Clinical Effects of Isoflavones and Berberine (Estromineral Lipid) in Menopausal Women with Borderline Dyslipidemia

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Abstract

Objective: During the menopause, oestrogen deficiency brings about changes in the lipid profile which increase the cardiovascular (CV) risk. The use of food supplements together with a healthy lifestyle and a varied, balanced diet is a logical approach for improving CV disease prevention.

A combination of nutraceuticals (EL) based on berberine (a natural extract that reduces total cholesterol and triglycerides), soya isoflavones (SI), Lactobacillus sporogenes and vitamin D3 was compared with the same formulation without berberine (E) in menopausal women with borderline dyslipidaemia.

Method: Randomised, controlled, parallel-group multicenter study in menopausal women with Tot-C >200 and <260 mg/dl on a controlled low-fat diet or not on treatment with lipid-lowering agents.

The study treatments were: 1 tablet of EL (Estromineral Lipid) or E (Estromineral®) daily by mouth for 3 months.

Typical menopausal symptoms, CV and metabolic parameters were evaluated at baseline and at the end of treatment.

Results: Fifty-nine gynecology centers treated 535 women, 287 with EL and 248 with E; mean age 53.8 years and body mass index 25.4 kg/m², in the menopause for 3.8 years, 6.7% with previous hormone replacement therapy use.

After 3 months, EL significantly reduced Tot-C (-9.2% vs. -4.9%; p<0.01), LDL-C (-16.7% vs. -9.9%; p<0.05) and triglycerides (-13.3% vs. -6.3%; p<0.06) compared with E.

Hot flushes, night sweats, palpitations, decrease in libido and vaginal dryness decreased significantly compared with baseline with both treatments (p<0.0001).

Three cases with EL (dyspepsia, gastric pain and erythema) and 1 case with E (gastric pain) experienced non-serious adverse events.

Conclusion: Berberine in EL and the SI in both formulations significantly improved, respectively, the lipid profile and vasomotor symptoms in menopausal women. EL has the rationale for a complete prevention of menopausal risk: osteoporosis (vitamin D3, calcium and folic acid), genitourinary dystrophy (SI) and CV diseases (berberine).

Keywords: Menopause; Isoflavones; Berberine; Dyslipidemia; Cholesterol; Menopausal symptoms; Plant extracts

Introduction

The progressive increase in mean life expectancy of the general population and of women in particular that occurred in the last century in all industrialized countries has in fact led to women spending over a third of their life in a condition of estrogen deficiency and being exposed to a higher risk of developing diseases associated with this condition.

Of these, diseases of the cardiovascular (CV) system assume special importance, as they are still the main cause of death in women over 50 years of age in developed countries. The prognosis of all coronary events is, moreover, much worse in women compared with their male counterparts.

Among the specific risk factors, which include, inter alia, obesity, hypertension, diabetes, smoking and sedentary lifestyle, hypercholesterolemia occupies a prominent place.

The sex hormones have significant effects on the CV system. The ovarian hormone insufficiency associated with the menopause plays an important role in the occurrence of CV disease in women, as shown by...
the results of the Framingham Heart Study, where the annual frequency of coronary artery disease in women increased depending on age and underwent a clear increase from 55 years of age [1].

The standard treatment for the management of the menopause should take into account not just disappearance of the vasomotor symptoms but also prevention of osteoporotic and especially CV damage.

Indeed, the menopause increases the CV risk as a result of the progressive physiological deficiency of estrogens, giving rise to an increase in low-density-lipoprotein cholesterol (LDL-C), triglycerides (TGs) and apolipoprotein B and A-1 as well as a decrease in high-density-lipoprotein cholesterol (HDL-C). The estrogen deficiency in the menopause is actually associated with weight increase and insulin resistance, independently of the duration of the menopause, with an increased risk of metabolic syndrome (MS) and CV disease [2]. The knowledge of the outcome of the treatments specifically proposed for CV risk prevention is not yet achieved [3,4].

The use of dietary supplements that control the lipid profile is a rational approach to consolidate the results of diet over shorter periods, to increase the subject's desire to improve lifestyle habits and to delay the need for blood glucose-lowering drugs.

These considerations led to the development of a food supplement (EL) specifically intended for menopausal women to control some of the commonest and clinically important CV risk factors such as cholesterol, TGs and homocysteine, along with the typical disturbances of the menopause characterised by episodes of CV lability (hot flushes, sweating, palpitations), progressive atrophy of the tissues of the genitourinary tract, and dryness and aging of skin.

EL (Estromineral Lipid) contains berberine, which reduces plasma levels of cholesterol and TGs [5], Si, the absorption of which is facilitated by the presence of Lactobacillus sporogenes (Ls) [6], which has recognized activity on menopausal vasomotor symptoms [7]; folic acid, which ensures control of plasma levels of homocysteine, another CV risk factor and also associated with osteoporosis [8]; vitamin D3 and calcium for bone metabolism [9]. In a controlled study versus functional placebo and diet, EL showed promising effects in relation to controlling blood cholesterol and TGs in combination with a varied, balanced diet and healthy lifestyle in the postmenopausal period [10].

The aim of the study was to evaluate the action of EL in management of the menopause and CV risk factors in menopausal women not requiring drug treatment for dyslipidemia versus a product with the same formulation but without berberine.

Gynecologists spread out uniformly throughout Italy who agreed to participate in the study received an individual balanced randomization list for each site, drawn up based on random number tables, which provided for 5 subjects per treatment group. In case a center did not recruit all the expected cases, the random ratio up to 2:1 was accepted.

Inclusion criteria were postmenopausal women (menstrual cycle absence for more than 12 months) with typical symptoms and borderline hypercholesterolemia (total cholesterol [Tot-C] >200 and <260 mg/dL).

Exclusion criteria were women with familial hypertriglyceridemia or severe diseases in an acute phase, concomitant HRT use, and other drugs interfering with the climacteric symptoms or treatments active on the lipid profile.

Eligible women were randomly assigned to oral treatment with 1 tablet daily of EL (Estromineral Lipid, Meda Group, Stockholm, Sweden) containing berberine 500 mg+E (isoflavones 60 mg+Ls 1 billion spores +vitamin D3 5 µg +calcium phosphate dihydrate 137 mg+ folic acid 0.2 mg or of E (Estromineral, Meda Group, Stockholm, Sweden) for 3 months. All subjects were recommended a low-calorie, low-fat, low-carbohydrate diet, in accordance with their clinical conditions.

To increase subject compliance with the study procedure (i.e. complying with the times of visits, adherence to the dose and dietary regimen, recording any prescribed concomitant treatments), each subject was given a letter for their general practitioner describing the study rationale, the CV risk factors, the importance of cholesterol control and the main operational features of the protocol.

The sample size was calculated, taking the reduction in Tot-C as the primary endpoint. Based on values from a previous study with EL (reduction in Tot-C at 4 weeks of -16% compared with -5% with diet alone), the approximate number of observations needed for the two percentages to be significant at the 5% level of probability with β=0.9 was 160 cases per group [10]. Effect size and statistical power were calculated on the differences observed in Tot-C at the end of the two treatments.

At baseline and after 3 months, the presence and severity of hot flushes, night sweats, palpitations, decrease in libido and vaginal dryness were evaluated.

In addition, the following CV parameters were to be determined: systolic and diastolic blood pressure, waist circumference, Tot-C, HDL-C, LDL-C, TGs and blood glucose level.

At the end of the study, doctors gave their overall assessment of the treatment and subjects expressed their opinion on the acceptability of treatment, using a semi-quantitative scale, with the following scoring system: 0=none; 1=modest; 2=moderate; 3=good; 4=excellent.

The statistical analysis of clinical efficacy was based on comparison between the differences at the various times versus baseline, obtained using all data available for that specific period in the two treatment groups and was undertaken using the χ² test and analysis of variance, applied where appropriate, using Excel statistical software. The level of significance was taken to be α=0.05 (type 1 error) and power β=0.90 (type 2 error).

Methods

The experimental design was a multicenter, prospective, randomised, controlled, parallel-group study coordinated by Prof. Vincenzo De Leo from the Department of Molecular Medicine and Development, Division of Obstetrics and Gynecology, University of Siena, Italy. The study was approved by the local ethics committee for clinical trials of medicinal products at Siena University Hospital, Italy on 21/10/2010 and was conducted in accordance with the recommendations of the 1964 Declaration of Helsinki and subsequent revisions up to 2013.

In particular, subjects provided written informed consent and could stop the study at any time without needing to give any justification.
Results

The study was completed in 59 of the 69 (85.5%) gynecology centers involve (Figure 1). Treatment was given to 535 women, 287 with EL and 248 with E, mean age 53.8 (± 0.3 SE) years and BMI 25.4 (± 0.3 SE) kg/m², who had been in the menopause for 3.8 (± 0.3 SE) years, 6.7% with previous use of HRT. The baseline characteristics of the lipid profile and menopausal symptoms were homogeneous in the two groups (Table 1).

At the end of treatment, EL had improved the lipid profile compared with E: -9.2% versus -4.9% for Tot-C (p<0.01), -16.7% versus -9.9% for LDL-C (p<0.05) and -13.3% versus -6.3% for TGs (p<0.06) (Figure 2).

The effect size (Es) of the main outcome Tot-C is 0.6 considered a medium Es, with a statistical power of 100% for double sided two samples analysis.

Both body weight and BMI fell by 1.4% with EL and by 1.2% with E, and waist circumference fell by 1.7% and 1.3%, respectively, although not in a statistically significant manner (Table 2).

There was a significant reduction in hot flushes, night sweats, palpitations, vaginal dryness and improvement in libido compared with baseline with both treatments (p<0.0001) but there were no statistically significant differences between treatments (Figure 3).

Discussion

Berberine presents in El brought about a significant reduction in Tot-C, LDL-C and TGs in women in the postmenopausal period and complements the potential for the overall risk prevention in the menopause. The activity of SI in improving menopausal vasomotor symptoms was not compromised by the presence of berberine.

The reductions, albeit not statistically significant, in body weight, BMI and waist circumference are an interesting finding because adherence to treatment is often hampered in menopausal women by the fear of weight gain, one of the commonest reasons for refusal or abandonment of HRT in a sample of 1398 menopausal women [11].

The results obtained are reliable and robust as they were obtained in a broad representative sample of the Italian population, with an appropriate experimental design and adequate treatment period. Lack of standardization of diet was aimed at reproducing how the product is used in everyday clinical practice and making the results easier to extrapolate to the general population.

Limitations of the study are the lack of menopause confirmation by serum estradiol and serum FSH estimation, the analytical determination of the lipid profile by a centralized laboratory, the non-blind design. The following considerations can be raised on the above mentioned weaknesses: the menopause was defined as usually done in

Table 1: Comparison of group characteristics at baseline (total no=535).

| Parameters     | EL Baseline | EL 3-mo | Within-group Δ vs. baseline (p) | E Baseline | E 3-mo | Within-group Δ vs. baseline (p) | Between-group comparison of absolute Δ (p) |
|----------------|-------------|---------|---------------------------------|------------|--------|---------------------------------|------------------------------------------|
| Weight (kg)    | 67.9 ± 0.6  | 66.8 ± 0.7 | NS                              | 67.6 ± 0.7 | 66.6 ± 0.7 | NS                              | NS                                       |
| BMI            | 25.4 ± 0.3  | 25.0 ± 0.4 | NS                              | 25.7 ± 0.4 | 25.3 ± 0.4 | NS                              | NS                                       |
| Waist circumference (cm) | 98.5 ± 1.1 | 96.8 ± 1.0 | NS                              | 96.9 ± 1.1 | 95.5 ± 1.0 | NS                              | NS                                       |
| Systolic BP (mmHg) | 128.0 ± 0.9 | 125.1 ± 0.8 | 0.0102                          | 124.5 ± 0.8 | 123.0 ± 0.8 | 0.2307                         | p< 0.05                                  |
| Diastolic BP (mmHg) | 81.0 ± 0.6  | 78.3 ± 0.5  | 0.0003                          | 78.6 ± 0.6  | 76.8 ± 0.5  | 0.0216                         | NS                                       |
| Blood glucose (mg/dL) | 102.9 ± 0.8 | 98.1 ± 1.1  | 0.004                           | 101.7 ± 1.7 | 94.1 ± 1.5  | 0.0001                         | NS                                       |

Table 2: Summary of patient characteristics and secondary endpoints before and after 3 months of isoflavones alone (E) or combined with berberine (EL).
real practice, the determination of cholesterol and triglycerides is based on a common routinely applied and agreed method,

The clinical activity of EL was initially compared with a functional placebo such as vitamin D3+calcium. The current study versus active treatment adds new knowledge about EL and provides better documentation of the role of the ingredients. Indeed, the active treatment used as the control (E) was chosen both to make it easier to check the actual contribution of berberine to the anti-dyslipidaemic action of EL and because of its efficacy on menopausal symptoms, which improved independently of their initial severity and treatment duration [11].

The results obtained with EL in this study agree with those in the previous controlled study versus diet alone [10]. In the two studies, in fact, the pre-treatment values of the lipid profile in the EL group were comparable (Tot-C 239.4 and 235.5 mg/dL in the 1st and 2nd study, respectively) and allow a reliable comparison of the clinical effect after 3 months: Tot-C -13.6% vs. -9.2%, TGs -22.0% vs. -13.3%. Body weight and BMI each fell by 1.7% compared with 1.4% with EL and waist circumference fell by 1.4% vs. 1.7%. Hot flushes, with night sweats, vaginal dryness and libido improved with EL in a comparable manner in the two studies.

The findings that in the menopause mean plasma Tot-C levels are 220 mg/dl, some 35% of menopausal women have borderline levels, i.e. with Tot-C between 200, and 239 mg/dl, backs up the need for a preventive measure for the lipid profile. Furthermore, TG concentrations progressively increase as women age, up to 70 years. In addition to paying attention to lifestyle and diet, use of food supplements with anti-dyslipidemic activity is appropriate as part of the management of the menopause for risk prevention (whether CV risk or not) to control with lipid profile and to postpone the need for actual blood glucose-lowering drugs that are more potent but less well tolerated. EL contains interesting ingredients of natural origin, the properties of which justify the clinical results observed.

First, berberine, an extract from the bark of Berberis aristata, reduces the blood cholesterol level because it stimulates expression of LDL receptors, which increases the levels of messenger RNA and availability of LDL-cholesterol receptors, thereby increasing hepatic uptake of plasma cholesterol, i.e. with lower amounts of cholesterol in the bloodstream [12].

In vitro and in vivo, berberine inhibits TG biosynthesis through activation of a key enzyme in the regulation of TG synthesis, AMP-activated protein kinase (AMPK). The biosynthetic chain leading to TG formation has malonyl-CoA formation from acetyl-CoA as its rate-limiting step. The enzyme that catalyzes this step is acetyl-CoA carboxylase (ACC). In its phosphorylated form, ACC is inactive. Inactivation of this enzyme leads to an increase in fatty acid oxidation, with a consequent decrease in TG synthesis. AMPK is able to regulate
phosphorylation of ACC and therefore its catalytic activity. In HepG2 human hepatoma cells, berberine activates AMPK and increases levels of phosphorylated ACC with a subsequent increase in fatty acid oxidation and reduction in TG synthesis [13].

Blood glucose levels and the degree of insulin resistance increase as the frequency of hot flushes increase [14]. Berberine is an insulin-sensitising agent and a reduction in insulin resistance is a useful effect in CV disease prevention. Berberine, in a nutraceutical combination, significantly reduced the HOMA-IR, fasting and post-prandial glucose levels and insulin in patients with MS [15].

SI, chemically classified as phytoestrogens, alleviate mild and moderate vasomotor symptoms in the menopause [16]. Epidemiological studies have shown that soy lowers the risk of CHD independently of its modest cholesterol-lowering effect, through an effect on many risk factors for CHD [17].

In a randomised vs. placebo study in healthy postmenopausal women, 6 months of treatment with E showed a statistically significant increase in arterial diameter and blood flow and a reduction in adhesion molecules, ICAM-1, VCAM-1 and E-selectin, markers of endothelial damage and found in atherosclerotic plaques [18].

SI reduces the CV risks of metabolic origin and correct various markers of MS [19].

Prolonged supplementation of the diet with SI is associated with a reduction in weight, BMI, waist circumference and total body fat, and thus has a role in the prevention of chronic diseases that lead to excessive obesity [20].

Vitamin D supplementation is also able to reduce CV risk [21].

The rationale for the addition of folic acid to the EL formulation is supported by evidence that folic acid supplementation in postmenopausal women resulted in the statistically significant reduction of plasma levels of homocysteine, a CV risk factor that is increased in the menopause [22]. Moreover, folic acid deficiency and hyperhomocysteinemia are recognized risk factors for osteoporosis in the postmenopausal period [23].

The food supplement sector is full of many products, the large number of which is due to the lack of production quality controls and because there is no requirement to document the activity and therapeutic safety with clinical trials. In herbal medicine, where the active substances are from plant extracts, it is especially important to standardise the composition to guarantee a consistent final formulation and reproducibility of the clinical result, as occurs for actual pharmaceuticals. For this reason, the data described here apply only to EL and not to products containing the same ingredients, because the manufacturing method and purity of the extracts make a difference.

Berberine in EL and the SI in both formulations significantly improved, respectively, the lipid profile and vasomotor symptoms in menopausal women. EL has the rationale for a broad spectrum of risk prevention in menopause: osteoporosis (vitamin D3, calcium and folic acid), genitourinary dystrophy (SI) and CV diseases (berberine).

Based on these considerations, Estromineral Lipid may be considered as an essential treatment that is an alternative to hormonal therapies in women in the postmenopausal period who do not tolerate HRT or for whom HRT is not indicated or contraindicated, or has already been given for 5 years, in accordance with the recommendations of the Food and Drug Administration.

The availability for the doctors of a product like Estromineral Lipid represents the solution to a problem: helping women to cope better with postmenopausal symptoms arising from estrogen deficiency and putting in place true prevention of CV disease by preventing the increase in, and/or reducing the levels of, cholesterol.

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