Endometrial safety assessment of a specific and standardized soy extract according to international guidelines

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Abstract

Objective: To assess the effects of an oral soy isoflavone extract (Phytosoya) on endometrium (evaluated by biopsy and ultrasonography) in postmenopausal women treated for 12 months.

Design: A total of 395 postmenopausal women were included in this international prospective, open-label study. The women were treated for 12 months with a specific standardized soy isoflavone extract (total of 70 mg/d). Endometrial biopsy and transvaginal ultrasonography were performed before and after 12 months of treatment according to European guidelines.

Results: A total of 301 assessable biopsy specimens were obtained from women treated for 12 months; the results were 99.67% atrophic/inactive endometrium and 0.33% proliferative endometrium. No case of hyperplasia or carcinoma was diagnosed, demonstrating the endometrial safety of this extract (point estimate: 0.0; upper limit of 95% CI: 0.012). Endometrial thickness did not show any increase after 12 months of treatment (2.2 mm at inclusion and 2.12 mm at the end of the study). Only eight women reported some kind of bleeding as an adverse event during the study.

Conclusions: These results of endometrial biopsy and endometrial thickness suggest that daily administration of 70 mg of a specific and standardized isoflavone extract for 12 months does not stimulate the endometrium.

Key Words: Endometrial safety – Isoflavones – Menopause.

Estrogen therapy is well established for the relief of climacteric symptoms in postmenopausal women as well as for the prevention of osteoporosis. However, according to conclusions from the Women’s Health Initiative trial, estrogen-progestin therapy (EPT) increases the risk of thromboembolic diseases and coronary events.1 Grodstein et al2 have suggested that the timing of EPT initiation in relation to menopause onset or to age could influence coronary risk.2 Long-term treatment with EPT may increase the risk of developing breast cancer.3 Small but statistically significant differences were found between previous EPT users and nonusers for most breast cancer risk factors.4

The Women’s Health Initiative estrogen-alone trial, which randomized hysterectomized women to conjugated equine estrogens only or placebo, was prematurely stopped because of an increased risk of stroke and absence of a reduction in risk of coronary heart disease.5 However, a subsequent analysis of these trial data demonstrated that therapy with conjugated equine estrogens alone for 7.1 years does not increase breast cancer incidence in hysterectomized women.6 These results are in contrast to the results of the Women’s Health Initiative trial with EPT.

Use of unopposed oral estrogen therapy for 1 to 2 years in nonhysterectomized women is associated with a relative risk of endometrial cancer of 2.4 compared with women who have never received this intervention.7 Therefore, the identification of an alternative that has the beneficial effects on climacteric symptoms but no cancer risk and side effects would be of considerable value.

Phytoestrogens are plant substances that are structurally and functionally similar to estradiol. These phytoestrogens seem to have more affinity for estrogen receptor (ER) β than for estrogen receptor α.8 Therefore, given the different tissue distribution of the α and β receptors, there is a clear potential that isoflavones could exhibit tissue-selective effects. The effects of isoflavones on the endometrium are less known, specifically whether prolonged exposure to phytoestrogens could promote endometrial hyperplasia or carcinoma as does estrogen. Some clinical studies on the effects on the
ENDOMETRIAL SAFETY OF A SOY EXTRACT

Endometrium in postmenopausal women have been carried out during the past few years, but their results are discordant. Therefore, the objectives of the present study were to provide a sufficiently precise estimate of the rate of endometrial hyperplasia or more serious adverse endometrial outcomes and to prove the overall safety/tolerability of 12 months of treatment with a specific and standardized soy extract (Phytosoya).

METHODS

Women were enrolled and treated at 34 centers across four countries (Australia, Belgium, France, and Spain) between June 2004 and September 2006. The trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. Ethical approval of the study protocol was obtained from the ethics committee from each country before the study start, and written informed consent was obtained from each participant at the screening visit.

Those eligible for inclusion were healthy postmenopausal women aged 45 to 65 years with an intact uterus and no natural menses for at least 2 years. At admission, menopause status was confirmed by serum follicle-stimulating hormone and estradiol levels in the postmenopausal range (follicle-stimulating hormone >30 IU/L and estradiol <35 ng/L).

The main exclusion criteria were undiagnosed vaginal bleeding, endometrial hyperplasia, submucosal myomas, endometrial polyps, estrogen-dependent tumors, uncontrolled hypertension, acute or history of a thromboembolic event, and obesity (body mass index >30). A washout period was observed for the following therapies: hormone therapy, dehydroepiandrosterone, tibolone, raloxifene (3 mos); isoflavones (2 mos); and local hormone treatment (1 mo). None of these medications was allowed throughout the study.

Women were not included if their endometrial thickness measured by transvaginal ultrasonography (TVU) was more than 4 mm and/or if the endometrial biopsy result was no tissue, endometrial polyp, or hyperplasia.

Endometrial assessments

The primary endpoint was the incidence of endometrial hyperplasia/cancer after 52 weeks of treatment, based on assessment of endometrial biopsy specimens. Endometrial biopsy specimens were obtained at study inclusion (M0) and after 12 months treatment (M12) using a cannula suction system (pipelle de Cornier samplers). A biopsy was also performed in cases of premature study withdrawal, whatever the reason, if the woman was treated for at least 12 weeks. Histological findings were assessed independently by two pathologists (C.B., F.N.), who were blinded to the time of biopsy. Biopsy specimens were classified as no endometrial tissue, tissue insufficient for diagnosis, atrophic endometrium, proliferative endometrium, secretory endometrium, menstrual type endometrium, hyperplasia (simple and complex, with or without nuclear atypia), or carcinoma. Endometrial polyps, if present, were fully histologically characterized. The protocol specified that in case of disagreement between the two readers on the presence or severity of hyperplasia, reading by a third specialist was required. If a unanimous decision among the three pathologists on discussion of the samples in dispute could not be achieved, the most unfavorable of the three competing diagnoses would be used for data analyses. In case of disagreement between two readers for other items of the classification, samples were reviewed by two experts during a consensus meeting.

TVU was performed on all women before the endometrial biopsy at entry and at 12 months. The endometrial thickness was measured at the thickest part of the endometrium in the longitudinal plane and included both endometrial layers.

Treatment

Phytosoya is a specific and standardized isoflavone extract containing 17.5 mg of isoflavones per capsule with the following distribution of isoflavones: 50% daidzin, 30% glycitin, and 20% genistin. The dose regimen was two capsules in the morning and two capsules in the evening, corresponding to a dose of 70 mg of isoflavones daily. The treatment duration was 12 months with the possibility for the women to continue the treatment for an additional 2 years.

Statistical analyses

Endometrial safety can be established if the incidence of hyperplasia/carcinoma does not exceed 2% after 1 year of treatment. According to the European Medicines Agency guidelines, a sample size of 300 women treated for 1 year was required to estimate the incidence rate of hyperplasia/carcinoma with the required precision (ie, that the upper limit of the one-sided 95% CI does not exceed the point estimate by more than 2%).

Assuming a dropout rate of approximately 20% and a nonassessable biopsy specimen rate of approximately 5%, 395 women were included to obtain 300 assessable biopsy specimens at the end of the study.

Women who took at least one dose of study medication were included in the safety set analysis. Stringent modified intent to treat (smITT) is defined as women from ITT who comply with the following conditions: final visit performed after at least 344 days and final biopsy less that 1 month after the last day of treatment.

RESULTS

A total of 499 outpatients were selected, and 104 were selected but not included because they did not meet all inclusion criteria, leading to a total of 395 postmenopausal nonhysterectomized women included in the study. The demographic and baseline clinical characteristics of the safety set are summarized in Table 1 (N = 395). Seventy-eight premature discontinuations were recorded (19 for adverse events, 6 lost to follow-up, 34 for lack of efficacy, 3 for protocol violation, and 16 for personal reasons). Among the 78 women who discontinued the study prematurely, 29 were treated for less than 12 weeks.

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TABLE 1. Demographic data of included women
(safety sample = 395)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>55.93 ± 4.24</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>64.14 ± 9.07</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.55 ± 3.26</td>
</tr>
<tr>
<td>Mean age at menopause, y</td>
<td>49.49 ± 3.30</td>
</tr>
<tr>
<td>Menopause duration, y</td>
<td>6.40 ± 4.03</td>
</tr>
</tbody>
</table>

Figure 1 depicts the distribution of women from selection to the final biopsy of assessable participants (305 women in smITT). This population had a mean study duration of 364.9 days (±11.54).

Endometrial biopsies

A total of 394 biopsy samples were available for analysis at inclusion (one biopsy was performed but not analyzed because the sample was lost by the carrier). The distribution of histological classification was as follows (Table 2): 1 woman had no endometrial tissue (0.25%), 39 had insufficient endometrial tissue (9.9%), 348 had atrophic/inactive (88.33%), and 6 had proliferative (1.52%). According to the European Medicines Agency guidelines revision 1, the endometrium with a biopsy result of insufficient tissue for diagnosis associated with an endometrial thickness less than 5 mm can be categorized as an atrophic endometrium. Because all endometrial thicknesses were less than 5 mm at inclusion, all results of tissue insufficient for diagnosis can be categorized as atrophic/inactive endometrium, giving a total of 387 atrophic endometria at inclusion (98.2%) (Table 3).

At the final visit, a total of 305 biopsy samples were analyzed for assessable women (compare with Fig. 1).

Biopsy samples were unavailable for six women who completed the study because of either a narrow cervix or refusal. However, these six women underwent TVU, and their endometrial thickness ranged from 0.5 mm to 2 mm and excluded any risk of hyperplasia. The distribution of histological classification for the 305 assessable women was as follows: 2 no tissue (0.66%), 47 insufficient tissue (15.41%), 255 atrophic/inactive (83.6%), and 1 proliferative (0.33%) (Table 2). Because no tissue (n = 2) and tissue insufficient for diagnosis with an endometrium thickness of 5 mm (n = 2) are not considered assessable biopsy samples, the total number of assessable biopsy samples at the final visit was 301 (Table 3). The biopsy result insufficient for diagnosis was associated with an endometrial thickness of less than 5 mm in 45 women, leading to a total of 300 atrophic/inactive endometria. The calculated 1-year incidence rate of endometrial hyperplasia or more serious adverse event outcome was 0% with an upper limit of 95% CI of 0.012.

Atrophic endometrial polyp was found in three biopsy samples at the final visit. In two cases the endometrial biopsy result was atrophic/inactive, and in one case there was no endometrial tissue. For one woman the conclusion of

TABLE 2. Biopsy results of assessable women

<table>
<thead>
<tr>
<th>Classification</th>
<th>Inclusion</th>
<th>Final visit (study duration: 364.9 ± 11.54 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tissue</td>
<td>1* (0.25%)</td>
<td>2* (0.66%)</td>
</tr>
<tr>
<td>Tissue insufficient for diagnosis</td>
<td>39 (9.90%)</td>
<td>47 (15.41%)</td>
</tr>
<tr>
<td>Atrophic/inactive</td>
<td>348 (88.33%)</td>
<td>255 (83.6%)</td>
</tr>
<tr>
<td>Proliferative</td>
<td>6 (1.52%)</td>
<td>1 (0.33%)</td>
</tr>
<tr>
<td>Secretory/vascular</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cancer</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>394</td>
<td>305</td>
</tr>
</tbody>
</table>

*The result of the first reading was inactive/atrophic (explaining the inclusion), but the presence of endometrial tissue was not found during the consensus meeting.

**Three atrophic endometrial polyps were diagnosed (two in an atrophic endometrium and one in a biopsy obtaining no tissue).

TABLE 3. Distribution of assessable biopsy samples according to European guidelines

<table>
<thead>
<tr>
<th>Category</th>
<th>Inclusion</th>
<th>Final visit (study duration: 364.9 ± 11.54 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total biopsy samples</td>
<td>394</td>
<td>305</td>
</tr>
<tr>
<td>Excluded biopsy samples</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>No tissue</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Tissue insufficient for diagnosis</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>accompanied by endometrium thickness = 5 mm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total of assessable biopsy samples</td>
<td>393</td>
<td>301</td>
</tr>
<tr>
<td>Atrophic/inactive</td>
<td>387</td>
<td>300</td>
</tr>
<tr>
<td>(insufficient tissue for diagnosis accompanied by endometrium thickness &lt;5 mm included)</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

FIG. 1. Distribution of women from selection to final biopsy. ND, no data; smITT, stringent modified intent to treat.
the histopathological report was that another sampling was recommended to confirm the histological result. Consequently, a hysteroscopy was performed for polyp resection and endometrium sampling. The atrophic feature of the polyp was confirmed as was atrophic endometrium.

Modification of histology patterns between baseline and study termination was observed for eight women. Six women had a proliferative endometrium at inclusion in the study that became atrophic/inactive or tissue insufficient at the end of the treatment (one premature discontinuation included). Conversely, one woman had an atrophic/inactive endometrium at entry that became proliferative with an endometrial thickness of 5 mm at the end of the treatment.

Among the 78 women who discontinued the study prematurely, 49 were treated for more than 12 weeks, and 24 of them refused to undergo a final endometrial biopsy. A total of 31 biopsy samples from nonassessable women were obtained: 25 from premature discontinuation and 6 from women who completed the study but did not have a biopsy performed within the time limit.

The histological results of the 31 biopsies from non-assessable women were 1 no tissue and 30 atrophic/inactive, leading to a total of 30 assessable biopsies. Taking into account all the assessable biopsy samples (231), the 1-year incidence rate was 0% with an upper limit of the 95% CI of 0.011.

**Endometrial thickness**

For the population studied (smIT), the average endometrial thickness was 2.2 mm with a SD of ±0.98 mm at inclusion and 2.12 mm (±1.10) at the end of the study. The mean change was −0.08 mm (±1.03), demonstrating that this specific and standardized isoflavone extract does not stimulate the endometrium after 1 year of treatment. The same pattern is found in the safety sample: 2.15 mm (±1.02) at inclusion and 2.08 mm (±1.1) at the final TVU. An endometrial thickness greater than 5 mm was found in two women at the final visit, ranging from 5.8 to 6.2 mm. Histology showed an inactive/atrophic endometrium in the two cases, and these women did not experience any bleeding. A hysteroscopy was performed for the woman who had an endometrial thickness of 6.2 mm and revealed an endometrial polyp with a regular surface, explaining the increase in endometrial thickness seen on TVU. The woman who had an endometrial thickness of 5.8 mm underwent TVU 3 months after last visit, and the endometrial thickness had decreased to 5.1 mm, which was judged by the investigator as not necessitating further examination.

**Bleeding episodes**

Eight women experienced some kind of bleeding episode during the study: three spotting, four mild metrorrhagia, and one severe metrorrhagia. All these women have completed the study, their endometrial thickness was less than 4 mm, and the biopsy result was atrophic endometrium or tissue insufficient for diagnosis at the final visit.

The woman who reported severe metrorrhagia lasting 7 days completed the study with no other bleeding episode during the 9 months of treatment; the endometrial thickness was 1.5 mm with an endometrium classified as tissue insufficient for diagnosis at final visit.

**General tolerance**

The only recurrent adverse event related to the study product was moderate gastrointestinal disorders, which were reported in 4.6% of women in the safety set. In the safety set, 68.9% of women found treatment to be excellent and 25.7% of women good in terms of tolerance.

**DISCUSSION**

The incidence of endometrial cancer is highest in developed countries, occurring predominantly in postmenopausal women. Although the etiology and risk factors are not yet fully understood, evidence suggests that estrogen might play a central role in the progression and development of the disease.

Numerous studies have shown that the use of estrogen for hormone substitution increases the risk of endometrial hyperplasia and endometrial cancer, as shown in the meta-analysis of Grady et al. The risk of developing endometrial cancer in users of unopposed estrogen is two- to eightfold higher than that of nonusers, and in most women endometrial hyperplasia due to estrogen effects precedes cancer formation. The major approach for preventing estrogen-induced endometrial hyperplasia consists of the addition of progestin, which exerts antiproliferative effects on the estrogen-primed endometrium. In women with an intact uterus, the risk is significantly reduced by the addition of progestogen.

Moreover, the clinical cardiovascular health benefits of estrogen-progestogen therapy for postmenopausal women remain controversial, and alternatives for hormone therapy have been sought. Soy is a rich source of the isoflavones genistein, daidzein, and glycitein. Studies have been reported in which foodstuffs such as soy produce a decrease in the incidence and severity of hot flushes. Some contradictory results could be due to the large chemical heterogeneity of soy derivatives used. No change in vaginal dryness has been observed in any of the studies. A double-blind, placebo-controlled study was conducted with our specific extract in 75 menopausal women. After 3 months of treatment, a significant decrease in the number of hot flushes was observed in the treated group.

Several studies have examined the effects of isoflavones on bone mass or bone turnover in postmenopausal women. As a result of limited relevant studies and differences in methodological approaches, there is a suggestion, but no conclusive evidence, that isoflavones have a beneficial effect on bone health.

Some studies have shown that soy isoflavones are able to decrease low-density lipoprotein cholesterol and increase high-density lipoprotein cholesterol, whereas others have failed to reproduce these results. The hypocholesterolemic effect of soy isoflavones may depend on the doses
Ingested and the duration of treatment as well as differences in isoflavone composition.

Considering soy extract as a substitute for hormone therapy in the treatment of postmenopausal women with a uterus, the question of the endometrial response to these phytoestrogens is raised. Several reports demonstrated no increase in uterine weight or increase in endometrial proliferation in ovarie-tomized animal models.29 Foth and Cline30 showed several potential mechanismic bases for the antiproliferative effects of soy in primate endometrium, a high concentration of serum sex hormone–binding globulin and therefore less free estradiol in plasma, inhibition of aromatase activity, and 17β-estradiol dehydrogenase, thus diminishing the formation of estradiol from other steroids.

Several studies were performed to determine the effects of phytoestrogens on the endometrium.31-34 The absence of endometrial effects was demonstrated, but the studies involved a small number of women or had a short study duration, and most of them used only TVU. Only one combined TVU and biopsy, but the treatment was adminis-
tered for only 6 months.31 However, Uner et al35 recently reported that long-term treatment with soy phytoestrogen was associated with an increased occurrence of endometrial hyperplasia compared with placebo. Because this study was conducted with high amounts of isoflavones formulated with 40% to 45% genistein (150 mg of isoflavones per day), these results could not be extended to all other isoflavone extracts.

Our clinical study was performed to assess the endome-
trial safety of our specific and standardized extract with the same dose used for an efficacy study (70 mg isoflavones daily; 50% daidzin, 20% genistin).36 No case of hyperplasia was diagnosed among the 305 postmenopausal women who completed the 1 year of treatment. These results clearly demonstrate the endometrial safety of this supplement with the precision required by European guidelines.

A low incidence of vaginal bleeding (2%) was observed in our study. Of the eight women reporting bleeding and/or spotting, all had an atrophic endometrium. Bleeding from an atrophic endometrium is not uncommon after menopause. Endometrial atrophy is found in 50% of women with postmenopausal bleeding.37 This phenomenon is thus far unexplained, although anatomical vascular variations or local abnormal hemostatic mechanisms in the uterus38 involve of acidic and basic fibroblast growth factor,39 or vascular endothelial growth factor,40 and preexisting pathologies41 have all been suggested.

CONCLUSIONS

Because no case of hyperplasia/carcinoma was diagnosed among the 301 assessable biopsy samples at 1 year, the endometrial safety of this extract was clearly demonstrated (point estimate: 0.0; upper limit 95% CI: 0.012). Furthermore, it does not exert a mitogenic effect on the endometrium, as demonstrated by the lack of change in the endometrial thickness associated with the histological results (99.67% inactive and 0.33% proliferative endometrium at 1 year).

Considering the constantly growing population of post-
menopausal women with their different symptoms and the potential adverse effects of long-term hormone therapy, phytoestrogen may be a safe alternative.

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REFERENCES


13. Person I, Adami HO, Bergkvist L, et al. Risk of endometrial cancer after treatment with estrogens alone or in conjunction with proges-

14. Person I, Yven J, Bergkvist L, Schairer C. Cancer incidence and mortality in women receiving estrogen and estrogen-progesterin replace-


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