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Estrogens as natural antioxidants of membrane phospholipid peroxidation

Katsuaki Sugioka, Yasuko Shimosegawa+ and Minoru Nakano

College of Medical Care and Technology, Gunma University, Maebashi, Gunma and +*Division of Neurosurgery, Institute of Brain Diseases, Tohoku University School of Medicine, Sendai, Japan*

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Estrogens, such as estrone, estradiol and estriol, were tested as possible antioxidants of lipid peroxidation induced by Fe^{3+}-ADP-adriamycin or Fe^{3+}-ADP-ascorbate. The estrogens with phenolic structure possessed substantial activities with respect to the inhibition of lipid peroxidation. Concentrations of estradiol and estriol required to achieve 50% inhibition of membrane phospholipid peroxidation were about 4- and 6-times that of α-tocopherol, respectively

Estrogen; Antioxidant; Lipid peroxidation

1. INTRODUCTION

It has been reported that thyroxine with a phenolic structure possesses a potent antioxidant activity on iron-induced phospholipid peroxidation [1]. In contrast to all other natural steroids, estrogens, estrone and its analog, have a phenolic structure in their molecules (ring A). The present work was undertaken to prove possible antioxidant activities of estrogens, using the Fe^{3+}-ADP-adriamycin complex system or the Fe^{3+}-ADP-ascorbate system as an initiator of lipid peroxidation and microsomal phospholipid liposomes as lipid source.

2. MATERIALS AND METHODS

2.1. *Preparation of liposomes*

Microsomal phospholipid was obtained from rat liver microsomes as described [2]. Liposomes were then prepared by mixing the phospholipid with or without the compound (estrone, estradiol, estriol, testosterone or DL-α-tocopherol) in chloroform-methanol (2:1, v/v), evaporating the organic solvents to dryness and sonicating the residue suspended in 0.1 M Tris-HCl buffer at pH 7.5 [2]. Estrone, estradiol, estriol and testosterone were purchased from Sigma. DL-α-Tocopherol was kindly by Eisai Co.

Correspondence address: M. Nakano, College of Medical Care and Technology, Gunma University, Maebashi, Gunma, Japan

2.2. *Incubation experiments*

The incubation mixture contained liposomes (0.85 μmol of lipid phosphorus/ml) with or without fatty soluble compound at a variety of concentrations, 0.1 mM Fe^{3+}-1.67 mM ADP-0.2 mM adriamycin and 0.1 M Tris-HCl buffer at pH 7.5 in a total volume of 3.8 ml (for O_2 consumption) and 6.0 ml (for lipid analysis). The reaction was initiated by the addition of adriamycin. In some cases, the mixture contained 0.1 mM Fe^{3+}-1.67 mM ADP-0.5 mM ascorbate, instead of the Fe^{3+}-ADP-adriamycin complex (IAAC) in the above mixture. The reaction was initiated by the addition of ascorbate. All incubation experiments were carried out at 37°C.

2.3. *Assay*

The lipid peroxidation was monitored mainly by

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oxygen consumption [2] and in some cases by the change in fatty acid composition [2]. The values obtained were compared with and corrected for those from adequate controls.

3. RESULTS

IAAC in Tris-HCl buffer, which is known to be converted to Fe^{2+}-ADP-adriamycin by self reduction during the incubation [2,3], consumed O_2, but to a lesser extent. The exposure of unsaturated phospholipid liposomes to IAAC caused a strong O_2 consumption, which increased linearly with increasing time after a short lag time. Estrone or estriol in the liposomes inhibited the O_2 consumption seen in the IAAC induced lipid peroxidation system. Testosterone, which has no phenolic structure in the molecule, had no inhibitory effect. However, the mode of inhibition produced by estrone or estriol is strikingly different from that displayed by a reference compound α-tocopherol in that the latter has a long lag time before reaching the maximal rate of O_2 consumption. These results are shown in fig.1.

To prove the ability of each steroid or α-tocopherol to inhibit the IAAC-induced lipid peroxidation, each compound at a variety of concentrations was incorporated into unsaturated phospholipid liposomes and exposed to IAAC for measuring O_2 consumption. An adequate control was also taken. Thus the inhibition by the compound could be expressed, in terms of %, according to the following equation:

$$\% \text{ of inhibition} = \frac{A - C}{A - B} \times 100$$

where A, B and C are the maximal O_2 consumption in the liposome-IAAC, IAAC and liposome-inhibitor-IAAC systems, respectively. Fig.2 shows the relationship between % of inhibition and concentration of a compound. From the curves in the figure, the concentrations of estradiol and estriol required to achieve 50% inhibition could be calculated to be 4-times and 6-times that of α-tocopherol, respectively. Estrone, however, did not elevate the degree of inhibition at concentrations above 25 μM, suggesting limited incorporation of this steroid into liposomes under our experimental conditions. To ensure the inhibition of lipid peroxidation by estriol, the liposomes with or without this steroid were exposed to IAAC or Fe^{3+}-ADP-ascorbate for 30 min, followed by the analysis of fatty acids in the liposomes. The results obtained are shown in table 1, indicating that a peroxidative cleavage of polyunsaturated fatty

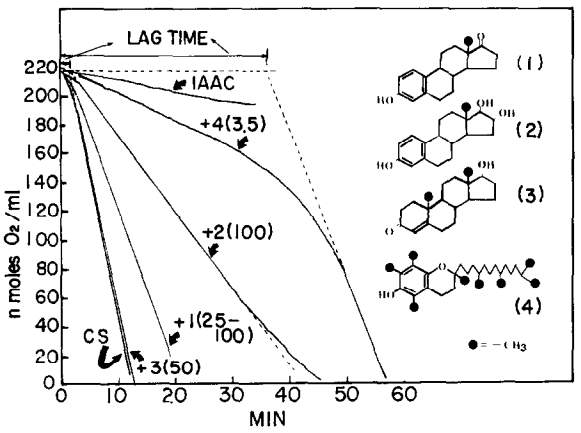

Fig.1. Oxygen consumption in IAAC (Fe^{3+}-ADP-adriamycin complex) system, CS (complete system containing liposomes and IAAC), or CS with the compounds 1–4. Number in parentheses represents the concentration of the compound, μM. Compounds were: 1, estrone; 2, estriol; 3, testosterone; 4, tocopherol.

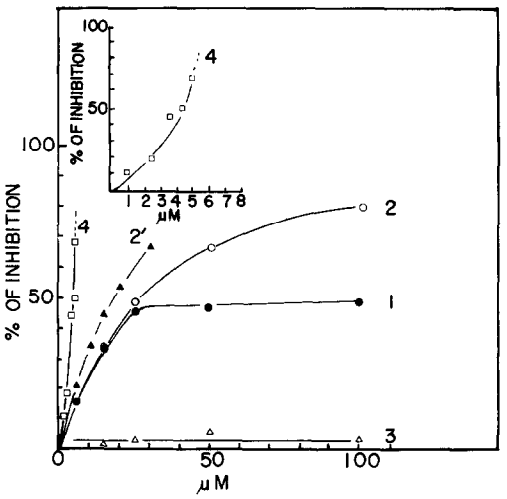

Fig.2. Relationship between % of inhibition and the concentration of the compound. Compounds were: 1, estrone; 2, estriol; 2', estradiol; 3, testosterone; 4, tocopherol.

Table 1

Fatty acid composition in phospholipids incubated with various components for 30 min

Incubation system	Fatty acid composition (mol/100 mol palmitic acid)					
	16:0	18:0	18:1	18:2	20:4	22:6
S1, basal [a])	100.0	85.7	37.6	54.2	101.3	30.9
S2, S1 + Fe^{3+}-ADP-adriamycin	100.0	84.5	37.9	48.4	65.9	16.1
S3, S2 + 100 μM estriol	100.0	88.7	40.2	51.3	90.9	26.8
S4, S1 + Fe^{3+}-ADP-ascorbate	100.0	89.7	39.1	50.5	86.3	24.1
S5, S4 + 100 μM estriol	100.0	85.4	39.4	54.9	102.1	30.5

[a]) Microsomal phospholipid liposomes (5.1 μmol lipid phosphorus) in 6 ml of 0.1 M Tris-HCl buffer (pH 7.5)

acids is significantly inhibited by estriol at the concentration used.

4. DISCUSSION

The concentration of total estrogens in peripheral blood is varied by the function of hormone-productive organs, but it is as low as about 1 μmol/l even at the last stage of pregnancy [4]. However, the hormones would be highly concentrated in cells of their target organs and hormone-productive organs. Furthermore estrone and estradiol in the cell membrane could be mainly metabolized to their catechol analogs [5], which have strong activities in inhibiting lipid peroxidation, identical to that of α-tocopherol [6]. In uterus, receptors of estrogens are known to be abundant in the nucleus [7] in which estrogens could be concentrated thereby protecting the nuclear membrane from peroxidative cleavage. The placenta at the last stage of pregnancy contains estrogens at 12.5 μmol/kg wet tissue [8], which would be enough to protect the cell membrane from phospholipid peroxidation under mild oxidative conditions.

Even though the mechanism for inhibiting lipid peroxidation by female hormones is unknown at present, estrogens may donate hydrogen atoms from their phenolic hydroxyl groups to lipid peroxyradicals for terminating chain reactions.

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