Effect of sequential estrogen/progestin treatment on biochemical vasoactive markers in postmenopausal women comparing oral and transdermal application

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Summary

Purpose: Estrogen replacement in postmenopause can elicit vasodilatory effects which may be important for cardiovascular protection. Progestin addition can antagonise this beneficial effect, but until now, only a few studies have been performed on this issue. In the present study the effect of estradiol as well as of added norethisterone acetate (NETA), a C19-progestin, on the renal excretion of various biochemical markers which can reflect vasoactive actions was investigated.

Methods: 37 postmenopausal women were treated for one sequential estrogen/progestin treatment cycle, i.e. two weeks with estradiol alone followed by two weeks with an estradiol/progestin combination. Both oral (n=20) as well as 'complete' transdermal (n=17) hormone substitution was applied, and the excretion of the following vasoactive substances or the stable metabolites, respectively, were measured in nocturnal urine prior to treatment, after 14 and 28 days: cGMP, which can reflect the production of nitric oxide, prostacyclin, thromboxane and serotonin.

Results: The excretion of cGMP was increased with both forms of administration during the estrogen phase as well as during the consecutive estrogen/progestin treatment. The prostacyclin-thromboxane quotient increased during estrogen phases, but decreased significantly by the addition of oral, but not with transdermal NETA, reflecting possible vasoconstrictory effects. Serotonin excretion increased, but this only was significant after the oral estrogen-only phase (2 weeks treatment), and after one cycle of complete transdermal treatment (4 weeks treatment), respectively.

Conclusion: The observed effects can be explained by vasodilatory actions during the estrogen phases which can be maintained or even increased during the consecutive estrogen/progestin treatment suggesting a time-dependent beneficial estrogen effect. However, oral progestin addition may antagonise this effect already within two weeks of treatment whereby the prostacyclin-thromboxane quotient seems to be the most sensitive marker surrogating on vasoconstrictory progestin action.

Key words: Estradiol; Progestin; Postmenopause; Vascular action; Biochemical markers; Application form.

Introduction

Estrogen substitution in postmenopause not only contributes to alleviating the menopausal syndrome, but also prevents osteoporosis and cardiovascular disease [1]. Cardiovascular protection by estrogens is ascribed partly to the positive influence of the lipid profile, but direct vessel effects are also partly responsible [2]. In vitro studies show that estrogens can increase the production of vasodilating substances occurring naturally, such as endothelial production of nitric oxide (NO) and prostacyclin [3-6]. Furthermore, estrogens also have a blocking effect on voltage-dependent calcium channels, reducing the intake of calcium, which leads to a vasodilatation of vascular smooth muscle cells [7].

These effects were confirmed in in vivo studies, demonstrating that estrogens are able to dilate vessels of the cardiovascular system [8-11].

In an earlier study, we could observe an increase in renal excretion of prostacyclin and thromboxane after unopposed estradiol treatment [12]. In estrogen substitution in postmenopause, where the uterus is intact, progestin is routinely added to avoid endometrial hyperplasia with the danger of developing a carcinoma. The object of the present study was to measure the influence of estradiol treatment on the renal excretion of various biochemical markers known to reflect vasoactive action, and the addition of the progestin norethisterone acetate (NETA), a C19-progestin with androgenic properties, added sequentially to estradiol replacement therapy. The question was, if there could be time-dependent effects and if NETA could antagonise beneficial estrogen actions, i.e. changes in the excretion of markers which surrogate on vasoconstrictory progestogenic effects whereby the oral administration form as well as complete transdermal hormonal replacement was investigated. The following biochemical markers, which can alter vasotonus by direct vascular action have been assessed: cGMP, which can reflect the systemic production of NO, prostacyclin, and thromboxane, as well as serotonin. So far little is known of the extent to which this addition of progestin influences the effect of estradiol on the vessels.

Patients and Methods

The study was carried out with 37 postmenopausal women with climacteric complaints. In all of the women, the last period was at least 12 months before. The serum values of 17ß-estradiol were in all cases below 70 pmol/l, and those of FSH above 40 IU/l.

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In an open study design, 20 women were treated for one cycle of usual estradiol/sequential progestin treatment, i.e., 2 weeks with oral estradiol valerate (2 mg/d) alone, and then 2 weeks with the combination of estradiol valerate (2 mg/d) and oral norethisterone acetate (NETA, 1 mg/d).

Seventeen women were treated transdermally testing also one typical treatment cycle by using estradiol patches for two weeks followed by estradiol/NETA patches for a further two weeks. With the transdermal treatment the daily dosage of estradiol was 0.05 mg and of sequential NETA 0.25 mg.

No wash-out phase was inserted between the estradiol treatment and the combined estradiol/NETA treatment, since in the case of postmenopausal hormone substitution the combined estrogen/progestin phase takes place immediately following the estrogen monophase. As estradiol has a short-life of about 30 min, any carry-over effect should be very small and thus negligible.

A placebo-controlled double-blind study was not carried out, as with hormone substitution the clinical hormone effect can quickly be recognised both by the patient and the treating physician due to different bleeding patterns, at least in women with an intact uterus.

Women who had taken medication able to influence the production of the various markers under investigation were excluded from the study. Also excluded were patients with neoplasias, thromboembolia and diseases of the liver or kidneys.

The urine excreted between 10 p.m. and 6 a.m. was collected before treatment and following each of the two phases of treatment. Aliquots were frozen at –20°C until measurement. The advantages of nocturnal collection of urine are that a convenient and reliable collection of samples is possible, and that definite comparable periods of time free of stress are involved.

Measurement of the markers in urine

cGMP: cGMP was measured directly from the urine using a commercially obtainable EIA kit (Cayman, USA). The inter- and intra-assay variations were 8.9% and 9.5%, respectively. The sensitivity of the assay was 5 nMol/l.

Prostacyclin/thromboxane system: Since prostacyclin and thromboxane are unstable compounds, the stable metabolites 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ and 11-dehydro-thromboxane B_2, respectively, were measured. Measurement of these metabolites took place following chromatographic separation by means of enzyme immunoassays. The prostacyclin metabolite was measured according to the working prescription of Hiramatsu *et al.* [13]. The inter- and intra-assay variations were 10.5% and 9.1%, respectively. The sensitivity of the assay was 10 pg/ml. The thromboxane metabolite was measured using a commercially obtainable kit from the firm Cayman, USA. The inter- and intra-assay variations were 9.9% and 8.9%, respectively. The sensitivity of the assay was 10 pg/ml.

Serotonin: As serotonin is also unstable, its stable metabolite 5-hydroxyindole acetic acid (5-HIAA) was measured. This was measured directly from urine using a commercial enzyme immunoassay (IBL, Hamburg). The inter- and intra-assay variations were 10.9% and 7.9%, respectively. The sensitivity of the assay was 0.6 μMol/ml.

The results were calculated per patient as percentage changes in excretion of the markers compared with the values before treatment = 100%. This transformation proved to be a more reliable indication compared with the absolute values because the individual absolute values are subject to very great variations for all the markers. The statistical evaluation was made after logarithmisation of the values using the Student's t-test.

Results

The basic data of the women, such as age, height, weight and menopause interval are given in Table 1. The data of the women in both groups are, as can be seen, clearly comparable.

The percent changes in the markers investigated compared with the values before treatment with oral or transdermal substitution are given in Table 2. The values before treatment are taken as a basis = 100%.

For cGMP excretion, in the case of both oral and transdermal hormone substitution, a significant increase following the estrogen and the estrogen/progestin phase compared with the values before treatment could be registered ($p<0.05$).

With respect to the vasodilator prostacyclin with both oral and transdermal treatment, an increase during the estrogen phase followed by a decrease during the estrogen/progestin phase was observed by measuring the excretion of the metabolite, whereas the vasoconstrictor thromboxane was found to be increased during the com-

Table 1. — *Basic clinical data of the postmenopausal women (mean values ± SD).*

	Oral HRT 20 pts.	Transdermal HRT 17 pts.
Age (years)	54.8 ± 4.4	53.6 ± 6.0
Height (cm)	163.3 ± 6.7	164.1 ± 6.2
Weight (kg)	73.6 ± 11.8	67.3 ± 7.8
Time since menopause (years)	4.8 ± 3.6	6.7 ± 7.5

Table 2. — *Percental changes in renal excretion of various markers in relation to the values before treatment (= 100%) following oral (n=20) and transdermal hormone treatment (n=17). Mean values ± SEM.*

	Without treatment	Oral treatment		Transdermal treatment	
Marker		Estradiol	Estradiol + NETA	Estradiol	Estradiol + NETA
cGMP	100	157.3 (21.2)*	177.0 (25.5)*	158.7 (22.9)*	183.8 (20.1)*
Prostacyclin-metabolite	100	143.6 (20.1)	86.9 (25.5)	144.3 (28.4)	103.4 (20.8)
Thromboxane-metabolite	100	110.2 (16.5)	187.8 (52.6)	116.2 (11.4)	138.9 (25.6)
Quotient					
Prostacyclin-metabolite to thromboxane-metabolite	100	163.4 (87.2)	67.2 (20.1)*	152.3 (25.9)	93.8 (22.9)
Serotonin-metabolite	100	184.9 (34.8)*	155.3 (51.0)	132.6 (19.7)	164.1 (21.4)*

* statistically significant difference to pretreatment values, $p<0.05$.

bined phase. However, this did not reach significance, perhaps due to high inter-individual variation. But on comparison of the quotient of prostacyclin to thromboxane, which is decisive for the regulation of the vasotonus, following oral treatment with estradiol/NETA this value decreased significantly compared with the value before treatment ($p<0.05$).

For the serotonin metabolite excretion, following oral estradiol treatment a significant increase was registered ($p<0.05$), which decreased again following treatment with estradiol/NETA. Following transdermal treatment with estradiol no change was registered, but consecutive further treatment for two weeks resulted in a significant increase ($p<0.05$) despite adding NETA to estradiol; both hormones were applied transdermally by using the combi-estradiol/NETA patch.

Discussion

Nitric oxide (NO), which is formed in the vascular endothelium, possesses strong vasodilatory and antithrombotic properties. As NO triggers the formation of cGMP, the measurement of cGMP can be adduced as a measure of NO production. Although this process takes place intracellularly, the measurement of cGMP occurring extracellularly permits conclusions concerning the influenceability of the NO/cGMP system [14-16].

The increased cGMP excretion both following the estrogen phase and the combined estrogen/NETA phase indicates that both forms of application can lead to vasodilatation by stimulation of cGMP, which can reflect NO production. Neither oral nor transdermal treatment with NETA added sequentially during one-cycle-treatments in this study appears to antagonise this effect of estrogen.

The tendency to a higher increase of cGMP during the second combined phase, i.e. week 3 and 4 of this study, can be explained by the longer total time of treatment with estradiol. On the other side it cannot be excluded that the longer treatment with added progestin could also antagonise this beneficial estrogen effect as observed for the measurements of the prostacyclin/thromboxane markers.

Prostacyclin has a vasodilatory and antithrombotic effect, while *thromboxane* possesses antagonistic effects, i.e. vasoconstrictory and thrombotic properties. Both substances are very unstable, for which reason the stable renal metabolites were measured, which reflect the production of these two parameters.

The effects on the two markers investigated were not found to be significantly different from the values preceding treatment. However, as shown in Table 2, an increase of prostacyclin-metabolite during the estrogen phase, and an increase of thromboxane-metabolite during combined phases were observed with both oral and transdermal administration, and significance failed perhaps due to high inter-individual variations. This may be speculative, but the decrease in the quotient of prostacyclin to thromboxane, which is decisive for the resulting effects on vessels and which took place following oral addition of NETA, can clearly be explained by a vasoconstrictory progestin effect, as progestins are thought to exert a vasoconstrictory effect on vessels [17].

For transdermal application, there was only a tendency to a similar picture; the decrease following the addition of NETA was presumably not strongly marked because, in contrast to oral treatment, a 75% lower dose of progestin was given. With oral treatment, thus, the higher dose of 1 mg NETA is able to antagonise the dilatatory estrogen effect. One may speculate that a negative influence of the prostacyclin/thromboxane system in the case of the transdermal progestin combination may not take place because of the lower dosage.

Serotonin secretion affects not only the psychological state, with the general feeling of well-being, but also the pathogenesis of cardiovascular disease by means of effects on the vessel system [18, 19]. The effects of hormone substitution on serotonin production were gauged by measuring renal excretion of 5-hydroxyindole acetic acid. Significant differences in serotonin production occurred between the oral and the transdermal hormone treatment inasfar as increased production was found with oral estradiol treatment, but in transdermal treatment an increase only after the second, i.e. combined phase. As for cGMP this could be explained by a time-dependent vasodilatory estradiol effect which was not antagonised by adding of transdermal but of oral NETA during week 3 and 4 of the study.

In conclusion the present study indicates a vasodilatory effect of oral and transdermal estradiol in postmenopausal women treated for 2 weeks. This beneficial effect may be time-dependent and thus may be enhanced after a further 2 weeks of treatment with combined estrogen/NETA treatment. However, a vasoconstrictory effect of oral NETA may partly antagonise this effect. Further studies are needed to investigate longer treatment durations.

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