Estradiol inhibits LDL oxidation:
Do the progestins medoxyprogesterone acetate and norethisterone acetate influence this effect?

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Summary

Estrogen replacement therapy in postmenopausal women must be combined with progestin to avoid endometrial cancer. However, progestin addition could antagonize cardioprotective effects of estradiol. Therefore we investigated the effect of the two most commonly used progestins-medoxyprogesterone acetate (progestosterone-derivative) and norethisterone acetate (nortestosterone-derivative)-alone and in combination with 17β-estradiol on copper-mediated oxidation of low density lipoprotein (LDL). Whereas 17β-estradiol alone inhibited the onset of LDL oxidation at the concentrations 0.5, 1.0, 5 and 10 μM, the progestins alone did not demonstrate any significant effect. In the estrogen-progestin combinations of 0.5μM 17β-estradiol with 0.5, 1.0, 5 and 10 μM progestin, respectively, the estradiol effect was not changed. These results suggest that medoxyprogesterone acetate as well as norethisterone acetate do not counteract the beneficial effect of 17β-estradiol on LDL oxidation when used in hormone replacement therapy.

Key words: Estradiol; Medoxyprogesterone acetate; Norethisterone acetate; LDL oxidation.

Introduction

A disturbed lipid metabolism, especially the oxidation of LDL, is generally considered to be an important factor in the etiology of cardiovascular diseases. Estrogens not only influence the concentrations of lipid subfractions i.e. reduce LDL-cholesterol and increase HDL-cholesterol [1], but also inhibit the oxidation of LDL [2, 3]. This effect may contribute to the well-established cardioprotective action of estradiol replacement therapy in postmenopausal women [4].

Treating women with unopposed estrogen enhances the risk of endometrial carcinomas. In combination with progestins, however, this can be avoided [5, 6, 7]. The progestins differ in their pharmacodynamic properties. The main criterion for the choice of the progestins is their efficacy with regards to endometrial action and tolerability. Two types of progestins are in common use: “C21 progestins”, which are derived from natural progesterone and are known to be particularly tolerable but less effective [8, 9], and “C19 progestins”, derived from nortestosterone, which are especially efficacious on the endometrium but less tolerable [10, 11]. For hormonal replacement the most frequently used progestins are medoxyprogesterone acetate (MPA), a C21 progestin, and norethisterone acetate (NETA), a C19 progestin.

The present study was designed to investigate the effect on LDL oxidation by the progestins MPA and NETA. Of particular interest was whether the beneficial estradiol-induced inhibition of LDL oxidation would be impaired by MPA and NETA. This could have consequences for hormonal replacement therapy in postmenopausal women notably in the case of patients with cardiovascular disease.

Materials and Methods

17β-estradiol (E2) and medoxyprogesterone acetate (MPA) were purchased from Sigma (Deisenhofen, Germany) and norethisterone acetate (NETA) from Ciba-Geigy (Basel, Switzerland).

LDL oxidation was investigated following the method described by Esterbauer et al. [12] using pooled blood serum from healthy premenopausal women containing the antioxidants EDTA (1 mg/ml) and butylated hydroxytoluene (BHT) (4.4 μg/ml). LDL was obtained by ultracentrifugation within 4 hours after the collection of blood samples. In brief: 1.638 g sodium bromide was added to 3 ml serum which was overlayed with saline. After ultracentrifugation at 105,000 g for 9 h (fixed angle rotor), the LDL-layer (density 1.02-1.05 ml) was aspirated by a syringe. Oxidation of LDL was started after ultracentrifugation as follows: LDL-solution was separated from BHT and EDTA by gelfiltration (Sephadex G-25, column 10x1.5 cm, eluens: saline). Samples of 150 μl of LDL (adjusted to 300 μg protein/ml) were each mixed with 850 μl saline containing 10 μM CuCl₂, and the test substances. The tested steroids, dissolved in ethanol, were added to this mixture to give final concentrations of 0.5, 1, 5 and 10 μM. E2, MPA and NETA alone were tested in each of these concentrations. In the estrogen/progestin-combinations estradiol was in each instance 0.5 μM, whereas MPA and NETA were added in the concentrations 0.5, 1, 5, 10 μM, respectively. Control values were obtained by the addition of alcohol alone in the same concentrations as in the test substances (final ethanol concentration in all samples = 1%).

The rise of conjugated diene formation, characteristic for the oxidation of LDL, was monitored spectrometrically at 234 nm. Tangents were drawn to the segments of the absorption curve corresponding to the lag phase and propagation phase of LDL oxid-
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Results

The control values for ox-LDL formation showed an average lag time of 98.5±11.2 min.

Table 1 shows the effect of 17β-estradiol (E2), medroxyprogesterone acetate (MPA) and norethisterone acetate (NETA) on the onset of LDL oxidation. E2 elongated the onset at the concentrations of 0.5 and 1 μM for 1.5±3.6 min and 73.6±63.3 min, respectively and exceeded the observation time of 300 min at 5 and 10 μM. Neither MPA nor NETA exhibited any significant effect on the onset of LDL oxidation in the concentration range tested.

The progestins combined with 0.5 μM E2, i.e. the lowest effective concentration of E2, did not antagonize the estradiol-induced elongation of the lag time over the entire concentration range tested (Table 2).

Table 1. — Increase of the lag time of LDL transformed into oxidized LDL after addition of 17β-estradiol, medroxyprogesterone acetate and norethisterone acetate, respectively. The values in minutes are expressed as means ± SD, n=9.

<table>
<thead>
<tr>
<th></th>
<th>0.5 μM</th>
<th>1 μM</th>
<th>5 μM</th>
<th>10 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol</td>
<td>24.5 (3.6)*</td>
<td>73.6 (6.3)**</td>
<td>&gt;300**</td>
<td>&gt;300**</td>
</tr>
<tr>
<td>Medroxy-</td>
<td></td>
<td></td>
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<tr>
<td>progesterone</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetate</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Norethisterone</td>
<td>5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
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<tr>
<td>acetate</td>
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</tbody>
</table>

* p<0.05, ** p<0.01 compared to control value.

Table 2. — Increase of the lag time of LDL transformed into oxidized LDL after addition of the combinations of 0.5 μM 17β-estradiol (E2) with 0.5, 1, 5 and 10 μM medroxyprogesterone acetate (MPA) and norethisterone acetate (NETA), respectively. The values in minutes are expressed as means ± SD, n=9.

<table>
<thead>
<tr>
<th></th>
<th>0.5 μM E2</th>
<th>0.5 μM E2</th>
<th>0.5 μM E2</th>
<th>0.5 μM E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
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<td>1 μM MPA</td>
<td>5 μM MPA</td>
<td>10 μM MPA</td>
</tr>
<tr>
<td>Estradiol</td>
<td>24.5 (3.6)*</td>
<td>24.1 (3.7)*</td>
<td>23.8 (3.5)*</td>
<td>25.9 (3.8)*</td>
</tr>
<tr>
<td>Medroxy-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>progesterone</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>acetate</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
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<tr>
<td>Norethisterone</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>acetate</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td></td>
<td>24.5 (3.6)*</td>
<td>23.6 (3.4)*</td>
<td>25.9 (2.9)*</td>
<td>26.8 (3.3)*</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01 compared to control value.

Discussion

The inhibition of oxidation of LDL is known to be an important step in preventing cardiovascular disease. Oxidized LDL is internalized by macrophages more efficiently than native LDL [13]. Lipid-laden macrophages accumulate in the intima of vascular vessels and form foam cells which are believed to be precursors of atherosclerotic lesions [13]. Furthermore, oxidized LDL is toxic to endothelial and smooth muscle cells of vascular vessels [14] and can impair the cardiovascular system by reducing nitric oxide synthesis [15], enhancing endothelin production [16] and increasing intracellular calcium in vascular cells [17].

The present study demonstrates that 17β-estradiol is able to delay the onset of LDL oxidation in vitro thus confirming the results of our previous study [18] and also of other groups [2, 3].

Whereas estradiol replacement therapy has been shown to have a beneficial effect on cardiovascular disease the effect of progestins is so far not clear. Progestins can counteract estrogens in many instances [19]. For example NETA decreases HDL-cholesterol [10, 20] and thereby antagonizes the HDL-increasing effect of estradiol. Nevertheless NETA is one of the most commonly used progestins, as it potentially transforms a proliferative endometrium into a secretory one and thus protects from the development of endometrial hyperplasia and cancer [6, 11, 21]. MPA does not counterbalance the HDL-increasing effect of estrogen [9] but has been shown to be less efficient concerning the secretory transformation of the endometrium [8].

Conclusion

Although the two progestins MPA and NETA show differences in their pharmacodynamic actions, e.g. concerning the influence on HDL-cholesterol and on the endometrium, the present results indicate that estradiol-induced inhibition of LDL oxidation is not influenced by either of the progestins. Even in high dosages they do not eliminate the beneficial estradiol effect. This is of clinical relevance for hormonal replacement therapy in postmenopausal women especially for prophylaxis and therapy of cardiovascular diseases.

References


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