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Effective treatment of vaginal atrophy with isoflavone vaginal gel☆

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ABSTRACT

Objetive: To assess efficacy and tolerability of a isoflavone (*Glycine max* L. Merr.) vaginal gel to the treatment of vaginal atrophy in postmenopausal women.
Methods: The double-blind, randomized, placebo-controlled, clinical trial. Ninety women were treated for 12 weeks with isoflavone vaginal gel 4% (1 g/day) and a placebo gel and conjugated equine estrogen cream (0.3 mg/day). After 4 and 12 weeks, the vaginal atrophy symptoms were classified at none, mild, moderate and severe and the vaginal cytology were taken to determine the maturation value. The endometrial safety (by transvaginal ultrasonography) was evaluated through at screening and the end of the trial.
Results: Isoflavone vaginal gel appears to be effective for relief of vaginal dryness and dyspareunia symptons and an increase in the intermediate and superficial cells was noted. These results were similar to the effects with use of conjugated equine estrogens and superior to placebo gel. No changes in endometrial thickness, sera FSH and estradiol levels were observed at study endpoint.
Conclusion: Glycine max (L.) Merr. at 4% vaginal gel on a daily basis in postmenopausal women led to improvements in vaginal atrophy symptoms and a significant increase in cell maturation values. Isoflavones proved good treatment options for relief of vulvovaginal symptoms especially in women who do not wish to use hormonal therapy or have contra-indications for this treatment.

1. Introduction

Postmenopausal estrogen deficiency results in numerous physiological changes that eventually affect virtually every organ system [1]. Vascular, musculoskeletal, and urogenital systems are especially vulnerable to continued estrogen decline. Sexual function and quality of life may also be diminished as a result of these changes [2]. The deficiency typically occurs in menopausal women, but can affect women of any age who experience a decrease in estrogenic stimulation of the urogenital tissues. In premenopausal women, hypoestrogenic states include the postpartum period, lactation and during administration of antiestrogenic drugs [3].

Vaginal atrophy (VA) is a common condition in postmenopausal women secondary to estrogen deficiency that causes involution of vaginal tissue, leading to itching, burning, dryness, irritation, and dyspareunia [1,4]. The reduction in estrogen levels is also associated with atrophy of the vulva and lower urinary tract, a condition commonly referred to as urogenital atrophy [5].

Unlike the vasomotor symptoms that typically accompany menopause, VA symptoms do not diminish over time and are unlikely to resolve without treatment [6]. The symptoms and consequences of VA can cause years of vulvovaginal discomfort, having a significant impact on quality of life for women in the postmenopausal stage of life [5].

Despite its high incidence, urogenital atrophy is an underreported and underdiagnosed condition. In fact, few women seek medical attention for vulvovaginal symptoms, often because they are uncomfortable talking about such a personal issue. According to The North American Menopause Society (NAMS), an estimated 10–40% of postmenopausal women experience symptoms related to urogenital atrophy but this aspect of menopause is often overlooked and undermanaged [1]. Only 20–25% of symptomatic women seek medical help, despite the availability of safe and effective options to treat vaginal and urological symptoms related to estrogen deficiency [7].

The proportion of women that experience symptoms such as vaginal dryness may increase five-fold as women advance through menopause [8]. Female bladder and urethral function also

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deteriorate with age [9]. Vulvovaginal atrophy can be especially problematic for women who want to be sexually active but find sexual intimacy uncomfortable because of vaginal dryness, itching, burning and dyspareunia [4,10]. Sexual activity itself has positive effects on vaginal elasticity, pliability, and lubrication while also promotes natural maintenance of urogenital health [2,4].

Hormonal replacement therapy (HRT), via systemic or topical routes, is widely used for the treatment of menopausal effects on urogenital tract. All local vaginal estrogen products have been recognized as being effective and well tolerated for treating vaginal atrophy [2,4,5,11]. In recent years, phytoestrogen supplements have become attractive as safer alternatives, and their efficacy has been investigated in experimental and clinical trials [12–14].

Isoflavones are the most studied of the phytoestrogens and some trials involving its oral form for treating climacteric symptomatology have shown no change in vaginal epithelium or endometrium. Similarly, topical preparations for the prevention and delay of skin maturation in postmenopausal women have shown satisfactory outcomes [15]. There are no studies available investigating the effects of vaginal administration of isoflavones on vaginal atrophy symptoms and endometrium in postmenopausal women as an alternative therapy for the relief of vaginal atrophy symptoms. The aim of the current investigation was to evaluate the efficacy of a new isoflavone vaginal gel compared to that of a placebo and conjugated equine estrogen.

2. Methods

2.1. Setting

The study commenced in January 2009 and was concluded in December 2010. The clinical trial was performed in accordance with the Declaration of Helsinki and International Standards of Good Clinical Practice (ICH-E6). The study protocol and patient informed consent form were approved by the Ethics Committee of the Irmandade da Santa Casa de Misericórdia de São Paulo hospital, in São Paulo – Brazil. All investigations were performed at this institution.

2.2. Study design

The double-blind, randomized, placebo-controlled, clinical trial comprised three phases. At the first visit, written informed consent was obtained and inclusion and exclusion criteria assessed. Eligible participants were randomly assigned to receive *Glycine max* (L.) Merr. Isoflavone vaginal gel, placebo gel or cream containing conjugated equine estrogens (CEE). Each one was vaginally administered daily for the entire 12 weeks. During both visits at four and twelve weeks, the definitions of vaginal symptoms were explained to participants and vaginal smears were taken for vaginal cytology. Endometrial safety (endometrial thickness by transvaginal ultrasonography) was evaluated at initial screening and trial endpoint.

2.3. Study drug

The isoflavone of *G. max* (L.) Merr. extract 4% and the placebo gel were manufactured by Hebron® Laboratory (in Caruaru, Pernambuco, Brazil), and the cream containing conjugated equine estrogens (CEE) 0.625 mg/g (Premarin®) was provided by Wyeth (Philadelphia, PA, USA). The extraction method of isoflavone was secreted industrial supplier. Each 1 g of isoflavone gel has 0.05 g of dry extract 10% soybean. The soybean dried extract 10% consists in: 3.2% Daidizin, 5.5% Genistin, 0.51% Glycitin, 0.35% Daidzein, 0.39% Genistein and 0.05% Glycitein. The chemical compounds can vary in the range of ±10.0%. All chemicals substances were characterized and quantified by HPLC/UV/DAD. The placebo formulation was the same of final product. The placebo gel consists in carbopol, methylparaben and propylparaben, sodium hydroxide and water. The three products were placed in similar tubes. Treatment instructions were to administer 1 g of isoflavone gel or 1 g of placebo or 0.5 g of CEE corresponding to 0.3 mg, vaginally at bedtime.

2.4. Patients

The inclusion criteria were: non-hysterectomized, postmenopausal (2 or more years since final menstrual cycle) women who were 45 years of age or older with symptoms of vaginal dryness and/or pruritus, pain/soreness, vulvar or vaginal burning, and dyspareunia. All participants were required to have serum E_2 levels less than 20 pg/mL, follicle-stimulating hormone levels greater than 40 mIU/mL, no superficial cells on vaginal cytology, an endometrial thickness of less than 4.0 mm as assessed by transvaginal ultrasonography, and a normal mammography during the 6 months leading up to study entry. Exclusion criteria were: use of any investigational drug or exogenous sex hormones within the 6 months leading up to study drug initiation, or current use of corticosteroids, known or suspected history of hormone-dependent tumor, breast carcinoma, genital bleeding of unknown cause, acute thromboembolic disorder associated with estrogen use, vaginal infection requiring treatment, allergy to the test drug or its constituents, hot flashes, and any serious disease or chronic condition that could interfere with study compliance.

2.5. Assessments

All women underwent medical examination (interview, hematology, biochemistry, urinalysis, gynecologic examination, mammography and transvaginal ultrasonography) in order to determine patient eligibility. During both visits at four and 12 weeks, patients reported by a questionnaire any symptoms of vaginal dryness, pruritus, pain/soreness, vulvar or vaginal burning and dyspareunia, which were subsequently classified as follows: (none = 0, mild = 1, moderate = 2, severe = 3). For vaginal cytology, vaginal smears were taken at four and 12 weeks from the upper third of the right lateral vaginal wall and analyzed at the Department of Pathology (Santa Casa Sao Paulo Hospital, Sao Paulo, Brazil). These samples were used to determine the Maturation Index, which describes the proportion of parabasal, intermediate and superficial cells. The maturation value (MV), or Frost Index, was calculated according to the formula: MV = (0 × % parabasal cells) + (0.5 × % intermediate cells) + (1.0 × % superficial cells) [20]. Maturation of the vaginal epithelium (a positive treatment effect) is evidenced by a decrease in parabasal cells and an increase in the proportion of superficial cells.

Serum FSH levels were analyzed using the chemiluminescence system ACS-180, Chiron – DL = 0.30 mIU/L and serum estradiol levels were analyzed using chemiluminescence/Chiron – LD = 10.0 pg/mL. The postmenopausal reference ranges used were serum FSH levels from 23.0 to 116.3 mIU/mL and estradiol levels from 0 to 19 pg/mL. The assay detection limits were 5 pg/mL for estradiol and 0.3 mIU/mL for FSH. Limits of quantitation were 500 pg/mL for estradiol and 200 mIU/mL for FSH. Blood for hormone analysis was collected at baseline and after 90 days of the study. After collection, blood was allowed to stand for approximately 30 min at ambient temperature and then centrifuged at >1200 × *g* (3500 rpm) to separate blood cells from serum. The serum was carefully transferred to a another serum container by means of pipette and analyzed for estradiol and FSH.

2.6. Statistical analyses

Without information on the vaginal effect of the isoflavone *G. max* (L.) Merr. a sample size of 90 patients was required. This sample size allowed treatment effect to be determined at $\alpha = 0.05$ with