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Salmon calcitonin plus intravaginal estriol: an effective treatment for the menopause

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Abstract

Objective: Intravaginal estriol (E3) effectively improves postmenopausal genito-urinary disturbances, without stimulating endometrial proliferation. The aim of the present study was to evaluate the effect of intravaginal estriol (E3) plus nasal spray salmon calcitonin (sCT), to improve neurovegetative symptoms and to prevent the decline of bone mineral density (BMD) of postmenopausal women. *Methods*: Two hundred and fourteen (214) healthy postmenopausal women were treated for 12 months with: (1) E3 (0.5 mg every other day) + Ca (0.5 g/day); (2) E3 + Ca + sCT (50 IU × 2/day); (3) sCT + Ca; (4) Ca. Climacteric complaints, such as hot flushes and sweating, BMD at the distal 1/10 of the radius, analyzed by dual photon absorptiometry, urinary excretion of hydroxyproline and serum alkaline phosphatase were evaluated at baseline and every 6 months. At the same time, patient compliance and drug tolerability were evaluated. *Results*: E3 but not sCT, improved hot flushes and sweating. E3 blunted but not completely counteracted the BMD decline observed in women treated with only Ca, and reduced urinary hydroxyproline excretion. sCT markedly increased BMD values and reduced both urinary hydroxyproline excretion and serum alkaline phosphatase. These effects were not potentiated by E3 coadministration. All treatments were well tolerated. *Conclusions*: Present data indicate that the combined administration of intravaginal E3 and sCT may represent an alternative therapeutic regimen for those postmenopausal women who do not accept or have contraindications to classical hormone replacement therapy.

Keywords: Salmon calcitonin; Intravaginal estriol; Bone mineral density; Postmenopausal women

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1. Introduction

Bone fractures consequent to postmenopausal bone demineralization, are one of the main causes of morbidity in industrialized countries [1]. In order to contain their high social cost, preventive measures capable of reducing postmenopausal bone loss have been developed. To this end, hormone replacement therapy undoubtedly offers the most effective and complete therapeutic response [2,8], although contraindications, low acceptance and low compliance have limited its widespread use.

Calcitonin inhibits bone resorption and is believed to prevent postmenopausal bone demineralization [9–11]. Salmon calcitonin, the most widely used calcitonin, can be administered either by injectable routes, with a high incidence of side effects and low compliance [12–14], or by non-injectable routes. Among the latter, the nasal spray formulation furnishes a good alternative, and in doses of 50 IU/day and 100 IU/day is capable of, respectively, exerting submaximal and maximal biological effects [15]. In spite of the relevant effect on bone, the widespread use of calcitonin has been limited by its inability to modify climacteric complaints, such as hot flushes, sweating, or vaginal atrophy.

Estriol is a weak estrogen that, when administered by an oral route, is capable of markedly improving climacteric symptoms [16,17]. Oral estriol is rapidly inactivated by the liver in its glucuronide derivative, and only 1–2% of the total administered dose enters the circulation in a bioactive form [18,19]. Furthermore, this massive inactivation is influenced by the time of administration and by foods, such that the circulating levels of the hormone are difficult to tailor in the required range [19]. Important pharmacokinetic advantages derive by intravaginal administration [18,19]. By this route, estriol bypasses the first hepatic inactivation, and a greater component (20%) of the administered dose is absorbed in a bioactive form. Furthermore, absorption is not modified by the time of administration or by foods, and more stable circulating levels are achieved. Following the intravaginal administration of 0.5 mg, estriol rapidly increases in the circulation and reaches values comparable or higher than those obtained with 8 mg, per os [18]. Furthermore doses of 0.5 mg/day for 2–3 weeks followed by 0.5 mg/day every 3 days are capable of markedly improving postmenopausal genito-urinary complaints [20–22], without stimulating endometrial proliferation [23–27]. The lack of endometrial stimulus eliminates the need to restore artificial menses with cyclical progesterone administration, and thus avoids one of the major causes of discomfort and withdrawal from estrogen replacement therapy [28]. Whether intravaginal estriol, is capable, like its oral administration, to improve neurovegetative symptoms, such as hot flushes and sweating is not yet known. Accordingly, in this multicentric study we investigated whether intravaginal estriol (E3), improves the occurrence of hot flushes and sweating, and whether in association with nasal spray salmon calcitonin (sCT) may constitute a combination capable of improving patients well-being and preventing postmenopausal osteoporosis.

2. Materials and methods

An open trial has been carried out in six Italian Research Institutes (Pavia, Bologna, Florence, Pisa and Naples). Among 240 planned, 214 healthy women in menopause for 1–2 years (FSH $>$ 40 IU/l and E2 $<$ 20 pg/m), with a mean age of 53.2 $\pm$ 1.1 years and with a body mass index $<$ 25, were enrolled into the study. Only women seeking a prevention of postmenopausal bone mineral loss and giving their informed consent to participate in the study were included. Exclusion criteria were, endometrial thickness $>$ 4 mm at a transvaginal ultrasound evaluation, smoking more than 15 cigarettes/day, drinking more than 500 ml/day of alcoholic beverages, drinking more than 2 doses/day of liquor, drinking more than 4 coffees/day, osteopenic diseases, using or having used drugs or hormones potentially interfering with calcium metabolism, and intense joint pain evaluated by a pain higher than 80 on a 0–100 points visuo-analogic scale. Women were allocated randomly to 4 groups, and received for 1 year, one of the following treatments for the prevention of postmenopausal bone mineral loss: (1) intravaginal E3, 0.5 mg every

other day + oral calcium, 0.5 g/day (Ca); (2) E3 + Ca + synthetic sCT nasal spray, 50 IU × 2/day; (3) sCT + Ca; (4) Ca.

No mention was made on the possible effects that some of the treatments may have had on neurovegetative symptoms.

At baseline and after 6 and 12 months of treatment, bone mineral density (BMD) at the distal 1/10 of the non-dominant radius was measured by dual photon absorptiometry (I^{125} and Am^{231}) on osteograph (Osteoden P, NIM, Verona, Italy) [4]. This method allows exclusion, more precisely than single photon absorptiometry, of soft tissue contribution to BMD. Calibration of any single densitometer was carried out in basal conditions and, every 3 months on a calibration phantom, common to all centres. The calibration curve obtained by means of 3 measurements in 3 standard points with a different density (9 measurements for each centre), showed an accuracy (CV) of 1.1%.

At baseline, and after 6 and 12 months, biochemical analysis of the following bone metabolism parameters were performed: serum alkaline phosphatase, by the 'ALP-kine test' kit (Sclavo, Siena, Italy) (intra- and interassay CV of 4.3% and 4.8%, respectively); urinary excretion of calcium, by atomic spectrophotometry (intra- and interassay CV of 3.2% and 5.5%, respectively), and urinary excretion of hydroxyproline, evaluated after hydrolysis by a colorimetric method (Hypronosticon kit, Organon Teknika BV, The Netherlands) (intra- and interassay CV of 6.2% and 7.1%, respectively). Urinary excretion of calcium and hydroxyproline were corrected for serum and urinary creatinine. At baseline, and at the end of treatment, the 24-h frequency and intensity of self-reported neurovegetative symptoms, such as hot flushes and sweating, were analyzed.

Patient compliance, drug tolerability and drug safety were evaluated at baseline and every 6 months. Poor compliance was considered the return of more than 20% of the provided E3, sCT and/or Ca. Drug tolerability was evaluated by the side effects subjectively referred to by patients, and drug safety was evaluated by a complete gynecological examination, with vaginal smear, an otorhinolaryngological evaluation of nasal cavities, and biochemical blood and urine analyses (complete blood count, PT, antithrombin III, blood glucose, bilirubin, SGOT, SGPT, γGT, LDH, uricemia, total and fractioned proteinemia, Na^+, K^+, Cl^-, creatinine clearance, standard urine analysis).

In a subset of subjects (n = 10 treated with E3 + Ca and n = 10 treated with E3 + sCT + Ca) endometrial thickness was measured by transvaginal ultrasounds at the end of the 12 months of treatment.

Statistical analysis was performed by analysis of variance (ANOVA) with two 'factors' (sCT and E3) and two levels (presence/absence). The 'centre' factor was taken into account in order to evaluate the variability deriving from the possible differences among the research centres involved in the study. Therefore, the complete model combines fixed (treatments) and random (centres) factors.

All results are expressed as the mean ± standard deviation (S.D.).

3. Results

In total, 206 patients completed the study (49 in the E3 + Ca group; 59 in the E3 + Ca + sCT group; 50 in the sCT + Ca group; and 48 in the Ca group).

Frequency and intensity of neurovegetative symptoms improved following E3 but not sCT administration (Table 1 and Table 3).

BMD values observed at baseline and after 6 and 12 months of treatment are reported in Table 2. In the 12-month period of treatment, BMD declined $-2.4 \pm 0.3\%$ in women with Ca, while only $-1.0 \pm 0.4\%$ in women treated with E3 + Ca (Fig. 1). By contrast, an increase of BMD was observed in women receiving sCT. BMD increased $3.7 \pm 0.6\%$ in women treated with sCT + Ca, and $3.0 \pm 0.6\%$ in women treated with E3 + Ca + sCT (Fig. 1). Statistical analysis shows that only E3 but not sCT administration significantly improved hot flushes and sweating (Table 3). By contrast, both factors positively influenced BMD (Table 3). The effect of sCT was

Table 1
Frequency in the 24 h period, and intensity of subjective hot flushes or sweating, subjectively referred to by postmenopausal women at baseline and after 12 months of treatment with intravaginal estriol (E3) + calcium (Ca) (n = 49), E3 + salmon calcitonin nasal spray (sCT) + Ca (n = 59), sCT + Ca (n = 50) or Ca (n = 48)

Treatment		Frequency							
		Absent		1–3/day		4–7/day		>7/day	
		n	(%)	n	(%)	n	(%)	n	(%)
E3 + Ca	Basal	12	(21.4%)	24	(42.9%)	17	(30.4%)	2	(3.6%)
	12 months	23	(45.1%)	24	(47.1%)	4	(7.80%)		
E3 + sCT + Ca	Basal	14	(22.6%)	24	(38.7%)	22	(35.5%)	1	(1.6%)
	12 months	34	(56.7%)	17	(28.3%)	8	(13.3%)	1	(1.7%)
sCT – Ca	Basal	20	(34.5%)	21	(36.2%)	15	(25.9%)	2	(3.4%)
	12 months	20	(38.5%)	26	(50.0%)	6	(11.5%)	0	(0%)
Ca	Basal	10	(17.9%)	31	(55.4%)	11	(19.6%)	4	(7.1%)
	12 months	17	(34.7%)	21	(42.9%)	6	(12.2%)	5	(10.5%)

Treatment		Intensity					
		Absent		Slight		Intense	
		n	(%)	n	(%)	n	(%)
E3 + Ca	Basal	12	(21.4%)	38	(67.9%)	5	(8.9%)
	12 months	25	(49.0%)	24	(47.1%)	2	(3.9%)
E3 + sCT + Ca	Basal	14	(22.6%)	41	(66.1%)	6	(9.7%)
	12 months	34	(56.7%)	24	(40.0%)	2	(3.3%)
sCT + Ca	Basal	20	(34.5%)	33	(56.9%)	5	(8.6%)
	12 months	21	(40.4%)	30	(57.7%)	1	(1.9%)
Ca	Basal	10	(17.9%)	44	(78.6%)	2	(3.6%)
	12 months	17	(34.7%)	28	(57.1%)	4	(8.2%)

already evident after 6 months, whereas that of E3 only after 12 months of treatment (Table 3). The significant interaction between E3 and sCT on BMD modifications, indicates that the adjunct of sCT, significantly increases the slight protective effect of E3 (Table 3). E3 and sCT significantly reduced the urinary excretion of hydroxyproline, but only sCT was capable of reducing alkaline phosphatase after 6 months of treatment (Table 3).

Treatment tolerability was considered good or very good by both physicians and patients. Only 32 out of the 214 enrolled patients showed side effects of mild or moderate severity. Side effects were equally distributed among the 4 different groups and were characterized by abdominal pain, dryness of the fauces, and nausea. Eight patients were withdrawn from the study (1 in the E3 + Ca and in the E3 + Ca + sCT groups, 2 in the sCT + Ca group and 4 in the Ca group), 3 for poor compliance and 5 for side effects of mild intensity.

All treatments were safe, and did not modify haematological and biochemical blood and urinary parameters (data not shown).

Endometrial thickness was not modified by E3 + Ca or E3 + sCT + Ca, and in all subjects investigated remained <4 mm at the end of the 12 months of treatment. Vaginal bleeding did not occur in any woman.

Table 2
Mean (± S.D.) bone mineral density (BMD) at the distal 1/10 of the non-dominant radius, urinary excretion of hydroxyproline corrected for creatinine (OHP/Cr ratio), and serum levels of alkaline phosphatase (Alk Ph), evaluated in postmenopausal women at baseline and after 6 and 12 months of treatment with intravaginal estriol (E3) + calcium (Ca) (n = 49), E3 + salmon calcitonin nasal spray (sCT) + Ca (n = 59), sCT + Ca (n = 50) or Ca (n = 48)

		E3 + Ca	E3 + sCT + Ca	sCT + Ca	Ca
BMD	Basal	370.9 ± 53.7	367.0 ± 64.3	367.3 ± 62.0	359.8 ± 59.4
	6 months	369.6 ± 51.2	372.5 ± 64.4	373.5 ± 61.9	355.7 ± 56.2
	12 months	367.1 ± 53.7	378.0 ± 64.8	380.9 ± 61.2	351.2 ± 55.7
OHP/Cr (mg/dl)	Basal	20.1 ± 10.1	20.1 ± 8.7	21.0 ± 10.2	20.7 ± 6.0
	6 months	19.4 ± 9.7	18.0 ± 8.3	17.7 ± 8.7	20.5 ± 6.7
	12 months	18.4 ± 9.4	17.3 ± 8.5	16.8 ± 7.3	21.0 ± 9.0
Alk Ph (mU/ml)	Basal	132.7 ± 53.3	145.6 ± 59.8	150.8 ± 52.8	123.4 ± 44.0
	6 months	130.6 ± 51.9	138.4 ± 53.1	138.6 ± 52.1	125.3 ± 46.0
	12 months	122.3 ± 50.0	138.4 ± 59.7	138.2 ± 51.5	125.7 ± 51.8

4. Discussion

Data obtained from this multicentric study confirm the effectiveness of 100 IU/day of nasal spray sCT, in reducing the postmenopausal BMD decline [9–11,15]. Treatment with sCT reduced the urinary excretion of hydroxyproline, serum alkaline phosphatase levels, and not only antagonized the decline, but increased BMD values. The increase of BMD observed in the first year of treatment, is similar to that obtained with other antiosteoporotic therapies, including estrogens [4]. The effect of sCT was not potentiated by the addition of E3, and thus, the BMD increase observed during sCT alone was similar to that during sCT plus E3. In previous studies it has been reported that E3 is not capable of potentiating the antiresorptive effect of more potent estrogens [29]. However, when given by itself, E3 exerted an effect on bone, resulting in a reduced urinary excretion of hydroxyproline, and in a blunted, but not abolished, decline of BMD. This, i.e. the first evidence showing a slight protective effect of intravaginal E3 on bone mineral loss, indicates that besides its known trophic genito-urinary activity [20–22], E3 may exert systemic effects. As a consequence, a stimulus to endometrial proliferation should be expected. Indeed both the present study, and previous experiences obtained with lower, similar or higher E3 doses, have consistently shown E3 incapable of stimulating the proliferation of the endometrium [23–27]. E3 has a weak affinity for estrogen receptors, and the receptor-E3 complex is rapidly removed from the nucleus [30]. It could be that the intermittent intravaginal administration of E3, influences basic cellular functions, such as enzymatic and trophic activities [31–33], without stimulating cell proliferation [30]. However, the agonist or antagonist effects exerted by estrogen-like molecules on different tissues is a complex phenomenon related to the ability of the different compounds to differentially regulate the estrogen receptor structure and activity [34]. This possibility may explain the capability of intravaginal E3 to slightly reduce bone mineral loss, without stimulating endometrial proliferation. It is noteworthy that a marked effect on bone, has been observed also with other weak estrogens or antiestrogens such as tamoxifen [35] and raloxifene [36,37]. In contrast to these 'antiestrogens', E3 is however capable of improving neurovegetative symptoms. It has been previously reported that the oral administration of 8 mg of E3 reduces hot flushes [16,17]. The present study is the first showing that also a low dose of intravaginal E3 is capable of improving neurovegetative symptoms. Hot flushes and sweating are among the most frequent climacteric complaints [38], and minimal estrogen doses are sufficient to improve them [39]. Accordingly, it is not surprising that the administration of a weak estrogen such as E3, is sufficient to reduce the neurovegeta-

tive complaints of postmenopausal women. Although the present study, was not controlled with placebo, each enrolled subject received at least one medicine, and none of them was aware of the potential beneficial effects on neurovegetative symptoms exerted by the provided anti-osteoporotic therapies. Thus, it is very likely that the positive effect on hot flushes and sweating observed only in the group of women receiving E3, cannot be ascribed to a placebo effect.

In conclusions, the pharmacokinetic advantages deriving from the intravaginal administration, the high effectiveness in reducing genito-urinary symptoms, the positive effects on neurovegetative complaints, and the inability to stimulate endometrial proliferation, make intravaginal E3 an attractive therapeutic tool for the postmenopausal period. The combination of intravaginal E3 and sCT, may offer a rather complete response to postmenopausal needs, giving the opportunity to furnish preventive and therapeutic measures to those postmenopausal women who do not accept or have contraindications for the classical hormone replacement therapy.

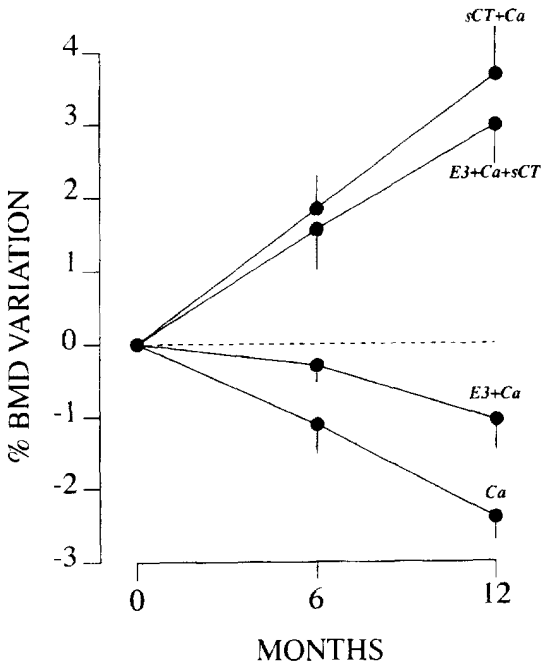

Fig. 1. Mean (± S.E.) percent variation of BMD values measured at the distal 1/10 of the non-dominant radius, by dual photon absorptiometry in postmenopausal women following the administration for 12 months of intravaginal estriol (E3) + calcium (Ca) (n = 49), E3 + salmon calcitonin nasal spray (sCT) + Ca (n = 59), sCT + Ca (n = 50), or Ca (n = 48).

Table 3
Results of the analysis of variance for the modifications induced in postmenopausal women by the administration of intravaginal estriol (E3) and nasal spray salmon calcitonin (sCT), in the frequency and intensity of hot flushes or sweating, distal radius bone mineral density (BMD), urinary excretion of hydroxyproline corrected for creatinine (OHP/Cr) and serum alkaline phosphatase (Alk Ph)

		E3	sCT	Interaction
Frequency	12 months	0.0253	n.s.	n.s.
Intensity	12 months	0.0434	n.s.	n.s.
BMD	6 months	n.s.	0.0001	n.s.
	12 months	0.0002	0.0001	0.0019
OHP/Cr	6 months	0.0249	0.0117	n.s.
	12 months	0.0157	0.0001	n.s.
Alk Ph[a]	6 months	n.s.	0.0155	n.s.

[a]Covariance analysis.

References

[1] Dempster DW, Lindsay R. Pathogenesis of osteoporosis. Lancet 1993; 341: 797–801.
[2] Notelovitz M. Osteoporosis: screening, prevention, and management. Fertil Steril 1993; 59: 707–725.
[3] Lindsay R. Estrogen therapy in the prevention and management of osteoporosis. Am J Obstet Gynecol 1987; 156: 1347–1351.
[4] Cagnacci A, Melis GB, Soldani R et al. Neuroendocrine and clinical effects of transdermal 17 β-estradiol in postmenopausal women. Maturitas 1991; 13: 283–296.
[5] Stevenson JC, Cust MP, Gangar KF, Hillard TC, Lees B, Whithead MI. Effects of transdermal versus oral hormone replacement therapy on bone density in spine and proximal femur in postmenopausal women. Lancet 1990; 335: 265–269.
[6] Field CS, Ory SJ, Wahner HW, Herrman RR, Judd HL, Riggs BL. Preventive effects of transdermal 17 β-estradiol on osteoporotic changes after surgical menopause: a two-year placebo-controlled trial. Am J Obstet Gynecol 1993; 168: 114–121.

[7] Weiss NS, Ure CL, Ballard JH, Williams AR, Daling JR. Decreased risk of the hip and lower forearm with postmenopausal use of estrogen. N Engl J Med 1980; 303: 1195–1198.
[8] Kiel DP, Felson DT, Anderson JJ, Wilson PWF, Moskowitz MA. Hip fracture and the use of estrogens in postmenopausal women. The Framingham Study. N Engl J Med 1987; 317: 1170–1174.
[9] Kanis JA, Johnell O, Gullberg B et al. Evidence for the efficacy of drugs affecting bone metabolism in preventing hip fracture. Br Med J 1992; 305: 1124–1128.
[10] Lindsay R. Prevention and treatment of osteoporosis. Lancet 1993; 341: 801–805.
[11] Di Renzo GC, Coata G, Cosmi EV, Melis GB, Maietta L, Volpe G. Management of postmenopausal osteoporosis. Eur J Obstet Gynecol 1994; 56: 47–53.
[12] Gennari C, Passeri M, Chierichetti SM, Piolini M. Side-effects of synthetic salmon and human calcitonin. Lancet 1983; 1: 594–595.
[13] Reginster JY, Franchimont P. Side effects of synthetic salmon calcitonin given by intranasal spray compared with intramuscular injection. Clin Exp Rheumatol 1985; 3: 155–157.
[14] M. Azria. Exogenous Calcitonin. In: Azria M, ed. The Calcitonins. Physiology and Pharmacology. Basel: Karger AG. 1989; 67–132.
[15] Overgaard K, Agnusdei D, Hansen MA, Maioli E, Christiansen C, Gennari C. Dose-response bioactivity and bioavailability of salmon calcitonin in premenopausal and postmenopausal women. J Clin Endocrinol Metab 1991; 72: 344–349.
[16] Schneider HPG. Oestriol and the menopause: clinical results from a prospective study. In: Fioretti P, Martini L, Melis GB, Yen SSC, eds. Serono Symposium, Vol. 39, The Menopause: Clinical, Endocrinological and Pathophysiological Aspects. London: Academic Press, 1982; 523–533.
[17] Tzingounis VA, Aksu F, Greenblatt RB. Estriol in the management of the menopause. J Am Med Assoc 1978; 239: 1638–1641.
[18] Schiff I, Wentworth B, Koos B, Ryan Kj, Tulchinsky D. Effect of estriol administration on the hypogonadal woman. Fertil Steril 1978; 30: 278–282.
[19] Heimer GM. Estriol in the postmenopause. Acta Obstet Gynecol Scand 1987; suppl 139: 5–23.
[20] Schmidbauer CP. Vaginal estriol administration in the treatment of postmenopausal urinary incontinence. Urologe A 1992; 31: 384–389.
[21] Van der Linden MC, Gerretsen G, Brandhorst MS, Ooms EC, Kremer CM, Doesburg WH. The effect of estriol on the cytology of urethra and vagina in postmenopausal women with genito-urinary symptoms. Eur J Obstet Gynecol Reprod Biol 1993; 51: 29–33.
[22] Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. N Engl J Med 1993; 329: 753–756.
[23] Kicovic PM, Cortes-Prieto J, Milojevic S, Haspels AA, Aljinovic A. The treatment of postmenopausal vaginal atrophy with ovestin vaginal cream or suppositories: clinical endocrinological and safety aspects. Maturitas 1980; 2: 275–282.
[24] Mattsson LA, Gullberg G. Clinical evaluation of treatment with estriol vaginal cream versus suppository in postmenopausal women. Acta Obstet Gynecol Scand 1983; 62: 397–401.
[25] Gerbaldo D, Ferraiolo A, Croce S, Truini M, Capitanio GL. Endometrial morphology after 12 months of vaginal oestriol therapy in postmenopausal women. Maturitas 1991; 13: 269–274.
[26] Heimer GM, Englund DE. Effects of vaginally-administered oestriol on post-menopausal urogenital disorders: a cytohormonal study. Maturitas 1992; 14: 171–179.
[27] Foidart JM, Vervliet J, Buytaert P. Efficacy of sustained-release vaginal oestriol in alleviating urogenital and systemic climacteric complaints. Maturitas 1991; 13: 99–107.
[28] Rees M. On menstrual bleeding with hormone replacement therapy. Lancet 1994; 343: 250.
[29] Christiansen C, Rodbro P. Does oestriol add to the beneficial effect of combined hormonal prophylaxis against postmenopausal osteoporosis? Br J Obstet Gynaecol 1984; 91: 489.
[30] Clark JH, Paszko Z, Peck Jr EJ. Nuclear binding and retention of the receptor estrogen complex: relation to the agonistic and antagonistic properties of estriol? Endocrinology 1977; 100: 91.
[31] Rodriquez Del Castillo A, Battaner E, Guerra M, Alonso I, Mas M. Regional changes on brain Na^+, K^+-transporting adenosine triphosphatase related to ovarian function. Brain Res 1987; 416: 113–120.
[32] Kundsen JF. Estrogen (EB) and EB + progesteron (P) induced changes in pituitary sodium-potassium adenosine-triphosphatase activity (ATPase). Endocrinol Res Commun 1976; 3: 281–282.
[33] Romanini C, Mazzanti L, Tranquilli AL, Valensise H, Cerster N. Effect of estriol on the transport of blood cells. In: Genazzani AR, Petraglia F, Volpe A, eds. Progress in Gynecology and Obstetrics. Carforth: Parthenon Publishing Group, Casterton Hall, 1990; 931–938.
[34] McDonnel DP, Clemm DL, Hermann T, Goldman ME, Pike JW. Analysis of estrogen receptor function in vitro reveals three distinct classes of antiestrogens. Mol Endocrinol 1995; 9: 659–669.
[35] Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP, DeMets DL. Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. N Engl J Med 1992; 36: 852–856.

[36] Turner CH, Sato M, Bryant HU. Raloxifene preserves bone strength and bone mass in ovariectomized rats. Endocrinology 1994; 135: 2001–2005.
[37] Draper MW, Boss SM, Huster WJ, Neild JA. Effects of raloxifene hydrochloride on serum markers of bone and lipid metabolism-dose response relationship. Calci Tissue Int 1994; 54: 339.
[38] Oldenhave A, Jaszman LJB, Haspels AA, Everaerd WTAM. Impact of climacteric on well-being. Am J Obstet Gynecol 1993; 168: 772–780.
[39] Melis GB, Paoletti AM, Bartolini R, Tosti Balducci M, Massi GB, Bruni V, Becorpi A, Ottanelli S, Foretti P, Gambacciani M, Spinetti A, D'Antona N, De Leo V, Agnusdei D, Camporeale A, Gennari C. Ipriflavone and low doses of estrogens in the prevention of bone mineral loss in climacterium. Bone Min 1992; 19: S49–S56.