteric symptoms of postmenopausal women who participated in our previous randomized, double-blind, EPT- and placebo-controlled study assessing the effect of genistein on bone loss. The study participants are described in detail there.[20] In brief, participants were healthy, ambulatory women, referred by the Department of Internal Medicine and the Department of Obstetrical and Gynecological Sciences at the University of Messina, Italy, who were 47 to 57 years of age, had not undergone surgically induced menopause, had not had a menstrual period in the preceding year, and had a follicle-stimulating hormone level greater than 50 IU/L and a serum 17β-estradiol level of 100 pmol/L or less.

Exclusion criteria were clinical or laboratory abnormalities that suggested cardiovascular, hepatic, or renal disorders; coagulopathy; use of oral or transdermal estrogen, progestin, androgen or other steroids in the preceding year; and smoking more than 10 cigarettes per day.

Measurement of circulating levels of genistein and 17β-estradiol

Estradiol (normal range 73-367 pmol/L for the follicular phase of the menstrual cycle) was evaluated using a solid phase immunoassay (Roche Diagnostics, Milan-Italy).

To evaluate genistein plasma levels, blood samples (0.5 mL) were collected in polypropylene tubes containing 50 µl of heparin (50,000 IU). After centrifugation at 3,000 × g at 4°C for 10 minutes, each sample was stored at −70°C until analysis. The assay was performed by using a high-pressure liquid chromatography method with ultraviolet detection, with some modifications.[20] The concentration of plasma genistein was expressed in µmol/L.

Diet

All participants received dietary instruction in an isocaloric, fat-restricted diet offering 30% energy from fat, less than 10% energy from saturated fatty acids, and a cholesterol intake of less than 300 mg/day. The consumption of soy products, legumes, or other nutritional supplements was prohibited.

Treatments

After a 4-week stabilization on the diet, study participants were randomly assigned (by a computer software that randomly allocated participants to the different groups) to receive continuous HT (n = 30; 1 mg/day 17β-estradiol combined with norethisterone acetate), the phytoestrogen genistein (n = 30; 54 mg/day), or pla-

TABLE 1. *Characteristic of 90 women at baseline*

	Placebo	Genistein	EPT
Age (y)	51 ± 0.73	52 ± 0.55	52 ± 0.91
BMI	24 ± 0.36	24 ± 0.55	23 ± 0.36
Years since menopause	6 ± 0.91	7 ± 1.09	7 ± 0.55
FSH (IU/l)	84 ± 4.2	79 ± 4.0	83 ± 4.9
Hot flush basal number	4.7 ± 0.58	4.6 ± 0.58	4.5 ± 0.56

All values are mean ± SEM. EPT, estrogen + progestogen therapy; BMI, body mass index; FSH, follicle-stimulating hormone.

cebo (n = 30). All the pills were identical in appearance. Genistein tablets, obtained from Lab Plants (Messina, Italy), contained 54 mg of total isoflavone. The purity of genistein was approximately 98%.

Study protocol

All participants were instructed to record in a diary the number of hot flushes each day for 2 weeks before each visit. The baseline, 3-, 6-, and 12-month hot flush counts were calculated as the mean of the last 14 days before treatment and before each visit. Only the number of hot flushes (including night sweats) was used as inclusion criteria, not the severity of hot flushes. We only analyzed participants who had at least five hot flushes (including night sweats).

Safety

Safety and tolerability were assessed by means of questions about symptoms and a physical examination during a clinic visit every 3 months. Moreover, laboratory analyses were performed every 6 months. Ultrasonographic endometrial thickness was evaluated at baseline and after 6 and 12 months in all participants included in our previous study.[20]

Statistical analysis

All analyses followed an intention-to treat design. Only 7 participants withdrew from the treatment but completed the study. Data are given as the mean ± SEM. The significance of difference was assessed using analysis of variance. The study had 90% power to detect a 2.5-mm difference in endometrial thickness among the three treatment groups. A P value of 0.05 or less was considered statistically significant.

RESULTS

Clinical characteristics

The clinical characteristics of the randomized women are reported in detail in Table 1. Participants in all the groups were of a similar age. No single partici-

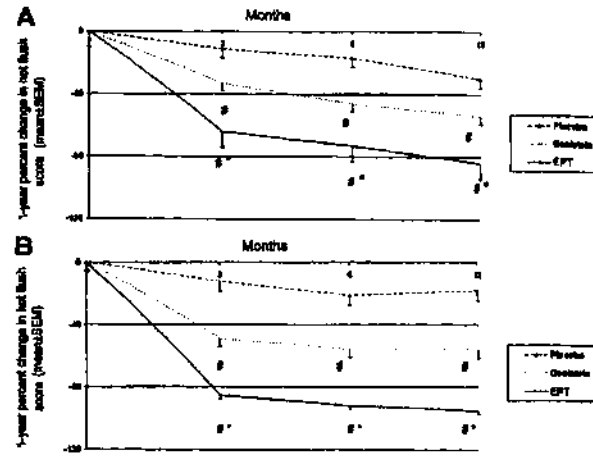

FIG. 1. **A.** Mean (± SEM) percent changes from baseline in hot flush score in all women. #, $P < 0.01$ versus placebo; *, $P < 0.05$ versus genistein. **B.** Mean (± SEM) percent changes from baseline in hot flush score in women having hot flush score > 5. #, $P < 0.01$ versus placebo; *, $P < 0.01$ versus genistein.

pant was less than 1 year postmenopausal when treatment started. Similar trends also were seen when the groups were subdivided according to smoking status and body mass index. The level of follicle-stimulating hormone was not significantly different in the three groups.

Hot flushes

Table 1 shows the number of hot flushes per day in the three groups at baseline. There was no difference in the hot flush count between the three groups at baseline (Table 1).

Figure 1A shows the hot flush score evaluated as a percentage change from basal values in all participants.

By comparison with placebo, daily flushes declined significantly, by a mean of 22% (95% CI: −38 to −6.2; $P < 0.01$) after 3 months, by a mean of 29% (95% CI: −45 to −13; $P < 0.001$) after 6 months, and by a mean of 24% (95% CI: −43 to −5; $P < 0.01$) after 12 months of genistein treatment.

Flush score decreased by a mean of 53% (95% CI: −79 to −26; $P < 0.001$) after 3 months, by a mean of 56% (95% CI: −83 to −28; $P < 0.001$) after 6 months, and by a mean of 54% (95% CI: −74 to −33; $P < 0.001$) after 12 months of EPT, as compared with placebo.

By comparison with genistein, daily flushes declined significantly, by a mean of 31% (95% CI: −57 to −4.7; $P < 0.05$) after 3 months, by a mean of 27% (95% CI: −53 to −0.26; $P < 0.05$) after 6 months, and by a mean of 30% (95% CI: −49 to −11; $P < 0.05$) after 12 months of EPT treatment.

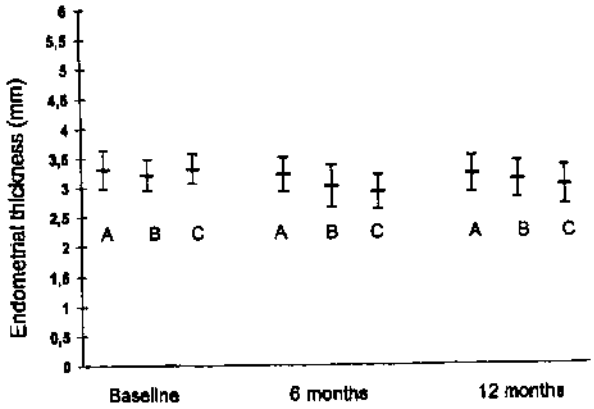

FIG. 2. Mean (± SEM) endometrial thickness at baseline, 6 and 12 months in placebo (A), genistein (B), and EPT (C) groups.

Genistein and EPT also significantly decreased the number of hot flushes in women having a hot flush score greater than 5, when compared with placebo (Fig. 1 B).

In our previous study,[20] we observed genistein administration resulted in a marked increase in plasma levels of the phytoestrogen, whereas the phytoestrogen did not change the plasma levels of 17β-estradiol. EPT significantly increased the circulating levels of 17β-estradiol, but it did not affect the plasma levels of the phytoestrogen genistein. Placebo treatment did not modify the circulating levels of either 17β-estradiol or genistein.

Safety

Genistein, EPT, and placebo were generally well tolerated and ingested with a high degree of compliance. There were no significant changes in routine biochemistry, liver function, or hematology results.

The daily administration of 54 mg of the phytoestrogen genistein did not cause any significant change in the endometrial thickness (Fig. 2). After a 1-year treatment, three women in both the placebo group and genistein group and two women in the EPT arm showed an endometrial thickness greater than 5 mm. Endometrial biopsies under hysteroscopic guidance were performed in these eight participants. No signs of endometrial abnormalities were observed.

DISCUSSION

Recently it has been demonstrated that phytoestrogens might have a positive impact on menopause with the benefit of no increased risk of breast and uterine cancer or cardiovascular disease.[20-26]

Furthermore, evidence from randomized clinical trials suggests that soy flower, which contains phytoestrogens, may relieve menopause-related vasomotor disturbances. However, it is not clear if isoflavones, especially genistein, administered as "pure" product might be more effective on these symptoms than soy foods.[21-24]

The conflicting results of previous studies could be attributed to poor study design with low participant numbers, lack of control of dietary intake of isoflavones, and the inclusion of participants with too few hot flushes per day at baseline.

Our data showed that EPT significantly reduced the number of hot flushes per day in postmenopausal women, thus confirming the positive role of estrogens in the prevention of vasomotor disturbances. In addition, our study clearly indicated that the number of hot flushes also decreased in postmenopausal women taking genistein.

The factors that may have contributed to the positive results of the current study might be identified in the "use" of 54 mg of pure genistein per day and in the inclusion criteria of more than five hot flushes per day.

Phytoestrogens cause important biological effects in the different target tissues, depending on the type of estrogen receptors (ER-α and ER-β) involved in the cellular response.[19]

The phytoestrogen genistein acts in cells by a classical genomic mechanism: first it enters by diffusion through the lipid bi-layer, then binds ER in the cytosol; this complex moves to the nucleus stimulating, the mRNA synthesis, and the production of tissue-specific proteins.[27] Genistein may also be termed a selective ER modulator (SERM) because it reveals both ER agonist and antagonist activity in a cell type and promoter specific manner. In fact, genistein shows full agonism for ER-α and only partial agonism for ER-β, but with higher affinity for ER-β than ER-α, indicating a possible way to clarify the genistein positive effects on the uterus.[28,29]

In fact, the low affinity for the specific receptor ER-α might explain the safety of this drug on the uterus. Indeed, our study showed no difference in endometrial thickness between placebo, genistein, and EPT groups. This finding confirms and extends recent published data[30] showing that the administration of 36 mg/day of genistein for 12 months did not induce endometrial growth when compared with placebo; however, our data are, at least in our opinion, more impressive because we have administered a higher dose (54 mg/day of genistein) and the isoflavone effect was controlled versus both placebo and EPT.

The mechanism by which genistein may reduce hot flushes is not fully understood. ER-α might play a key role in the genesis of vasomotor symptoms. Therefore, it could be speculated that genistein reduces hot flushes by a mechanism involving the ER-α receptor in the central nervous system.

Finally, it is important to underline that, in our study, we did not observe significant side effects after 1 year of genistein administration, although we administered a high dose (54 mg/day) of pure genistein. This confirms the good safety profile of this natural drug.

CONCLUSION

The present data collectively suggest that the use of genistein at the dose of 54 mg per day is effective in alleviating the acute hot flush symptoms of menopause.

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