Estradiol inhibits LDL oxidation: Do the progestins medroxyprogesterone 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-medroxyprogesterone acetate (progesterone-derivative) and norethisterone acetate (nortestosterone-derivate)-alone and in combination with 17ß-estradiol on copper-mediated oxidation of low density lipoprotein (LDL). Whereas 17ß-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 17ß-estradiol with 0.5, 1.0, 5 and 10 µM progestin, respectively, the estradiol effect was not changed. These results suggest that medroxyprogesterone acetate as well as norethisterone acetate do not counteract the beneficial effect of 17ß-estradiol on LDL oxidation when used in hormone replacement therapy.

Key words: Estradiol; Medroxyprogesterone 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 medroxyprogesterone 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

17ß-estradiol (E2) and medroxyprogesterone 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 g/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 $CuCl_2$ 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 oxi-

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dation. The length of the lag phase = lag time was determined as the intercept of the two tangents.

The antioxidative effect of the substances tested is expressed as the elongation of the lag time after the ox-LDL formation of the control. The increase in lag time was measured in minutes up to a maximum of 300 min. Each test substance, alone or in combination, was tested in duplicate in nine different LDL-pools.

Protein content of LDL was determined by a colorimetric protein assay (Bio-Rad, Müchen, Germany). Statistical analysis was performed using the Student's t-test.

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 17ß-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 24.5±3.6 min and 73.6±6.3 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 oxidated LDL after addition of 17ß-estradiol, medroxyprogesterone acetate and norethisterone acetate, respectively. The values in minutes are expressed as means ± SD, n=9.*

	0.5 µM	1 µM	5µM	10µM
Estradiol	24.5 (3.6)*	73.6 (6.3)**	>300**	>300**
Medroxy-progesterone acetate	<5	<5	<5	<5
Norethisterone acetate	<5	<5	<5	<5

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

Table 2. — *Increase of the lag time of LDL transformed into oxidated LDL after addition of the combinations of 0.5 µM 17ß-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.*

E2	0.5 µM E2 + 0.5 µM MPA	0.5 µM E2 + 1 µM MPA	0.5 µM E2 + 5 µM MPA	0.5 µM E2 + 10 µM MPA
24.5 (3.6)*	24.1 (3.7)*	23.8 (3.5)*	25.9 (3.8)*	26.1 (3.2)*

E2	0.5 µM E2 + 0.5 µM NETA	0.5 µM E2 + 1 µM NETA	0.5 µM E2 + 5 µM NETA	0.5 µM E2 + 10 µM NETA
24.5 (3.6)*	23.6 (3.4)*	25.9 (2.9)*	26.8 (3.3)*	24.3 (3.2)*

* 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. Oxidated 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, oxidated 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 17ß-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.

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