Pulsed estrogen therapy in prevention of postmenopausal osteoporosis. A 2-year randomized, double blind, placebo-controlled study

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Abstract The aim of this study was to evaluate the efficacy of pulsed estrogen therapy (intranasal 17β-estradiol) in the prevention of postmenopausal bone loss. A total of 386 women (40–65 years old), less than 5 years past menopause, were randomized to intranasal placebo, 17β-estradiol 150 µg, or 300 µg daily for 2 years. Women with an intact uterus received micronised progesterone 200 mg per day, 14 days of each 28-day cycle. Women randomised to placebo-treatment received placebo progesterone. The primary endpoints were changes in BMD at the spine (L2–L4) and femoral neck. Secondary endpoints were changes in bone turnover markers: serum osteocalcin (sOC) as a marker of bone formation and urinary C-terminal telopeptides (uCTX) as a marker of bone resorption. BMD increased at all measured sites in women receiving active treatment in a dose-related manner, the difference compared to placebo being 5.2% and 6.7% at the spine, and 3.2% and 4.7 % at the hip, respectively, with 150 µg and 300 µg ($P < 0.001$). On the other hand, a decrease versus baseline of −3.2% and −3.3% at the spine and hip, respectively, was observed in women receiving placebo ($P < 0.001$). In the patients with at least one risk factor for osteoporotic fracture, the difference between placebo and 150 µg or 300 µg was even higher at the spine (5.4% and 7.4%, respectively), and at the femoral neck (4.0% and 5.2%, respectively). Correspondingly, uCTX decreased from baseline by 39% and 46 %, and sOC by 22% and 27%, in the 150 µg group and 300 µg group (all $P < 0.001$ versus placebo). A strong correlation was found between variations of bone turnover markers after 1 year and BMD after 2 years, emphasizing that bone markers can predict BMD response during hormonal treatment. Acceptability and general tolerance were good. This study demonstrates that pulsed estrogen therapy at the dose of 150 µg and 300-µg per day prevents bone loss in a dose-dependant manner at each site studied, and normalizes bone turnover markers to premenopausal levels.

Introduction

Menopause-related estrogen deficiency accelerates the rate of bone resorption and decreases the rate of bone formation, causing a rapid loss of bone mass and leading to an increased risk of osteoporosis and fractures [1]. Indeed, osteoporosis is a major cause of morbidity and mortality in postmenopausal women throughout the world [2]. Hormone replacement therapy (HRT) is well established for the prevention of postmenopausal estrogen deficiency symptoms such as vasomotor disturbances and genitourinary atrophy. In addition, use of HRT prevents long-term development of postmenopausal bone loss [3] and the occurrence of osteoporotic fractures at vertebral or non-vertebral sites [4]. Recently, some studies have demonstrated that the favorable effect of estrogen on BMD persists for many years after stopping treatment [5, 6].

S21400 (17β-estradiol by intranasal route) introduces a new concept in the treatment of estrogen deprivation symptoms: pulsed estrogen therapy which is a brief exposure of target tissues to an appropriate dose of 17β-estradiol administered by the non-oral route, avoiding continuously elevated plasma estrogen levels [7]. In contrast to oral or transdermal therapies, S21400 gives a pronounced peak serum concentration of estradiol that is reached 10–30 min after

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administration. The serum concentration of estradiol returns to 10% of the peak value after 2 h and to untreated postmenopausal women levels within 8–12 h [8]. Intranasal application avoids the first-pass hepatic metabolism seen during oral therapy, which necessitates a higher dose of estradiol. In clinical trials, it has been shown that S21400 300 µg/day is at least as effective as 2 mg oral estradiol or a patch delivering 50 µg/day in reducing climacteric symptoms, but with a better gynaecological tolerance [9, 10]. The 300 µg/day dose provides significant relief of vasomotor symptoms compared to placebo from week 2 of treatment, whereas the dose of 150 µg/day is significantly effective after 8 weeks of treatment [11].

The aim of this 2-year randomized, double blind, placebo-controlled study was to demonstrate the efficacy of pulsed administration of estrogen, using two different doses of S21400 (150 µg/day and 300 µg/day) in the prevention of early postmenopausal bone loss. Primary study end-points were changes in BMD at the lumbar spine and total hip, and secondary study end-points were changes in the bone markers, serum osteocalcin (sOC) and urinary degradation products of the C-terminal telopeptides of type I collagen (uCTX) after 2 years of treatment.

Materials and methods

Patients

The women, 40–65 years of age at baseline, were randomized if they were less than 5 years past menopause at study entry. Menopause was defined as amenorrhea for more than 12 months or for more than 6 months and a concomitant serum level of estradiol below 0.16 nmol/l and a follicle stimulating hormone (FSH) level above 42 IU/l. All women who had undergone hysterectomy had menopause confirmed by measurement of serum estradiol and FSH at least 2 months before study entry. Women who had undergone surgical menopause (bilateral ovariectomy) could be included, provided the operation was performed at least 6 weeks before study entry. Women who previously had received estrogen replacement therapy, had to go through a 6-month wash-out period before randomization. Bone mineral density (BMD) at the lumbar spine (L2–L4) had to be between 1.0 SD above and 2.5 SD below the mean value for normal premenopausal women (upper limit of T-score initially set at –1 was extended to +1 by amendment). To be eligible for the study, all women had to be in good general health with no clinical or laboratory evidence of systemic disease or conditions with known influence on bone metabolism. This included hip fracture due to osteoporosis, radiographic vertebral fracture ≥25%, severe lumbar osteoarthritis or scoliosis, secondary osteoporosis, or bone disease such as Paget's disease or osteomalacia. Furthermore, subjects with nasal disease incompatible with nasal therapy such as frequent epistaxis, chronic rhinitis or sinusitis, severe allergic rhinitis, or frequent nasal treatment were excluded. Subjects with contraindications for the use of HRT were also excluded. This included a suspicious breast lump, an abnormal mammogram, progressive uterine disease such as endometrial hyperplasia, fibromyomas, or undiagnosed vaginal bleeding. Women with a history of significant cardiovascular disease or uncontrolled hypertension were also excluded. The trial was approved by the local ethical committees and performed in accordance with the Declaration of Helsinki. All women gave a written informed consent to participate in the study.

Study design

A 2-year, double blind, placebo-controlled, randomized study was performed at two centers in Denmark (Ballerup and Aalborg). Five hundred and seventy-seven women were selected and recruited using questionnaires sent out with the aid of their social security numbers. Following a 4-week run-in period (selection), 386 patients were randomized to receive either S21400 150 µg/day ($n = 129$) or 300 µg/day ($n = 129$) (Aerodiol, Servier Laboratories, Courbevoie, France) or placebo ($n = 128$) for a 2-year period. Randomization was performed due to the order of inclusion. Thus, the first patient received the lowest numbered therapeutic unit in the block of units supplied to the center concerned. The treatment allocation lists were drawn up and encoded by Servier Laboratoires. All treatments were supplied as identical intranasal sprays to be administered once daily in the evening (one spray in each nostril). Women with an intact uterus also received 200 mg of micronized progestogen (Effik; Effik Laboratories, Madrid, Spain), or progestogen placebo (placebo patients), combined with the nasal spray for the last 14 days of each 28-day cycle. No dose modifications were allowed during the study. Study visits were scheduled at screening (week −4), and then at week 0 (baseline), 4, 12, 26, 52, 78, and 104.

At each visit, the women were questioned about gynecological and local acceptability (such as vaginal bleeding, mastalgia, genitourinary, and nasal symptoms) and a general physical examination was performed. A pelvic (including cervical smear) and a breast examination as well as transvaginal ultrasound were performed at baseline and at week 52 and 104. Bilateral mammography was assessed at baseline and at week 104. Women who stopped their treatment prematurely were asked to continue the study without treatment with all planned examinations.

Measurement of bone mineral density

Lumbar spine and hip BMD were measured at each study center by dual-energy X-ray absorptiometry (DXA) using a Hologic system (Hologic, Waltham, Mass., USA). BMD at the forearm was measured by using the DTX 200 device (Osteometer MediTech, Rødovre, Denmark). For a given patient, the same scanner was used throughout the study. Quality control scans (Hologic Spine Phantom) were performed daily. Cross calibration phantom scans were used to assess the equivalence of scanner performance between centers. All scans were read centrally by SYNARC (Rødovre, Denmark). At selection, two consecutive DXA scans were performed after repositioning of the woman between the scans. At subsequent visits, only one scan was performed. The in-vivo short-term precision error of the measurement was 1.0% at the spine and 1.5% at the femoral neck. BMD measurements were taken at the anterior-posterior projection of the lumbar spine and the left hip (total hip, femoral neck, and trochanter). If treatment was discontinued prematurely (unless the previous scan was performed within 6 months earlier), a termination scan of the spine and hip was performed. "Responders to treatment" were defined as women who had gained bone mass (BMD increase greater than 0%) and "non-responders to treatment" were defined as women with a significant loss of bone mass (BMD decrease greater than twice the in vivo short-term precision error of the BMD measurement, i.e. 2.0% at the spine or 3% at the femoral neck) at the end of the study compared to baseline.

Assessment of biochemical markers of bone turnover

At baseline and each subsequent visit, blood and urine samples were drawn between 8 and 10 a.m. after an overnight fast. Each sample was determined in duplicate and the mean value was given as the result. In the study we measured the N-terminal mid-fragment (1–43) of OC (N-mid osteocalcin one-step ELISA-assay; Nordic Bioscience, Herlev, Denmark). The intra- and interassay coefficients of variation were 3.4% and 5.3%, respectively.

Measurements of uCTX were performed by ELISA (CrossLaps ELISA; Nordic Bioscience, Denmark). The uCTX concentration was corrected for creatinine excretion. The intra- and interassay coefficients of variation were 5.1% and 4.0%, respectively.

Statistics

Analysis of bone mass was performed on the intention-to-treat (ITT) population, which was defined as all randomized patients who received at least one dose of treatment and who had an evaluation of lumbar spine BMD at baseline and once again under study treatment. Missing values at W104 were replaced by the last post-baseline values reported. The per protocol (PP) population, which was used to analyse the bone turnover markers and their correlations with BMD variations, consisted of all randomised patients with an evaluation of lumbar spine BMD at baseline and at the last planed visit (W104). A subpopulation of early post-menopausal patients at increased risk of osteoporosis (high risk population) which consisted of women in the PP population with at least one osteoporotic risk factor [osteopenia at either the spine or the hip, low calcium intake (< 500 mg/day), heavy smoker (> 20 cigarettes/day), low BMI (< 23 kg/m^2) or early menopause (before age 45)], and a duration of menopause less than 5 years was planned to confirm efficacy results. The comparison between each dose of S21400 and placebo at study end (ITT population) or W104 (PP and high risk population) was studied according to a one-tailed hierarchical step-down procedure under the clinical assumption of a monotonic dose-related effect.

In the ITT population, the percentages of responders and non-responders to treatment at the end of the study were compared between each dose of S21400 and placebo using a one-tailed hierarchical step-down procedure with the Cochran-Mantel-Haenszel test adjusted by center. A simple linear regression model was used to assess whether long-term changes in BMD could be predicted on the basis of changes in sOC or uCTX.

The type I error was set at 5% for all two-tailed tests and at 2.5% for all one-tailed tests (hierachical procedures).

All safety analyses were performed in the safety set population, which consisted of all randomized patients who received at least one dose of treatment.

Results

Of the 577 women who were initially selected for inclusion in the study, 386 women were randomized to the three study groups. The remaining women did either not meet the inclusion criteria or had personal reasons for not participating. Overall, 345 (89.4%) women completed the 2-year follow-up, and 110 (28.5%)

discontinued prematurely the study treatment, 80 patients for adverse events, and 30 for non-medical reasons. Fifty-one randomized patients were excluded from the ITT analysis because they had never taken their treatment ($n = 10$) or had no post-baseline lumbar spine BMD measurements ($n = 41$). The PP population consisted of 270 women: 104 patients in the placebo group, 86 in the group treated with S21400 150 μg per day, and 80 in the group treated with S21400 300 μg per day. The "high risk population" consisted of 255 women.

Baseline characteristics were similar among groups as shown in Table 1.

Bone mineral density and bone turnover markers

Mean lumbar spine BMD increased from baseline to the end of the study in both S21400 groups and decreased in the placebo group. The percent change from baseline to end was greatest in the S21400 300 μg group ($3.53 \pm 4.10\%$, mean $\pm$ SD). This increase was significantly higher with S21400 300 μg and 150 μg ($P < 0.001$) than with placebo, in a dose-dependent manner, with an adjusted estimated difference (SE) of 6.72% (0.48) and 5.19% (0.44), respectively (Table 2). Most of the gain in spine and hip BMD in the groups receiving active treatment occurred during the first year (Fig. 1). After 1.5 years, spine BMD stabilized in the 150 μg group, whereas it still increased in the 300 μg group. Regarding the other BMD sites evaluated, total hip, femoral neck, femoral trochanter, distal and ultra-distal forearm BMD, a significantly increase was seen in both S21400 groups compared to placebo, at each visit from W26. These increases were significantly higher with S21400 300 μg and 150 μg ($P < 0.001$) than with placebo with adjusted differences (SE) of 4.70% (0.39) and 3.18% (0.37) for total hip BMD of 4.82% (0.50) and 3.60% (0.47) for femoral neck BMD of 5.74% (0.50) and 3.82% (0.46) for trochanter BMD of 3.08% (0.55) and 3.17% (0.57) for distal forearm BMD of and 5.50% (0.75) and 5.30% (0.74) for ultradistal forearm BMD, respectively (Table 2). The mean percentage BMD increase compared to baseline was even higher whatever the site measured in the high risk population receiving

Table 1 Baseline characteristics in the randomised population (mean $\pm$ SD). *SD* standard deviation; *BMI* body mass index

	Placebo ($n = 128$)	S21400 150 μg ($n = 129$)	S21400 300 μg ($n = 129$)
Age (years)	52.8 ± 2.0	52.6 ± 1.6	52.8 ± 1.8
BMI (kg/m^2)	24.9 ± 3.6	25.4 ± 4.0	25.4 ± 4.1
Years since menopause	2.3 ± 1.4	2.4 ± 2.5	2.0 ± 1.2
Previous ERT (%)	10.2	4.7	4.7
Hysterectomy (%)	7.8	4.7	4.7
Osteopenia[a] (%)	89.8	87.6	89.9
Current smoker (%)	25.8	27.9	33.3
Daily intake of calcium (mg)	694.0 ± 468.2	693 ± 433.8	724.8 ± 422.1
Spine BMD (g/cm^2)	0.957 ± 0.089	0.961 ± 0.100	0.957 ± 0.099
Femoral neck BMD (g/cm^2)	0.735 ± 0.084	0.724 ± 0.084	0.722 ± 0.080
Osteocalcin (ng/ml)	20.9 ± 7.3	22.7 ± 7.4	22.0 ± 9.3
Urinary CTX (μg/mmol creatinine)	292 ± 112	291 ± 111	304 ± 123

[a] Osteopenia at the lumbar spine or hip was defined as BMD T-score ≤ -1

Table 2 BMD values and mean percentage changes from baseline after 2 years of therapy in the ITT population

	Placebo ($n = 118$)	150 μg/day ($n = 114$)	300 μg/day ($n = 103$)
Lumbar spine (%)	−3.2 ± 3.5	2.0 ± 3.5	3.5 ± 4.1
Estimated difference versus placebo		5.2 ± 0.4	6.7 ± 0.5
P-value versus placebo		$P < 0.001$	$P < 0.001$
Total hip (%)	−3.3 ± 3.1	−0.1 ± 2.5	1.4 ± 2.8
Estimated difference versus placebo		3.2 ± 0.4	4.7 ± 0.4
P-value versus placebo		$P < 0.001$	$P < 0.001$
Femoral neck (%)	−3.8 ± 3.6	−0.1 ± 3.5	1.1 ± 4.1
Estimated difference versus placebo		3.6 ± 0.5	4.8 ± 0.5
P-value versus placebo		$P < 0.001$	$P < 0.001$
Trochanter (%)	−3.6 ± 3.8	0.3 ± 3.4	2.2 ± 3.7
Estimated difference versus placebo		3.8 ± 0.5	5.7 ± 0.5
P-value versus placebo		$P < 0.001$	$P < 0.001$

S21400 150 μg or 300 μg compared to placebo, with an estimated difference (SE) between 150 and 300 μg/day, respectively, and placebo group of 5.40% (0.52) and 7.36% (0.55) at the spine, 3.58% (0.42) and 5.38% (0.43) at the total hip, 3.96% (0.55) and 5.18% (0.58) at the femoral neck, 4.24% (0.53), and 6.44% (0.58) at the trochanter. A corresponding response was seen in the bone turnover markers (Fig. 1). After an initial increase observed after 4 weeks of treatment, the bone formation markers (sOC) decreased in both S21400 groups. sOC was normalized in most of the women after 1 year of treatment. In contrast, in the placebo group, sOC tended to increase in most women. After 2 years of treatment sOC was significantly decreased ($P < 0.001$) in the 150 μg and 300 μg treatment groups (−21.8% and −27.3%, respectively) compared to the placebo group (+31.7%). Regarding bone resorption markers (uCTX), the decrease occurred from the first month of treatment, to reach a plateau after 6–12 months of treatment in both S21400 groups. In contrast, uCTX remained stable in the placebo group throughout the study. After 2 years of treatment, uCTX levels decreased significantly ($P < 0.001$) in the 150 μg and 300 μg treatment groups (−39.0% and −46.1%, respectively) compared with the

Fig. 1 Mean % change in BMD at the spine BMD for the ITT-group (*left top*), femoral neck BMD for the ITT group (*left bottom*), mean % change in serum osteocalcin (*sOC*) for the PPS-population (*right top*), and urinary C-terminal telopeptidedes of type I collagen (*uCTX*) for the PPS population (*right bottom*). Values are mean ± SEM
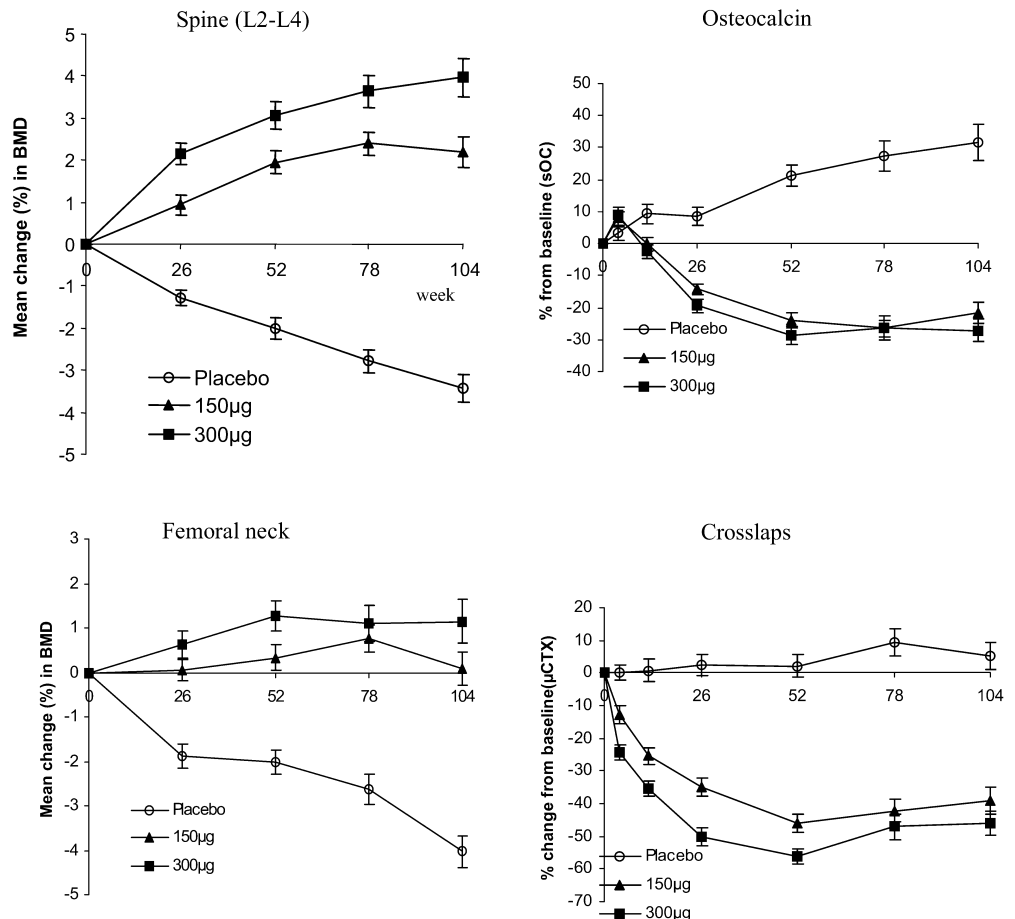
placebo group (+5.3%) (Fig. 1). In term of responders to treatment, most of the women gained bone at the end of the study with the 150 and 300 µg doses of S21400, respectively: 78% and 82%, compared to 16% with placebo (Table 3). Interestingly the proportion of non-

Table 3 Number of patients with BMD gain or loss after 2 years of treatment in the ITT population
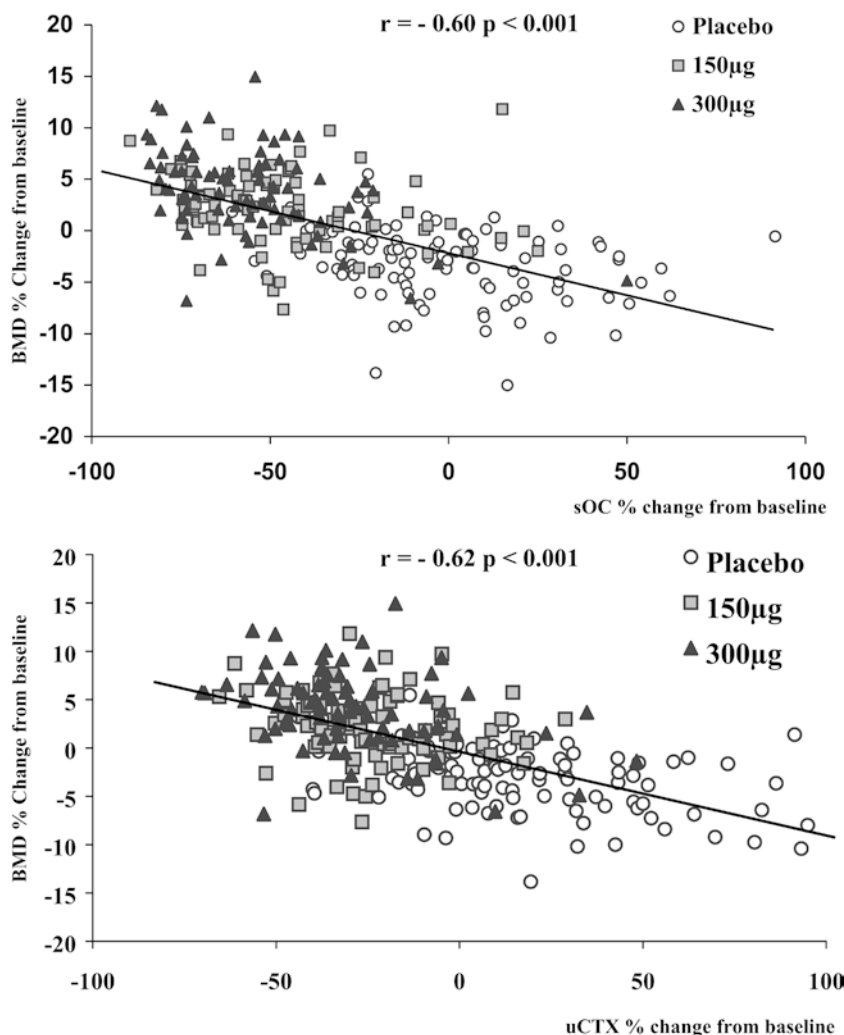
	Placebo (n=118)	150 µg/day (n=114)	300 µg/day (n=103)
Lumbar spine			
Responders[a]	19 (16%)	89 (78%)*	84 (82%)*
Non-responders[b]	67 (57%)	11 (10%)*	10 (10%)*
Femoral neck			
Responders[a]	15 (13%)	52 (46%)*	61 (59%)*
Non-responders[b]	53 (45%)	10 (9%)*	8 (8%)*

[a]Responders: patients with a gain in bone, i.e. BMD variation (end value−baseline value) >0%
[b]Non-responders: patients with a gain in bone, i.e. BMD variation (end value−baseline value) of more than twice the in vivo short-term coefficient of variation of DXA measurements at the corresponding site
*$P < 0.001$ versus placebo

responders to treatment was similar in both S21400 groups and very low regardless of the site. A strong correlation was found between variations in the bone resorption marker (uCTX) or the bone formation markers (sOC) and response in BMD after 2 years ($r = -0.62$ and $r = -0.60$, respectively). The more the bone markers decreased (after 12 months), the higher was the response in BMD (after 2 years) both in women receiving placebo and in women receiving active treatment (Fig. 2). A similar trend was also seen using 6-month changes in sOC and uCTX (data not shown).

Safety

Overall, 353 women (94%) reported at least one adverse event during the treatment period with the most frequently reported adverse events being those which were possibly related to the route of administration of the treatment (rhinitis, sneezing, and application site reaction) and to the estrogen effects (breakthrough bleeding) (Table 4). The majority of local symptoms occurred during the first month of treatment and their frequency

Fig. 2 Correlations between change in spine BMD after 2 years and bone markers

Table 4 Adverse events within the study groups during the study. Evaluated for the safety set ($n = 376$)

	Placebo ($n = 125$)	150 µg/day estradiol ($n = 128$)	300 µg/day estradiol ($n = 123$)
Rhinitis	39 (31%)	47 (37%)	34 (28%)
Rhinorrhea	5 (4%)	5 (4%)	9 (7%)
Sneezing excessive	5 (4%)	13 (10%)	25 (20%)
Breakthrough bleeding	20 (16%)	26 (20%)	33 (27%)
Vaginal discharge	1 (1%)	9 (7%)	10 (8%)
Excessive mastalgia	3 (2%)	6 (5%)	10 (8%)
Headache	15 (12%)	23 (18%)	17 (14%)
Abdominal pain	8 (6%)	14 (11%)	14 (11%
Hot flushes	12 (10%)	7 (6%)	5 (4%)

decreased thereafter. Intensity was rated as mild or moderate. Breakthrough bleeding was experienced by 33 patients (27%) in the 300 µg group, 26 women (20%) in the 150 µg group, and 20 patients (16%) in the placebo group. Of these women, only five experienced severe breakthrough bleeding: one patient in the 300 µg group, two women in the 150 µg group, and two women in the placebo group. Between baseline and the end of the study, moderate to severe mastalgia was more frequent during active treatment compared to placebo (eight and six patients in the 300 µg group and 150 µg group, respectively, versus three women in the placebo group). There were two cases of death considered not to be related to the study treatment (lung carcinoma and glioblastoma). Additionally, four breast neoplasms were reported, one in the placebo group, one in the S21400 150 µg group, and two in the S21400 300 µg group. For three of them, suspect findings were seen retrospectively on the baseline mammogram. No clinically relevant changes from baseline or between groups were detected in the haematological and liver enzyme blood tests. After 2 years of treatment there was a decrease in mean (SD) body weight of -0.5 (2.8) kg in the groups receiving active treatment as compared to an increase of 0.3 (2.5) kg in the placebo group. The estimated difference in weight between the groups receiving active treatment and placebo was statistically significant ($P = 0.024$). No significant or clinically relevant difference was seen between the study groups in terms of blood pressure variables. In all, 80 women were withdrawn from the study due to adverse events: 15 women (11.7%) in the placebo group, 28 women (21.7%) in the S21400 150 µg group and 37 women (28.7%) in the S21400 300 µg group. Of these, 15/80 were due to local nasal symptoms (one woman in the placebo group, six women in the S21400 150 µg group and eight women in the S21400 300 µg group). Ten out of 80 withdrew due to vaginal bleeding (four women in the S21400 150 µg group and six women in the S21400 300 µg group), 10/80 withdrew due to headache or migraine (four women in the placebo group, three women in the S21400 150 µg group and three women in the S21400 300 µg group). Only 5/80 withdrew due to hot flushes (three women in the placebo group and two women in the S21400 150 µg group).

Discussion

This is the first study to show that S21400 (pulsed estrogen therapy provided by nasal administration of estradiol) significantly increases bone mineral density compared to placebo after 2 years of treatment. The increases in bone mineral density observed with S21400 were of the same magnitude as those observed after a 2-year treatment with other hormonal replacement therapies currently registered for osteoporosis prevention such as conjugated equine estrogens, 0.625 mg [12], 2 mg oral micronized 17β-estradiol [13], or a 50 µg transdermal patch [14]. In addition, the increase in lumbar spine BMD seen during pulsed estradiol therapy is greater than that reported following long-term treatment with raloxifene 60 mg or tibolone 2.5 mg [15, 16]. Furthermore, as already observed with estrogens [17], the mean percentage increase in bone mineral density was even higher for the subset of women who had a high risk of osteoporosis. In our study, a dose-related effect on BMD was observed with both doses of estradiol. Increasing the daily dose from 150 µg to 300 µg 17β-estradiol per day resulted in a 1.8% additional average gain in BMD at the spine, but without significant difference in terms of responders to treatment, showing adequate efficacy in preventing bone loss with 150 µg intranasal 17β-estradiol.

In terms of response to treatment, most women treated with S21400 (150 or 300 µg) responded favorably, whatever the bone sites. Interestingly, although the mean BMD gain at the spine or the femoral neck increased with the dose, the percentage of non-responders did not increase when the dose of S21400 was decreased, contrary to what is seen with low dose estradiol patches [14]. In the current study, all women with an intact uterus received micronized natural progesterone, chosen because of its lack of a confounding effect on BMD [12].

The effect of pulsed estrogen therapy on bone turnover markers and BMD was consistent with previous report with S21400 [18]. Concerning serum osteocalcin, the decrease with S21400 was detectable after 12 weeks. The delayed response in osteocalcin normalisation in the S21400 groups has previously been reported with transdermal estradiol and intranasal estradiol [18], and this is probably due to the lack of a digestive first bypass effect on serum insulin-like factor achieved after non-oral administration of estradiol. The continuous increase of bone formation markers in the placebo group was probably linked to the fact that the women were early postmenopausal (>80% of the study population had a menopause duration < 3 years) and experienced high rate of bone loss (> 3% after 2 years).

The observed changes in BMD reflected the changes in the bone turnover markers that were normalized to premenopausal levels during treatment. In both treated and untreated women, there was a strong reciprocal relationship between the short-term changes in the biochemical markers of bone turnover and the long-term

changes in BMD, which is consistent with several previous reports [19]. The highest average BMD response was thus seen in women treated with estradiol and having the most suppressed bone turnover markers, and the lowest average BMD response was seen in women treated with placebo and having the least suppression in the bone turnover markers.

The local or general acceptability of S21400 was good and consistent with previous findings [7, 10, 20]. The dropout rate was similar to other HRT clinical trials and the adverse events responsible for premature termination were of a mild nature (nasal symptoms, vaginal bleeding and headache). Only a few discontinued due to hot flushes.

In summary, this study demonstrates that pulsed estrogen therapy achieved after S21400 administration prevents postmenopausal bone loss. The sustained "plateau kinetics" seen with oral and transdermal formulations are therefore not a requirement for efficacy and acceptability in the treatment of postmenopausal symptoms, and prevention of postmenopausal bone loss. So far, the results suggest that S21400 is a promising alternative to conventional postmenopausal HRT. An initial dose of 300 µg per day is recommended for an optimal efficacy/tolerability ratio.

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