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Serum placental protein 14: a novel marker of selective oestrogen receptor modulator action on the postmenopausal endometrium

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The primary objective of this study was to investigate whether changes in the serum level of an endometrial secretory protein, placental protein 14 (PP14), can reflect endometrial adverse events induced by selective oestrogen receptor modulators (SERMs). A randomized, double-blind, placebo-controlled trial was used. Participants were healthy postmenopausal women aged 45–65 years, who received either various doses of raloxifene (30, 60 or 150 mg day^{-1}) or levormeloxifene (1.25, 5, 10 or 20 mg day^{-1}) or placebo for 12 months. Serum PP14 and endometrial thickness (ET) were monitored by radioimmunoassay and transvaginal ultrasonography, respectively. In the levormeloxifene trial, endometrial status at 12 months was assessed by hysteroscopy. Raloxifene induced only slight increases in serum PP14 and ET. Levormeloxifene, however, induced marked increases in both study parameters at all the does tested. The 6 month changes in PP14 showed a positive correlation with both the 6 and 12 month changes in ET ($P < 0.001$). Marked stromal oedema, pseudocysticity with or without hypervascularity and endometrial proliferation were seen on hysteroscopy in those showing the largest increases in serum PP14. These results suggest that the PP14 assay used on a group basis may provide useful information on the endometrial effects of SERMs administered in a given dose range, and thereby could assist future clinical trials aiming to find the optimal dose range of new SERMs.

Keywords: endometrial adverse events, levormeloxifene, postmenopausal women, raloxifene, serum placental protein 14.

Introduction

Osteoporotic fractures, cardiovascular diseases and malignancies of the endometrium and breast are still the most frequent causes of death among elderly women (Hill 1996). Selective oestrogen receptor modulators (SERMs) were originally designed to prevent these diseases by exhibiting potent oestrogen-like effects on the skeletal and cardiovascular systems, while acting as oestrogen antagonists on the breasts and genital tract (Cosman and Lindsay 1999). Based on these features, there is an ongoing interest in finding potent and safe SERMs from various medical fields associated with women's health.

Although considerable efforts have been made in the past few decades, the ideal compound for such purposes has yet to be found. The main limitation in SERM development has always been adverse effects affecting the endometrium (Bese *et al.*

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1996, Mitlak and Cohen 1999, Andia *et al.* 2000, Cano and Hermenegildo 2000, Plouffe 2000, Marttunen *et al.* 2001, Cline *et al.* 2001). The current golden standard, raloxifene, has been associated with negligible endometrial adverse events in the 30–150 mg dose range (Delmas *et al.* 1997). Yet a structurally very similar SERM, levormeloxifene, has recently been shown to induce marked changes in endometrial thickness (ET) and concomitant genitourinary complications that ultimately led to the discontinuation of further development of the drug (Alexandersen *et al.* 2001).The observation that no clear-cut dose–response relationship could be demonstrated when studying the effects of 1.25–20 mg day^{-1} levormeloxifene on bone markers and serum lipids suggested that the gynaecological adverse events could be due to inappropriate dosing of the drug. The unfortunate outcome of the trial draws increased attention to the fact that there is still no laboratory marker that can reflect the risk for endometrial adverse events when testing novel SERMs in a given dose range.

Previous studies have suggested that secretory proteins may provide useful help when characterizing the impact of steroid hormones and SERMs on hormone reactive cells, including endometrial cells (Komm *et al.* 1986, Sheen and Katzenellenbogen 1987, Takeda and Shimizu 1988, Bell and Drife 1989, Dietze *et al.* 1996). We have previously developed a sensitive radioimmunoassay (Byrjalsen *et al.* 1989) to measure serum placental protein 14 (PP14), a well-known endometrial secretory protein (Seppala *et al.* 1988), in human serum. Our previous studies (Byrjalsen *et al.* 1989, 1992), supported by others (Okon *et al.* 1998, Seppala *et al.* 1987), suggest that PP14 in certain aspects can provide useful information on endometrial status both in pre- and postmenopausal women.

In the present study, we aimed to investigate (i) whether changes in serum PP14 are able to reflect the biological effects of second-generation SERMs on the endometrium; (ii) whether short-term changes in serum PP14 are associated with the simultaneous and long-term changes in ET as measured by transvaginal ultrasonography; and (iii) how the changes in serum PP14 relate to endometrial status as assessed by hysteroscopy.

Participants and methods

Study participants

The two randomized, placebo-controlled, double-blind SERM trials were originally designed to investigate the effects of various doses of raloxifene and levormeloxifene on bone mineral density, bone turnover markers, serum cholesterol and uterine endometrium in postmenopausal women (Delmas *et al.* 1997, Alexandersen *et al.* 2001) Participants were aged 45–65 years, were at least 1 year postmenopausal (serum oestradiol below 25 pg ml^{-1}) and had an intact uterus. Exclusion criteria ensured that none of the participants had chronic diseases or were taking medication known to influence the study parameters. All women gave written informed consent to their participation in the study in accordance with the ethical principles stated in the Declaration of Helsinki II and the European Standard for Good Clinical Practice. The Ethical Committee of Copenhagen and North Jutland counties approved the study protocols.

Random number tables were used in both studies as a way of allocating participants to the double-blinded treatment. In study I, participants received therapy with raloxifene (30, 60 or 150 mg day^{-1}) versus placebo, whereas in study II they were treated with levormeloxifene (1.25, 5, 10 or 20 mg day^{-1}) versus placebo. All participants in both studies received a calcium supplement of 400–600 mg day^{-1} during the whole study period.

Measurements

Body weight and height were measured to the closest 0.1 kg and 0.1 cm, respectively, with participants wearing light indoor clothes and no shoes.

Specialists in gynaecology or trained research physicians performed transvaginal ultrasonography at baseline and following 6 and 12 months of therapy with the respective SERM. The uterus was scanned using a Sonoline SI-250 (Siemens AG, Erlangen, Germany) or an Aloka SSD-1100 (Simonsen & Weel, Denmark) device with a vaginal probe, operating at a frequency of 5–7.5 MHz. The thickest anteroposterior diameter of the double-layer endometrial stripe was measured with callipers on the display. On the basis of reported data, we selected 4 mm as the maximal normal ET (Omodei *et al.* 2000, Mortakis and Mavrelos 1997).

Serum PP14 was measured using a previously described competitive radioimmunoassay (Byrjalsen *et al.* 1989). Serum samples from the two trials were analysed in duplicate in two separate assays. The amount of PP14 was determined with reference to standard curves constructed by analysis of PP14 solutions of known concentration. The detection limit of the assay was 0.3 $\mu g\,l^{-1}$. Intra-assay and inter-assay variations were 4% and 10%, respectively (Byrjalsen *et al.* 1989).

In the levormeloxifene trial, hysteroscopic examination was performed on an outpatient basis using paracervical blockade as local anaesthesia and a slow saline perfusion of the uterine cavity to ensure good visualization. The cavity was thoroughly searched for abnormalities, which were recorded on videotape for later review and description. The same gynaecologist, who was blinded to treatment allocation during both performance of the hysteroscopic examination and the description process, performed all the examinations. Hysteroscopic findings were classified into the following categories: (i) normal endometrium; (ii) unspecific changes – fluid in the uterine cavity, polyps and proliferative endometrial changes; (iii) single specific changes – either stromal oedema or pseudocysticity; and (iv) combined specific changes – stromal oedema and pseudocysticity with or without hypervascularity and proliferative endometrial changes.

Data analysis

Statistical analysis was carried out using the GraphPad Data Analysis Software version 2.01 (GraphPad Inc, San Diego, California, USA). The baseline characteristics of the various treatment groups and the changes in PP14 and ET in the intervention group compared with the placebo group were analysed by one-way analysis of variance (ANOVA) followed by Bonferroni's test. Spearman's correlation coefficient was used to establish the univariate relationship between the changes in PP14 and the changes in ET. Differences between the changes in PP14 and ET found in patients with normal compared with abnormal endometrial status were analysed using the Student's t-test for unpaired observations. Differences were considered as significant if $p < 0.05$. Sensitivity and specificity were determined from 2×2 tables including all the data from the levormeloxifene trial, unless the change in serum PP14 concentration or ET was 0.

Results

Study participants

Table 1 shows the characteristics of the participants at entry to the trial. There were no significant differences between the intervention groups either in the raloxifene or the levormeloxifene trial.

Time-dependent changes in serum PP14 and ET

Raloxifene induced only slight increases in the serum level of PP14 during the treatment period (figure 1A). Compared to the changes seen in the placebo group, none of these responses reached statistical significance. Similarly, the raloxifene-induced changes in ET were also clinically insignificant (figure 1B). At 6 months, only five (3%) of the raloxifene-treated participants had increased ET exceeding the 4 mm limit value.

In contrast, the levormeloxifene-induced changes in serum PP14 and ET were markedly more prominent compared with those induced by placebo and raloxifene (figures 1C and 1D). None of these study variables showed a clear-cut dose–response relationship (figures 1C and 1D). After 6 months of treatment, 43 (70%)

Table 1. Characteristics of the participants at entry to the trial.

	Raloxifene					Levormeloxifene					
	Placebo ($n = 31$)	30 mg ($n = 44$)	60 mg ($n = 48$)	150 mg ($n = 42$)	ANOVA	Placebo ($n = 21$)	1.25 mg ($n = 23$)	5 mg ($n = 12$)	10 mg ($n = 13$)	20 mg ($n = 13$)	ANOVA
Age (years)	54.9 ± 3.7	54.6 ± 3.2	55.9 ± 3.0	54.9 ± 3.2	NS	57.5 ± 4.0	57.7 ± 4.6	56.7 ± 3.1	58.4 ± 4.3	58.2 ± 3.4	NS
Height (m)	1.65 ± 0.06	1.64 ± 0.05	1.64 ± 0.07	1.65 ± 0.06	NS	1.62 ± 0.04	1.62 ± 0.06	1.65 ± 0.05	1.63 ± 0.06	1.63 ± 0.06	NS
Weight (kg)	70.0 ± 11.0	69.3 ± 12.2	69.2 ± 9.6	68.6 ± 9.5	NS	67.2 ± 9.2	67.3 ± 9.0	70.0 ± 10.1	70.7 ± 8.5	69.0 ± 10.4	NS
Age at menopause (years)	50.4 ± 3.5	49.5 ± 3.0	50.7 ± 2.8	50.0 ± 3.1	NS	49.1 ± 5.9	50.2 ± 3.3	50.0 ± 3.9	50.1 ± 3.9	50.3 ± 4.5	NS
Serum PP14 ($\mu g l^{-1}$)	3.4 ± 0.7	3.5 ± 0.6	3.6 ± 0.8	3.6 ± 0.7	NS	2.8 ± 0.5	2.8 ± 0.8	2.8 ± 0.5	3.1 ± 0.7	2.8 ± 0.4	NS
ET (mm)	1.4 ± 0.5	1.3 ± 0.6	1.4 ± 0.6	1.7 ± 1.0	NS	2.5 ± 1.2	2.5 ± 1.0	2.1 ± 0.6	2.2 ± 1.0	2.8 ± 1.6	NS

Results shown are mean ± SD.

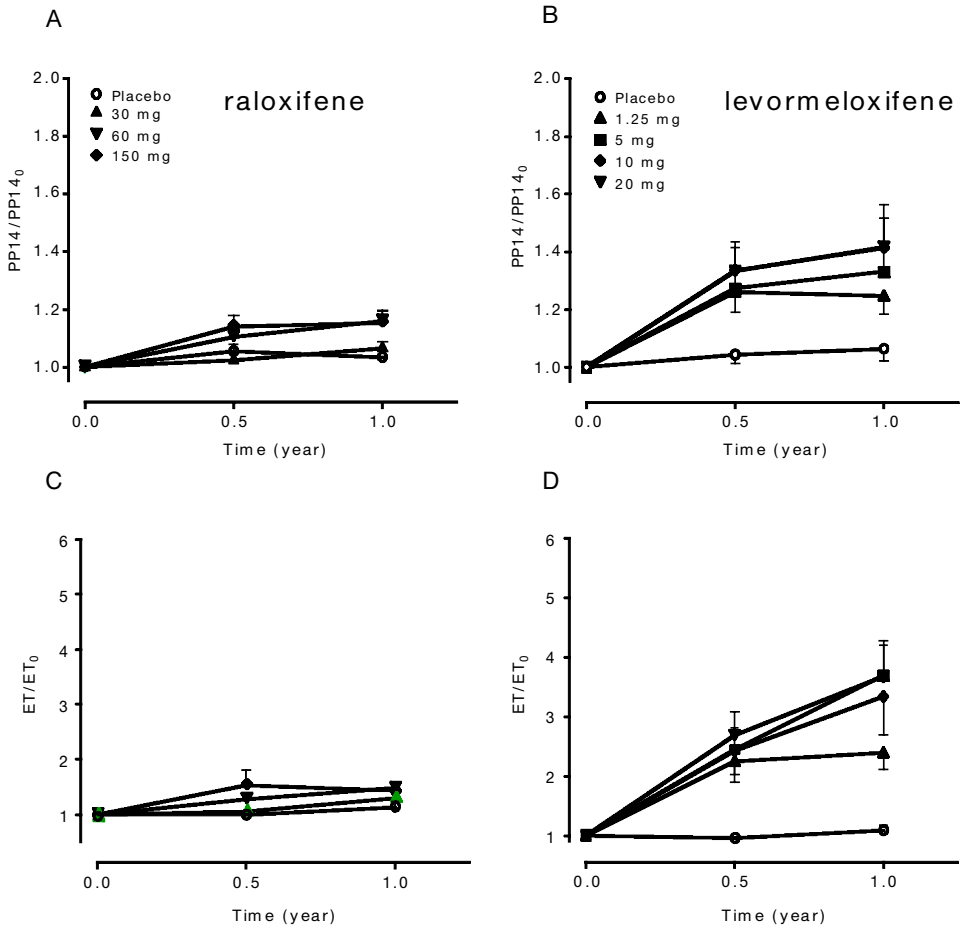

Figure 1. Changes in serum PP14 concentration and ET during treatment with various doses of raloxifene (A and B) or various doses of levormeloxifene (C and D). Changes are expressed as percentage increases relative to baseline values ($PP14_0$). The number of patients in the various intervention groups is given in table 1.

of the levormeloxifene-treated participants already showed an ET larger than 4 mm.

Associations between serum PPI4 and ET

Baseline levels of serum PP14 showed no statistically significant association with ET in either the raloxifene or the levormeloxifene trial.

Changes in PP14 at 6 months in the raloxifene trial were significantly correlated with the simultaneous changes in ET ($r = 0.431$, $p < 0.001$, $n = 165$). The correlation between these study variables was even stronger in the levormeloxifene trial, where the changes in these variables were on a much larger scale compared with those seen with raloxifene (figure 2A). The correlation was characterized by an r value of 0.67 ($n = 82$, $p < 0.0001$). When analysing the data from those receiving treatment with levormeloxifene ($n = 61$), the diagnostic sensitivity and

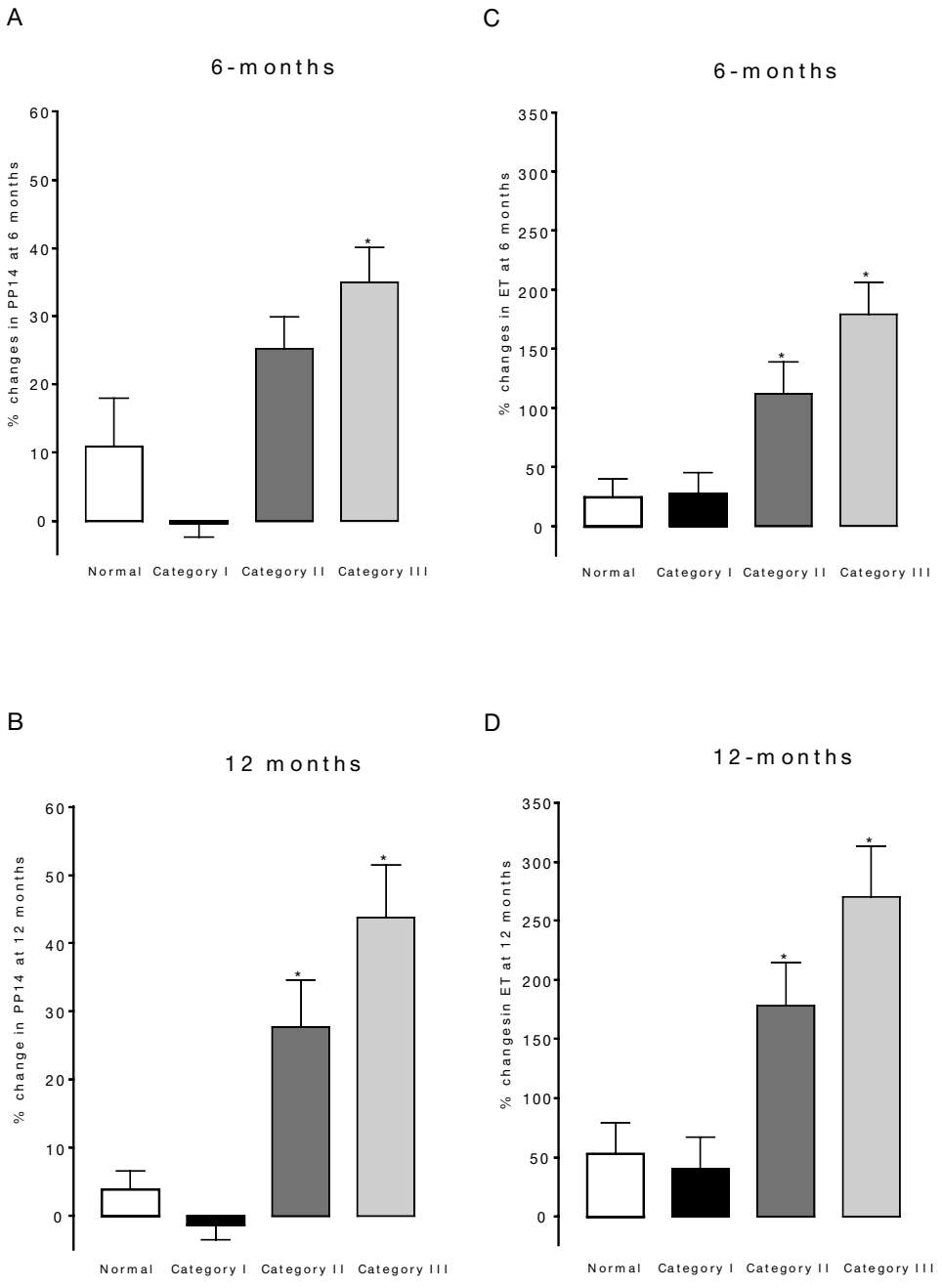

Figure 2. Six month changes in serum PP14 versus 6 month (A) and 12 month (B) changes in ET. (C) Six month changes in ET versus 12 month changes in ET. Data are obtained from 82 postmenopausal women receiving treatment with levormeloxifene ($n = 61$) or placebo ($n = 21$).

specificity of the 6 month change in PP14 for indicating the simultaneous change in ET were 98% (54 out of 55) and 83% (five out of six), respectively. The 6 month change in serum PP14 also showed a weaker, but still significant association with the 12 month change in ET ($r = 0.36$, $n = 82$, $p = 0.001$; figure 2B). The diag-

nostic sensitivity and specificity of the 6 month change in PP14 for the prediction of the 12 month change in ET were 93% (55 out of 59) and 50% (one out of two), respectively. When analysing the association between 6 and 12 month changes in ET in the same trial, the correlation was characterized by an r value of 0.58 ($n = 82$, $p < 0.0001$; figure 2C).

Endometrial status by hysteroscopy

The 6 and 12 month changes in PP14 according to the endometrial changes are indicated in figure 3. Normal endometrial status and unspecific endometrial changes such as fluid in the uterine cavity, polyps and proliferative changes *per se* were not associated with increases in PP14. Stromal oedema and pseudocysticity were, however, accompanied by considerable increases in PP14. The simultaneous presence of these changes with or without proliferation was associated with further increases in the serum level of PP14 (figures 3A and 3B). The changes in ET at 6 and 12 months showed similar trends (figures 3C and 3D).

Discussion

The clinical development of SERMs is complicated by the fact that these drugs act on various organ systems simultaneously and each of these effects can be characterized by a different dose–response relationship. While there are well-established laboratory assays to estimate the impact of these compounds on the skeletal and cardiovascular systems, until now no laboratory assay has been introduced for the estimation of the biological responses of the endometrium to these agents. In the present study, we demonstrated that monitoring changes in the serum level of the endometrial secretory protein PP14 appears to be able to serve such a purpose.

When using the PP14 assay, we found that the serum level of this secretory protein increased only slightly with raloxifene administered in the 30–150 mg day^{-1} dose range. These weak responses are in line with previous studies reporting similar changes in secretory proteins with raloxifene (Komm *et al.* 1986, Sheen and Katzenellenbogen 1987, Takeda and Shimizu 1988, Bell and Drife 1989, Dietze *et al.* 1996). The simultaneous measurements of ET, in accordance with previous clinical studies (Delmas *et al.* 1997), however, indicated that these slight changes in serum PP14 do not appear to have much clinical significance. Thus these results provide further evidence that the recommended 60 mg day^{-1} dose of raloxifene is indeed appropriate and has negligible effects on the endometrium.

Although levormeloxifene belongs to the same class of SERMs and possesses a fairly similar chemical structure, it exhibited prominent adverse effects on the endometrium. The biological response of the endometrium was indicated by marked changes in ET and serum PP14, which showed a strong positive correlation during the treatment period. Neither the changes in ET nor those in serum PP14 showed a clear-cut dose–response relationship, which, in accordance with previous observations (Plouffe 2000), further supports the notion that the frequent adverse effects induced by levormeloxifene can be largely attributed to an overdosing of the drug. The association between the 6 month change in PP14 and the 12 month change in ET suggest that the assay applied on a group basis might have

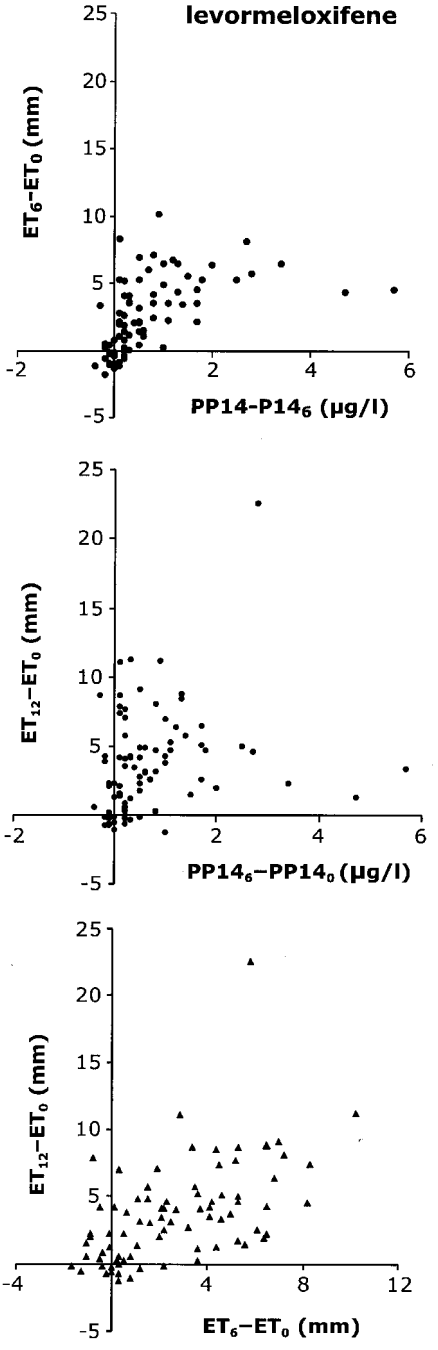

Figure 3. Percentage changes in serum PP14 and ET compared with baseline according to the endometrial status as seen on hysteroscopy. Normal endometrium, $n = 16$; category I (non-specific changes) – fluid in the uterine cavity, polyps and proliferative changes, $n = 5$; category II (single specific changes) – stromal oedema or pseudocysticity, $n = 22$; category III (combined specific changes) – stromal oedema and pseudocysticity with or without hypervascularity and proliferative changes, $n = 28$. $^*p < 0.05$ compared with normal endometrial status. There were no significant differences between the different categories in terms of the baseline level of serum PP14 or ET.

provided a sign of concern as to the inappropriateness of the dose range selected for the trial. In the light of the impressive bone and lipid effects (Alexandersen *et al*. 2001), it was particularly unfortunate that the development of levormeloxifene had to be discontinued due to the severe drug-related endometrial adverse effects, which might have been avoided if better tools had been available to plan a more targeted clinical trial.

The hysteroscopic examinations revealed a wide range of abnormalities following 12 months' administration of levormeloxifene. The unspecific endometrial changes, also seen in the placebo group, were not associated with increases in PP14. However, the apparent SERM-specific endometrial changes (stromal oedema and pseudocysticity), also seen in tamoxifen trials (Bese *et al*. 1996, Andia *et al*. 2000, Marttunen *et al*. 2001, Cline *et al*. 2001), were accompanied by considerable increases in the serum level of PP14. At the present time it is not possible to explain the mechanisms responsible for the changes in serum PP14, but the responses do seem to be associated with SERM-specific endometrial abnormalities.

In conclusion, the results of the present study suggest that the PP14 assay may serve as a useful supplement to conventional tools used to estimate the effects of SERMs on the endometrium. Used on a group basis, the assay might be of assistance in short pilot trials aiming to find the safest dose range of novel SERMs. On an individual basis, it may act as an adjunct to ultrasonographic examinations in identifying endometrial changes in SERM-treated patients, especially if hysteroscopy and biopsy are difficult to carry out.

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