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Prevention of postmenopausal bone loss by pulsed estrogen therapy: comparison with transdermal route

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Abstract

Objective: To compare the efficacy of pulsed estrogen therapy following intranasal 17 β -estradiol (E2) (S21400) with patch E2 in preventing postmenopausal bone loss and on bone turnover. **Methods:** In this multinational open study, 361 postmenopausal women aged 51.5 (S.D. 4.6) years were treated with S21400 300 μ g per day or patch E2 (delivering 50 μ g per day), two patches per week, for 56 weeks. Bone mineral density (BMD) was assessed at the spine and hip using dual X-ray absorptiometry at baseline and week 56 (W56). Bone turnover markers (osteocalcin, bone alkaline phosphatase, urinary type I collagen C-telopeptides) were measured at baseline and weeks 12, 28 and 56. **Results:** Spine and hip bone mineral density significantly increased in both groups ($P < 0.001$ versus baseline). Mean (S.D.) percent increases were 2.1 (3.0) at the spine (both groups), and 1.2 (2.4) and 1.1 (2.2) at the hip in the S21400 and patch E2 groups, respectively. Bone mineral density also significantly increased ($P < 0.001$ versus baseline) in osteopenic patients following S21400 and patch E2: 3.1 (3.5) and 2.4 (3.5) at the spine, and 2.0 (2.6) and 1.2 (2.7) at the hip, respectively. Bone metabolism was normalized at week 56 with a significant decrease ($P < 0.001$) from baseline in all markers: 56% and 53% for type I collagen C-telopeptides, and 24% and 25% for osteocalcin in the S21400 and

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patch E2 groups, respectively. *Conclusion:* Pulsed estrogen therapy was as effective in normalizing bone turnover and preventing postmenopausal bone loss as a reservoir patch.

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Keywords: Postmenopausal; Estrogen therapy; Bone loss

1. Introduction

Postmenopausal osteoporosis is a serious age-related disorder and is a major cause of morbidity and mortality in postmenopausal women. Approximately 40% of women will experience one or more fractures after the age of 50 years [1]. It is well established that estrogens inhibit bone resorption and that estrogen replacement therapy is widely used to prevent postmenopausal osteoporosis. Such therapies have been shown to reduce the risk of osteoporotic fracture by approximately one-third compared to placebo [2].

Intranasal administration of 17 β -E2 (S21400) leads to a specific E2 plasma kinetic profile which differs totally from that achieved by oral or transdermal routes [3]. This formulation introduces a new concept in hormone replacement therapy (HRT), the “pulsed estrogen therapy”, a brief once-daily exposure of target tissues to a small dose of natural 17 β -estradiol, producing continuous therapeutic efficacy over 24 h, thus avoiding continuously elevated plasma estrogen levels. The efficacy of pulsed estrogen therapy achieved after S21400 administration has been validated against placebo [4] and the 300 μ g daily dose has been shown to be equivalent in climacteric symptom reduction to 2 mg oral micronized or E2 50 μ g per day transdermal E2 delivery system, but with a better gynecological tolerance [5,6]. The effects of pulsed estrogen therapy on bone mineral density have not been previously studied.

The aim of the current study was to compare pulsed estrogen therapy achieved after S21400 administration with a reference reservoir patch, administered at comparable doses, for effectiveness in the prevention of postmenopausal bone loss, as assessed by their effects on bone mineral density and bone turnover markers. Long-term tolerability of the two routes of administration and their acceptability to women were also assessed.

2. Methods

Caucasian female outpatients, aged 40–65 years, were enrolled in the study if they had been postmenopausal for less than 10 years, had no prior exposure to nasal or patch HRT, and had undergone a washout period of at least 6 weeks if they had previously taken percutaneous or oral HRT. Women included in the study had been amenorrheic for at least 6 months and had hormonally confirmed menopause (i.e. E2 <30 pg/ml and follicle-stimulating hormone (FSH) >40 mIU/ml). Further inclusion criteria included absence of clinically significant abnormalities on recent mammography (<12 months) or cervical smear (<18 months), along with normal serum glucose, lipids, and liver enzyme levels. Patients were excluded if they had any ear, nose, and throat or skin disease incompatible with the study treatments. Contraindications for the use of estrogen and/or progesterone, were osteoporotic or had concomitant bone or endocrine diseases liable to affect bone metabolism, or were receiving concomitant treatment with non-authorized drugs (i.e. agents likely to affect bone metabolism or used for symptomatic treatment of hot flushes). The trial was conducted with local ethics committee approval in accordance with the respective regulations for each country, the Declaration of Helsinki and good clinical practice. Written informed consent was obtained from all patients before participation in the trial.

This controlled study was undertaken in 51 centers in seven European countries (France, Italy, Spain, Poland, Portugal, Belgium, and Netherlands). Within each country, patients were randomized to receive either S21400 300 μ g per day (AERODIOL[®], Servier Laboratories, Courbevoie, France) as one spray in each nostril once daily, or continuous administration via a reservoir patch (Estraderm TTS[®] 50, Ciba Laboratories) delivering 50 μ g per day, replaced twice weekly for 12 weeks. This initial treatment period was fol-

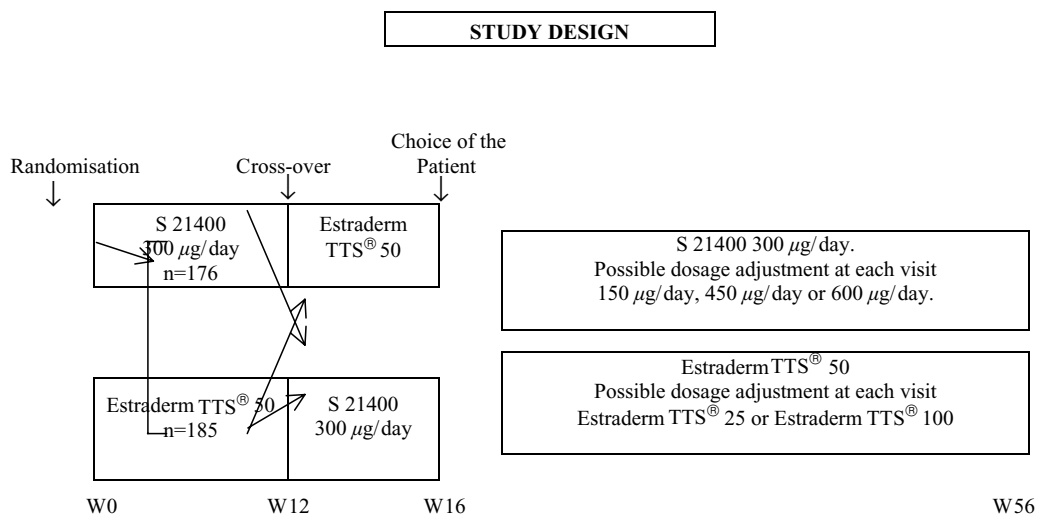


Fig. 1. Treatment periods.

lowed by a further 4-week period in which each group received the alternative therapy. After this 16-week period, patients received the treatment of their choice (either S21400 or patch E2) for further 40 weeks (W16 to W56) (Fig. 1). The study design for the initial 16-week period was previously detailed by Lopes et al. [6]. Dose adaptation was allowed from W16 for each therapy in case of clinical signs of hypoestrogenism (S21400 dose increased to 450 µg or 600 µg per day; patch E2 dose increased to 100 µg per day, replaced twice weekly) or hyperestrogenism (S21400 dose reduced to 150 µg per day; patch E2 dose reduced to 25 µg per day, replaced twice weekly). All patients with an intact uterus received dydrogesterone (10 or 20 mg per day according to local guidelines) during the last 14 days of each 28-day cycle to ensure endometrial shedding.

The primary efficacy endpoint at week 56 was the percentage change from baseline in lumbar spine bone mineral density. Secondary efficacy criteria included bone mineral density changes from baseline at the femoral neck and total hip and the percentage change in biochemical markers of bone turnover. Bone mineral density was measured by dual-energy X-ray absorptiometry, using Hologic or Lunar systems. Quality control scans were run in accordance with the manufacturer's recommendations. The same scanner was used for any given patient and the same protocol was applied for any given centre throughout the study.

Two consecutive scans were carried out at baseline and again at W56 with a repositioning between measurements. Bone mineral density was measured at lumbar level (L2–L4) in the anterior–posterior projection and at the hip level (femoral neck, trochanter and intertrochanteric region, Ward's triangle and whole area). A final quality control of the measurements was carried out by a central laboratory (CELAM, Lyon, France).

Bone markers (urine and serum) were measured at W0, W12, W28, and W56 by a central laboratory (SYNARC, Lyon, France). Radioimmunoassay was used to analyze osteocalcin and bone-specific alkaline phosphatase in serum (ELSA-OSTEO™, CIS Biointernational, France, and Ostase™, Hybritech®, USA, respectively) and type I collagen C-telopeptide (urinary cross laps™; Osteometer Biotech®, Denmark) corrected for creatinine in urine (urinary type I collagen C-telopeptides). The intra-assay variability for osteocalcin, bone-specific alkaline phosphatase and urinary cross-laps was <6%, <12%, and <8%, respectively.

The Kupperman Index was used to measure climacteric symptoms (hot flushes, night sweats, sleep disturbances and nervousness) at W–4, W0, W12, W16, W28, W40, and W56, using an 11-point questionnaire in which symptoms are graded (0–3) according to their frequency or severity [7]. The frequency and intensity of hot flushes and the number of night sweats

were recorded in patient diaries. The intensity of hot flushes was defined as mild (hot flushes without sweating), moderate (bothersome hot flushes with or without sweating) or severe (hot flushes with sweating that interrupted patients' activities or awoke them). Genitourinary symptoms were evaluated at each visit and included day or night-time polyuria, dysuria, urgency, stress incontinence, and dyspareunia. At W12, W16, and W56 patients graded their satisfaction with the treatment from 0 to 100 using a visual analogue scale including general satisfaction, convenience, speed of administration, effectiveness, discretion, and hygiene.

Lipids were assessed at W0, W12, W28, and W56 on a Hitachi 717 automated analyzer: total cholesterol (colorimetric enzymatic test), high-density lipoprotein(s) (HDL; precipitation and colorimetric enzymatic test), low-density lipoproteins (LDL; enzymatic kinetic test) and triglycerides (colorimetric enzymatic test). Anti-thrombin III (activity test) and fibrinogen (optical detection) analyses were also carried out at W0, W12, W28, and W56.

The safety determinations of the study included gynecological, local nasal/dermal and general tolerability of the treatment, and blood chemistry analysis (alanine transaminase, aspartate aminotransferase, alkaline phosphatase, and γ -glutamyl transpeptidase). Full gynecological and breast examinations were carried out at enrollment. Further, breast examinations were carried out at all other visits, along with assessments of vaginal trophicity and cervical mucus. The occurrence of mastalgia and vaginal bleeding (withdrawal or breakthrough bleeding) were recorded in the patients' diaries. Nasal tolerability of treatment was assessed using a non-suggestive questionnaire that evaluated the presence/absence of the following symptoms: prickling sensation, runny nose, sneezing, nose bleeds, and rhinitis. Dermal tolerability (erythema, oozing lesions, skin pigmentation, and pruritis) was assessed by the investigator. General adverse events (rated as mild, moderate or severe) and their relation to the treatment were determined by the investigator at each visit.

Statistical analyses were performed on the two groups, defined according to the treatment taken by each patient for the longest period of time between inclusion and W56. All statistical tests were two-sided with a type I error of 5%. Quantitative variables were described by sample size, mean and

standard deviation (S.D.) and qualitative variables in terms of frequency and percentage per category. All efficacy analyses were performed on the Full Analysis Set, which consisted of all randomized patients who received at least one dose of treatment and who had an evaluation of lumbar spine bone mineral density at W0. Bone mineral density analyses were also performed on a subset of osteopenic patients at baseline. The efficacy results were confirmed in the subgroups of patients who received the alternative treatment only between W12 and W16. Changes over time were studied within each group using a two-tailed Student's *t*-test for paired samples (bone mineral density at lumbar spine, total hip and femoral neck) and one-way analysis of variance with repeated measures followed, in case of a significant time effect, by a Dunnett's test versus baseline (bone markers, Kupperman index, lipids, and hemostasis parameters). The difference between groups at W56 was assessed using a covariance analysis on the W56 percentage change with baseline as covariate (bone mineral density) or a Student's *t*-test for independent samples on the W56 percentage change from baseline (bone markers, lipids and haemostatis parameters). Ninety-five percent confidence intervals of estimated differences between groups (patch—S21400) at W56 were calculated. Missing values at W56 were replaced by the last values including baseline (bone mineral density analyses) and by the last values reported from W28 (bone markers and Kupperman index analyses, and lipids and hemostasis between-groups analyses). Missing values of the Kupperman index at W40 were replaced similarly. The difference between groups was also assessed on each aspect relative to treatment satisfaction at W56 using a Student's *t*-test for independent samples.

All safety analyses were performed on the Safety Set, which consisted of all randomized patients who received at least one dose of treatment. The incidence of moderate to severe mastalgia between W0 and W12 was compared between groups using a chi-square test.

3. Results

Of the 454 women screened for the study, 361 were randomized to receive the study medication. Of these patients, 176 were randomized to receive

S21400 during W0–W12, followed by the patch during W12–W16, and 185 patients received the patch during W0–W12 followed by S21400 during W12–W16. Among the 340 patients who were chosen between the patch and the spray at W16, 66% (224 patients) chose the spray versus 34% (116 patients) who chose the patch [6].

When defined according to the longest duration of treatment, 234 patients were included in the S21400 group and 127 in the patch E2 group. Fifty-one patients stopped the treatment prematurely with the same frequency in each group, but more frequently for adverse events in the patch E2 group than in the S21400 group, respectively 15/127 (12%) and 12/234 (5%). In more than half the reasons were for gynecological disorders (more frequently in the patch E2 group) or local symptoms: rhinorrhea, nasal discomfort, and nose bleed for S21400, and erythema, eruption, and abnormal pigmentation for the patch. Twenty one patients withdrew for non-medical reasons and three for major protocol deviation. One patient was lost to follow-up at W56 (Fig. 2). The mean total duration of exposure to the treatment was similar in the two groups (373 and 351 days in the S21400 and patch E2 groups, respectively) with a mean exposure to the alternative treatment of around 15% of the total duration in both treatment groups. The baseline characteristics of patients were comparable for all factors analyzed (Table 1). Approximately 20% of patients who received S21400 for the longest period during the study had at least one dose change between W16 and W56 compared with around 11% in the patch E2 group. In each group the most common dose change was a dose increase (58% in each group).

Treatment with both S21400 and the patch E2 was associated with increased bone mineral density in all areas analyzed (Table 2). Mean lumbar spine, femoral neck and total hip bone mineral densities were significantly increased ($P < 0.001$) between baseline and the end of treatment in both groups. No significant differences in the percentage increases in bone mineral density in the lumbar spine, femoral hip or total hip were noted between treatment groups. These results were confirmed in the subgroup of patients who received the alternative treatment only during the W12–W16 period (mean duration exposure to the alternative treatment <8% of the total treatment duration period). In this subset of patients (102 and 51 in the S21400 and

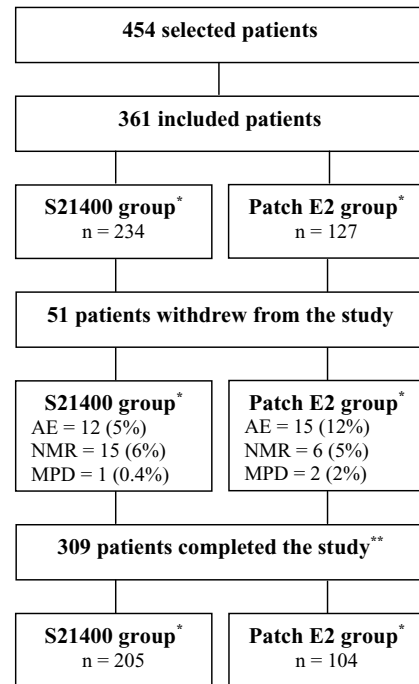


Fig. 2. Patients disposition. Asterisk (*) indicates the treatment groups which were defined according to the treatment received (either S21400 or patch E2) for the longest period during the 56 weeks of treatment, double asterisk (**) indicates one patient in S21400 group was lost to follow-up at visit W56, AE, adverse event; NMR, non-medical reason; and MPD, major protocol deviation.

patch E2 group, respectively), the S21400 group experienced mean (S.D.) percentage increases in bone mineral density of the lumbar spine of 2.3 (3.2) and the total hip of 1.8 (2.7), with similar increases seen in the patch E2 group (2.4 (3.6) and 1.7 (2.9), respectively). There was no significant difference in bone mineral density between the two treatment groups in any other site evaluated. S21400 and patch E2 treatments resulted in mean (S.D.) percentage increases in bone mineral density of the Ward's triangle (1.52 (4.67) and 1.68 (5.16), respectively), the trochanteric region (1.56 (2.91) and 1.39 (2.86)) and the intertrochanteric region (0.95 (2.76) and 0.72 (2.6)).

Patients who were osteopenic (T score <−1) at baseline experienced a higher increase in bone mineral density than in the general study population: 3.1 (3.5) in the S21400 group ($n = 71$) and 2.4 (3.5) in the patch E2 group ($n = 36$) at the lumbar spine, and

Table 1
Demographic data of randomized patients at baseline

	S21400 ^a (<i>n</i> = 234)	Patch ^a (<i>n</i> = 127)
Age (years)	51.4 ± 4.6	51.6 ± 4.3
Body mass index	25.1 ± 4.3	25.6 ± 4.5
Age at menopause (years)	49.1 ± 3.8	49.1 ± 3.9
Time from menopause (years)	2.9 ± 2.7	3.0 ± 3.3
Menopause type, <i>n</i> (%)		
Natural	189 (81)	92 (72)
Surgical	43 (18)	35 (28)
Other	2 (1)	0 (0)
Hysterectomy, <i>n</i> (%)	71 (30)	43 (34)
Previous HRT, <i>n</i> (%) ^b	83 (35)	38 (30)
Wash-out duration (days)	225 ± 486	325 ± 454
BMD spine (g/cm ²)	0.974 ± 0.142	0.969 ± 0.133
BMD total hip (g/cm ²)	0.876 ± 0.122	0.883 ± 0.112
BMD femoral neck (g/cm ²)	0.775 ± 0.113	0.778 ± 0.111
CTX (μg/mmol creatinine)	307 ± 154	305 ± 146
Osteocalcin (ng/ml)	24 ± 9	24 ± 9
B-ALP (ng/ml)	11 ± 4	11 ± 3

HRT, hormone replacement therapy; BMD, bone mineral density; CTX, urinary type I collagen C-telopeptide; and B-ALP, bone-specific alkaline phosphatase. Values are expressed as mean ± standard deviation unless otherwise stated.

^a Treatment groups were defined according to the treatment received (either S21400 or patch) for the longest period during the 56 weeks of treatment.

^b No patient had previously been treated by patch or S21400.

2.0 (2.6) in the S21400 group (*n* = 70) and 1.2 (2.7) in the patch E2 group (*n* = 29) at the total hip. These increases between baseline and the end of treatment were statistically significant (*P* < 0.001) within each group but there was no significant difference between treatment groups in any bone mineral density site examined. However, the percentage of these patients at risk of osteoporosis who increased their BMD at end was higher in the S21400 group as compared to the patch E2 group, respectively 76% and 64% at lumbar spine, 79% and 45% at total hip.

Similar efficacy was found with both treatments in urinary and serum markers of bone turnover during W0–W56 (Fig. 3). Bone resorption markers (urinary type I collagen C-telopeptides) significantly decreased (*P* < 0.001) between baseline and the end of treatment in both groups. The mean (S.D.) percentage decrease at end relative to baseline was 56.2 (28.4) in the S21400 group and 53.3 (30.9) in the patch E2 group, with no statistically significant difference between groups. Bone formation markers (osteocalcin, bone-specific alkaline phosphatase) significantly de-

Table 2
Bone mineral density—percentage change after 1 year of treatment vs. baseline

	Mean percent change from baseline to end (S.D.)		Difference (S.E.) (95% CI) ^a
	S21400 (<i>n</i> = 228) ^a	Patch (<i>n</i> = 125) ^a	
Lumbar spine	2.1 (3.0)*	2.1 (3.0)*	−0.07 (0.33) (−0.71; 0.58)
Femoral neck	1.3 (2.8)*	1.6 (2.9)*	0.39 (0.31) (−0.21; 1.00)
Total hip	1.2 (2.4)*	1.1 (2.2)*	−0.08 (0.26) (−0.58; 0.42)

Estimated difference between groups (patch—S21400) at end, adjusted on baseline, for the mean percentage change relative to W0. S.D., standard deviation; S.E., standard error; and CI, confidence interval.

^a Treatment groups were defined according to the treatment received (either S21400 or patch) for the longest period during the 56 weeks of treatment.

* *P* < 0.001 vs. baseline.

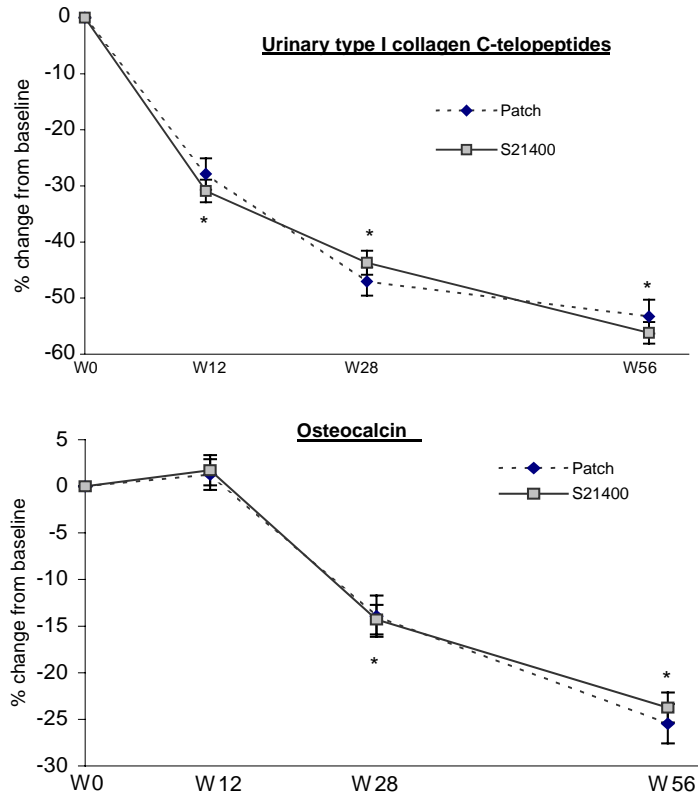


Fig. 3. Markers of bone turnover after 1 year of treatment. Treatment groups were defined according to the treatment taken for the longest duration. Values are expressed as standard error of the mean (S.E.M.) percentage of change from baseline. Urinary type I collagen C-telopeptide. * $P < 0.05$ vs. baseline.

creased ($P < 0.001$) between baseline and the end of treatment in both treatment groups. The mean (S.D.) percentage decrease according to baseline was 23.7 (24.6) in the S21400 group and 25.5 (22.0) in the patch E2 group for osteocalcin, and 12.9 (24.5) and 14.4 (23.9), respectively, for bone alkaline phosphatase. There was no statistically significant difference in bone formation markers between treatment groups. Similar results were obtained from the analysis performed in the subgroup of patients who received the alternative treatment only during the W12–W16 period.

The mean Kupperman Index score was significantly decreased ($P < 0.05$) at each time point from W12 to the end of treatment in both groups, with a mean percentage decrease from baseline at end of 81% in the S21400 group and 79% in the patch E2 group. There were concomitant decreases in the mean numbers of both total and moderate-to-severe hot flushes per day

and in the number of night sweats per day between baseline and the end of treatment in both groups.

Although few patients in either group had genitourinary symptoms at baseline, during the study the frequency of such symptoms decreased in both groups. The percentage of patients with normal vaginal trophicity increased from 27% at selection to 91% at W56 in the S21400 group, and from 27% to 85% in the patch E2 group.

The mean concentration of total cholesterol decreased significantly at each time point from W12 to W56 in S21400 and from W28 in the patch E2 group, with no significant difference at end between the two groups (Table 3). The mean LDL concentration also decreased significantly between baseline and W56 in both groups. This decrease in mean LDL was significantly ($P < 0.05$) more pronounced in the S21400 than in the patch E2 group. HDL concentrations in-

Table 3
Effect of S21400 and patch on lipid parameters after 1 year of treatment

	S21400 ^a (<i>n</i> = 214)	Patch ^a (<i>n</i> = 107)	Difference (S.E.) (95% CI) ^b
Total cholesterol			
Baseline value (mmol/l)	6.0 (0.9)	5.9 (1.0)	
Percent change at end	−4.6 (10.4)	−2.7 (11.1)	1.89 (1.26) −0.58; 4.37)
Time effect	<i>P</i> < 0.001	<i>P</i> < 0.001	ns
HDL cholesterol			
Baseline value (mmol/l)	1.7 (0.4)	1.7 (0.4)	
Percent change at end	8.0 (18.1)	6.5 (15.6)	−1.43 (2.05) (−5.47; 2.60)
Time effect	<i>P</i> < 0.001	<i>P</i> < 0.001	ns
LDL cholesterol ^c			
Baseline value (mmol/l)	3.7 (0.8)	3.7 (0.9)	–
Percent change	−10.6 (15.1)	−5.6 (16.1)	5.02 (1.83) (1.42; 8.63)
Time effect	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001
Triglycerides			
Baseline value (mmol/l)	1.1 (0.5)	1.2 (0.5)	–
Percent change at end	4.8 (34.1)	−1.7 (32.9)	−6.54 (3.99) (−14.38; 1.31)
Time effect	ns	ns	ns

S.D., standard deviation; S.E., standard error; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and ns, not significant.

^a Treatment groups were defined according to the treatment received (either S21400 or patch) for the longest period during the 56 weeks of treatment.

^b Estimated difference between groups (patch—S21400) at end for the mean percentage change relative to W0.

^c *n* = 213 in the S21400 group.

creased significantly between baseline and the end of treatment in both the S21400 and patch E2 groups. No clinically or statistically significant changes in triglyceride levels were detected.

Fibrinogen significantly decreased in the S21400 group (*P* < 0.001) and in the patch E2 group (*P* < 0.037) between baseline and W56 compared to baseline, with no noticeable difference at end between the groups. The mean (S.D.) percentage changes in fibrinogen in the S21400 and patch E2 groups were −4.8 (31.3) and −3.3 (31.7), respectively. No clinically relevant modifications were found in the anti-thrombin III activity at end in either group.

All patients who had received at least one dose of treatment were included in the safety analysis (*n* = 358). A total of 14 patients had at least one serious adverse event during the treatment period: 10 in the intranasal group (traumatic fracture of femoral neck, traumatic fracture of elbow olecranon, ovarian carcinoma, basal cell carcinoma, anal fistula surgery, tooth extraction, bartolinitis, angina pectoris, chest pain, wound due to fall) and four in the patch E2 group (pulmonary embolism after surgery for hernia

congenital, nasal septum operation, manic depressive syndrome, hemorrhoidectomy). All were considered unrelated to the study treatment by the investigators. No deaths were reported. There was no clinical difference between the two treatment groups regarding the incidence of emergent adverse events. Application site disorders (prickling and sneezing with S21400, erythema and pruritus with patch), respiratory system disorders (rhinitis, pharyngitis, bronchitis) and general disorders (headache, influenza-like symptoms) were the most frequently reported emergent adverse events in both the groups. Most emergent adverse events (90%) had mild-to-moderate intensity in each group. The incidence of moderate-to-severe mastalgia during the W0–W12 period was the only clinically relevant difference observed between the two treatments during the study: significantly fewer events were reported in the S21400 group than in the patch E2 group (7.2% versus 15.5%; *P* = 0.02).

During the W0–W12 period, there were less breakthrough bleeding episodes with S21400 than with patch E2, although the difference did not reach statistical significance (6% of cycles as compared to 8%).

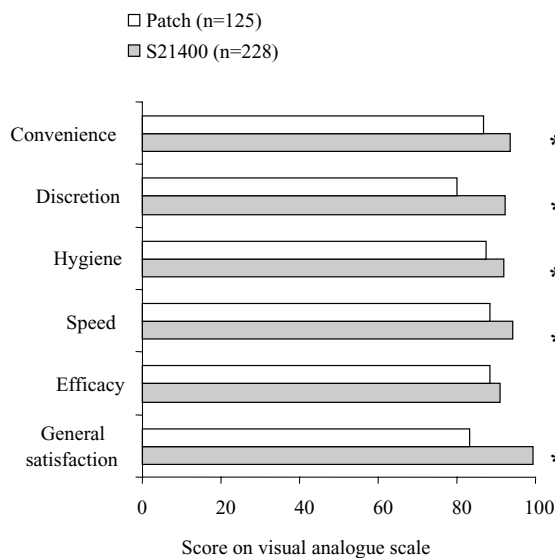


Fig. 4. Patient satisfaction with S21400 or patch treatment at week 56 evaluated on a visual analogue scale. Treatment groups were defined according to the treatment received for the longest period. * $P < 0.05$ between treatments.

Up to W52, this difference was maintained with breakthrough reported in 5% of the cycles in S21400 group and in 7% of cycle with patch E2 (non-significant).

The mean scores achieved on the questionnaire evaluating patient satisfaction with treatment (rated on a 100 mm visual analogue scale) were significantly ($P < 0.05$) higher for the S21400 group at W56 than for the patch E2 group on almost all aspects assessed: 99.5 versus 83.2 for general satisfaction, 94.1 versus 88.4 for speed, 91.8 versus 87.5 for hygiene, 92.3 versus 79.9 for discretion, and 93.5 versus 86.8 for convenience, respectively (Fig. 4). Patient satisfaction was also higher in the S21400 group than the patch E2 group on the efficacy assessment (91.0 versus 88.4), though this difference did not reach significance.

4. Discussion

This is the first study to show that pulsed estrogen therapy provided by nasal administration of E2 significantly increases bone mineral density in a similar manner to that seen following transdermal E2 therapy. The increases in bone mineral density, observed after 1 year of treatment with S21400, were of the same

magnitude as those observed after a 1-year treatment with other hormonal replacement therapies currently registered for osteoporosis prevention, such as conjugated equine estrogens, 0.625 mg [8], micronized 17β -estradiol, 2 mg [9] or a 50 μ g transdermal patch [10–12]. Furthermore, as has already been observed with estrogens [11], the mean percentage in bone mineral density increase compared to baseline was even higher for the subset of patients who were osteopenic at baseline (T score < -1) than for the general study population.

The results of this study show that S21400 300 μ g has a very similar activity profile on bone turnover as a 50 μ g transdermal patch. Moreover, the findings confirm the efficacy of S21400 in normalizing bone turnover to the premenopausal range, comparable to that seen with transdermal E2 therapy [11,12]. The decreases in urinary type I collagen C-telopeptides seen following intranasal and transdermal E2 therapies were also similar; reductions were significant within 12 weeks of treatment and levels continued to fall over 56 weeks by 56% and 53% relative to baseline values, respectively. These findings are in agreement with a previous report on the long-term effects of S21400 on biochemical bone turnover markers, which demonstrated a return to premenopausal levels after 1 year of treatment [13]. In the case of serum osteocalcin, a lag phase of about 12 weeks was seen with both treatments before decreases were detectable. This delayed response of osteocalcin normalization has been reported with transdermal E2 [11,12,14] and intranasal E2 [13], and is probably linked to the lack of digestive first pass effects on serum insulin-like growth factor (IGF1) observed after non-oral administration of E2 [13]. The particular design of this study offers some advantages as it allowed to draw several practical conclusions from only one study: on the equivalence of both treatments for climacteric symptoms, for assessing the preference for intranasal preparation after the women have used both means of administration and finally the similar efficacy on bone metabolism.

Furthermore, the possibility for choosing the treatment by the patient and for adjusting the dose according to the signs of under or over dose reflects the daily clinical situation and gave the benefit of a rather low rate of premature withdrawal. On the other hand, the groups analyzed after 1 year were determined by their duration of treatment and not by randomization, as

usually advised by statisticians. Nevertheless, conclusions regarding bone metabolism may be considered as valid because main baseline characteristics related to bone were similar in both groups (Age, BMI, time since menopause, bone mineral density and bone markers), and change in bone markers after 12 weeks in groups determined by randomization were strictly similar.

Lopes et al. have reported that S21400 and transdermal E2 have equivalent efficacy in reducing climacteric effects during the first 16 weeks of the study [6]. The findings of the present study demonstrate that this efficacy is maintained for up to 56 weeks, with a further slight improvement between weeks 16 and 56.

Over the 56-week treatment period, both intranasal and transdermal E2 were associated with significant decreases in total cholesterol and LDL levels, though statistically, the effect on LDL was significantly more pronounced in the S21400 as compared to the patch group. HDL concentrations significantly increased in both treatment groups, with no clinically or statistically significant modification of triglyceride levels, contrary to oral route where triglycerides have been shown to increase [5].

A number of studies have suggested that the risk of venous thromboembolic events is increased in women receiving estrogen replacement therapy [15–17]. However, such effect may be dependent on the route of administration of E2: as a consequence of the first-pass effect of oral estrogens on hepatic protein synthesis and especially on the modification of haemostasis factors such as anti-thrombin III, has been found after oral E2 administration [18–20]. In the current study no clinical modification of anti-thrombin III was found in either group after 56 weeks of treatment. Furthermore, fibrinogen decreased throughout the study in both groups, resulting in a statistically significant reduction from baseline. Thus, it appears that the thromboembolic risk associated with S21400 should be comparable to that of transdermal administration, for which it was recently found that it is not increased, contrary to the oral route [19].

The equivalence of efficacy of S21400 and transdermal E2 on postmenopausal symptoms (climacteric symptoms or postmenopausal bone loss) over 1 year of treatment demonstrated in this study was seen despite that fact that the two formulations of E2 have different pharmacokinetic profiles. S21400 provides a

pulse-like delivery of E2 producing high transient increases in E2, whereas the transdermal patch provides a sustained delivery of E2. These results validate the concept of pulsed E2 therapy for the treatment of postmenopausal bone loss and demonstrate that continuous administration of E2 is not mandatory for continuous efficacy on short- or long-term E2 deficiency consequences.

Both intranasal and transdermal E2 were well tolerated, with similar incidences of adverse events. During the first 12 weeks of the study, the frequency of moderate-to-severe mastalgia was significantly lower in the S21400 group than in the transdermal group ($P = 0.02$) [6]. Intranasal E2 therapy has also been shown to be associated with a lower incidence of mastalgia and uterine bleeding than oral E2 treatment [5].

At the end of the first 16-week period of the study, twice as many patients opted to continue treatment with S21400 as with the transdermal E2 patch, indicating the general acceptability of the intranasal formulation. Patients' satisfaction with the intranasal formulation continued during the follow-up period, with higher scores being achieved in the intranasal group for almost all aspects evaluated (general satisfaction, hygiene, speed, discretion, and convenience). This acceptability of S21400 over a long duration of treatment has been demonstrated in another study, in which 85% of women were still on treatment after 1 year [21].

Several prospective studies have shown the benefits of estrogen supplementation in preventing postmenopausal osteoporosis. The duration of estrogen treatment appears to be the determining factor in this effect since the benefit seems to disappear after estrogen withdrawal [22]. Moreover, at least 5 years of therapy are required to achieve a lasting effect on bone mass sufficient to decrease the risk of hip fracture several years after treatment withdrawal [23]. The use of an intranasal form of E2 that improves patient satisfaction may be associated with greater compliance in the long-term.

This study has some limitations: mainly it addresses a surrogate end point, bone mineral density, after a relatively short period, 1 year and results should be confirmed on hard end point on the fracture rate. Indeed, strong correlation has been shown between increase in bone mineral density and further reduction

in osteoporotic fractures (and significant decrease in the rate of vertebral and hip fractures has already been demonstrated with estrogens. Nevertheless, the demonstration of a positive benefit/risk ratio in prevention of osteoporosis should be highly desirable on the long-term with this preparation, especially in the context of the recent data of the WHI study.

The WHI study was the first randomized controlled study to have demonstrated the effectiveness of estrogens in the reduction of hip fracture in postmenopausal women. Nevertheless, the excess of breast cancers and cardiovascular events observed in these obese, hypertensive postmenopausal women after 5.2 years of treatment with oral combination of medroxy progesterone and conjugated estrogens challenged the place of estrogens for osteoporosis prevention. Besides the characteristics of the population, not particularly at risk for osteoporosis, the deleterious effects of quite high doses of estrogens orally administered on thromboembolic risk should be taken into account before extrapolating the conclusions to all means of hormone therapy.

The results of this 1 year study demonstrate that 300 µg S21400 has a very similar activity profile to a 50 µg transdermal E2 patch in increasing bone mineral density and reducing bone turnover markers. Furthermore, they confirm the efficacy of pulsed estrogen therapy achieved after administration of intranasal E2 in normalizing bone turnover to premenopausal levels and in preventing postmenopausal bone loss. This study also highlights the potential benefit of this formulation for long-term use in comparison with the transdermal route, because of its good gynecological tolerance (significantly lower incidence of mastalgia and a low rate of unexpected vaginal bleeding) and significantly greater patient satisfaction.

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