Acta Biochimica Polonica

Vol. 41 No. 2/1994

QUARTERLY

Inhibitory effect of 2-hydroxyestradiol on lipid peroxidation in placental microsomes

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Key words: 2-hydroxyestradiol, lipid peroxidation, placental microsomes

It has been reported previously that estrogens exhibit substantial protecting activity against lipid peroxidation in model systems [1] and *in vivo* [2].

A number of reports indicate that preeclampsia is associated with elevated blood levels of lipids peroxidation products [3, 4]. Placental microsomes are highly susceptible to lipid peroxidation *in vitro* [5], and recent data suggest that the placental tissue may be a major source of lipid peroxidation products in pregnancy [6]. Lipid peroxide accumulation *in vivo* increases with advancing gestation, and lipofuscin pigment has been found in higher concentration in term placenta than in the placenta earlier than on the 32th week of gestation [7].

Little is known about the antioxidant status in normal and preeclamptic pregnancies. The antioxidant activity of serum was found to be significantly higher in women during normal pregnancies as compared to non-pregnant ones [8].

Placental microsomes synthesize 2-hydroxyestradiol (2-OH E) which is the main catabolite of estrogens [9]. The present work was undertaken to check a possible antioxidant activity of 2-OH E in placental microsomes.

Lipid peroxidation was initiated by *t*-butylhydroperoxide (Bu^tOOH) as a potential source of peroxyl radical, commonly considered a propagating molecule [10]. Tissue from term human placenta was homogenised at $0^{\circ}C$ in 10 vol. of 20 mM Tris/HCl buffer, pH 7.4, containing 150 mM KCl. The homogenate was centrifuged for 10 min at $10000 \times g$, and the supernatant was recentrifuged at $105000 \times g$ for 60 min.

The sedimented microsomal fraction was resuspended in the homogenisation medium and used for the examinations.

Lipid peroxidation was determined by the thiobarbituric acid test using a molar absorption coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$ at 535 nm for the malondialdehyde-thiobarbituric acid adduct [11]. Addition of Bu^tOOH to the microsomal suspension resulted in formation of malondialdehyde (MDA), a final lipid peroxidation product. Low concentrations of 2-OH E were found to inhibit effectively MDA formation (Fig. 1). On the other hand, the parent molecule estradiol had no significant effect on

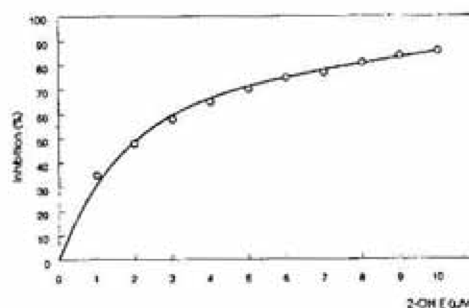

Fig. 1. Inhibitory effect of 2-hydroxyestradiol on lipid peroxidation in microsomes from term placenta.
The incubation was carried out for 30 min at 37°C under aerobic conditions with constant shaking in 2.0 ml medium containing 50 mM KPO_4 buffer, pH 7.4, and 2.2 mg of microsomal protein. Lipid peroxidation was initiated by addition of Bu^tOOH to final concentration of 5 mM. Malondialdehyde was measured after [11].

lipid peroxidation under these conditions (not shown).

The dimethylpyrroline-N-oxide (DMPO) spin trapping method successfully differentiates the radicals arising from the cytochrome P450-mediated Bu^tOOH cleavage [12]. We have found that addition of Bu^tOOH to the suspension of human placental microsomes resulted in formation of peroxyl ($ROO^\bullet$) alkoxyl ($RO^\bullet$) and carbon centred ($C^\bullet$) spin adducts with DMPO (Fig. 2). 2-OH E was found to inhibit formation of such adducts (Fig. 2).

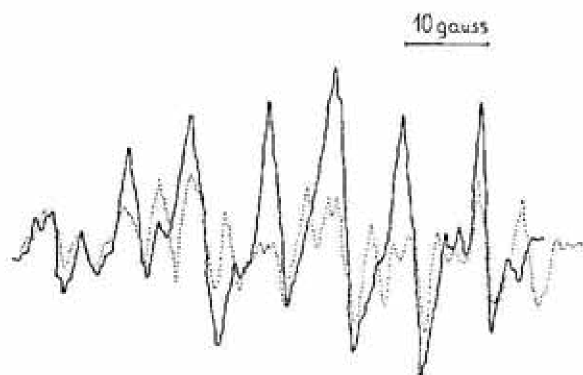

Fig. 2. E.s.r. spectra observed on reaction of placental microsomes (5 mg protein) with 40 mM Bu^t-OOH in the presence of 40 mM DMPO, in the presence (.....) or absence (—) of 2-hydroxyestradiol. Scan time 4 min, receiver gain 4×10^4, modulation amplitude 0.5×1 G. For detection of e.s.r. spectra a Varian model E4 spectrometer was used.

Our results indicate that 2-OH E can act as a natural endogenous strong chain breaking antioxidant in placental tissue.

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