Comparative Study of Cardioprotective and Antiradical Activity of Estrogens and Their Nitro Derivatives

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Cardioprotective and antiradical activities of estrogens and their nitro derivatives are compared. Antiradical activity was observed in estradiol, ethanol estradiol, and estradiol nitrate, but not in nystranol, which exhibited antiradical properties only after acid hydrolysis. The data obtained on hearts from rats with experimental myocardial infarction show that estrogens and their nitro derivatives restrict the area of myocardial infarction due to antiischemic and/or antinecrotic activities.

Key Words: *antiradical activity; nitro estrogens; experimental myocardial infarction*

Considerable attention is now focused on clinical application of estrogens and their synthetic analogs for preventing and treating cardiovascular diseases not only in women, but also in men [2,6].

There is evidence that natural estrogen 17β-estradiol possesses a cardioprotective effect and restricts the area of myocardial infarction (MI) [5].

In this connection it is of interest to study modified synthetic estrogens carrying an ONO_2 group, potential donor of nitric oxide. These derivatives are nystranol and estradiol nitrate.

Chemical structure of nystranol and estradiol nitrate suggests that they could affect the cardiovascular system more efficiently than classical estrogens due to generation of bioactive NO group.

Natural estrogens have a pronounced antiradical activity [3]. There are no such data on the nitro estrogens. Bearing in mind the important role of lipid peroxidation in the pathogenesis of MI, examination of estrogen nitro derivatives for potential antiradical activity would have a practical resonance.

Our aim was to compare cardioprotective and antiradical activity of estrogens and their nitro derivatives and to reveal possible individual effects related to the presence of a nitro group.

MATERIALS AND METHODS

Experiments were carried out on outbred albino rats of both sexes weighing 200-300 g. Estradiol (17β-estradiol), ethanol estradiol, nystranol (9α-oxy-11β-nitroxyethynylestradiol diacetate), and estradiol nitrate (17 estradiol nitrate) were injected intraperitoneally in a dose of 10 mg/kg (in 20% ethanol) one hour before coronary occlusion. MI was provoked by ligating the descendant branch of the left coronary artery immediately below the auricula [7].

The effect of steroids on the size of experimental MI was assessed after 4-h coronary occlusion.

Estrogens and their nitro derivatives were tested for antiradical activity in the reaction of azo-bis-isobutyronitrile-induced oxidation of isopropylbenzene (cumol) as described elsewhere [9,10]. Test substance was added to cumol in a concentration of 5×10^{-3} M. Nystranol was hydrolyzed with 0.1 M HCl. Oxidation kinetics was assessed by oxygen consumption measured using a gasometric setup. To determine the rate constant of interaction of the inhibitor with cumol peroxide radicals (K_7), the kinetic curve of oxygen absorption was plotted in the semilogarithmic scale convert-

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ing it to a straight line. When measuring oxygen consumption (in ml), the rate constant for intaraction between the inhibitor and cumol radicals (l/M×sec) was calculated from the tangent of the plot slope:

$$K_7 = \frac{2.3 \times K_3 \times V}{tg\alpha \times 5.74 \times 10^{-3}},$$

where V is the volume of the reaction mixture (in ml) at 25°C; 5.74×10^{-3} is a conversion coefficient, which serves to obtain K_7 in liter/M×sec in the measurement oxygen consumption (in ml); K_3 is the rate constant of chain growth; K_7 is the rate constant of the reaction of inhibitor with cumol peroxide radicals.

The experimental value of K_7 describes the reaction capacity of any free radical inhibitor and serves as a qualitative characteristic of the antioxidant.

The results were statistically analyzed using Student's t test at $p \leq 0.05$

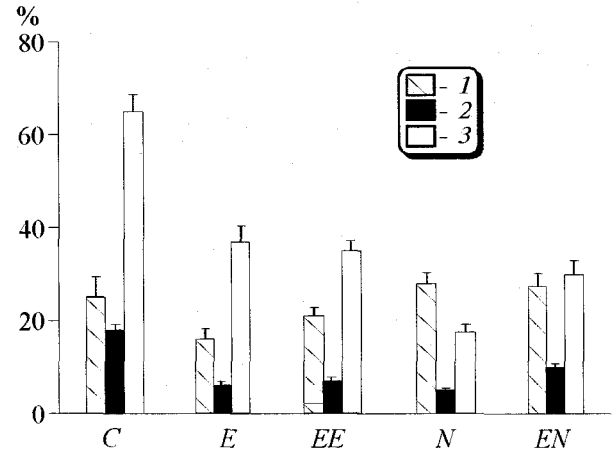

Fig. 1. Effect of estrogens and nitro estrogens on experimental myocardial infarction in rats. *C*: control; *E*: estradiol; *EE*: ethynyl estradiol; *N*: nystranol; *EN*: estradiol nitrate; *1*) ischemic zone, % of total weight; 2) necrotic zone, % of total weight; *3*) necrotic zone, % of ischemic zone. *$p<0.05$ compared to the control value.

RESULTS

The experiments showed that the antiradical activity is characteristic of only estrogens with phenol hydroxyl in the third position (17β-estradiol, ethynyl estradiol, and estradiol nitrate, Table 1). Nystranol exhibits the antiradical properties only after acid hydrolysis (Table 1).

Therefore, antiradical activity of nitro derivatives of estrogens depends on a mobile hydrogen atom in the third position as in natural estrogens [1,3]. Despite the absence of antiradical activity in the model reactions, nystranol could be a potential antioxidant *in vivo*, because metabolic transformation proceeds through hydrolysis of the ester bond with the formation of active substance. Therefore, nystranol could be a prodrug, but this hypothesis needs further testing on biological models.

There is evidence that incorporation of electroacceptor groups [4,8] (in particular, nitro group with its negative inductive and mesomeric effects) into antioxidant molecules significantly decreases the rate constant of reaction between inhibitor and peroxide radicals. Our findings suggest that the inhibition constants for estrogens and their nitro derivatives are similar (Table 1). The absence of the antiradical effect in nystranol is related not to the nitro group, but to acetate in the third position in ring A. This hypothesis is corroborated by the appearance of antiradical activity in nystranol molecule after acid hydrolysis and by the fact that the inhibition constant for nystranol is similar to that for other estrogens.

TABLE 1. Antiradical Activity of Estrogens and Their Nitro Derivatives Against Cumol Peroxide Radicals ($M \pm m$)

Substance	$K_7 \times 10^{-4}$, liter/mol×sec
Estradiol	2.0±0.1
Ethynyl estradiol	1.8±0.2
Nystranol	—
Nystranol after acid hydrolysis	1.9±0.1
Estradiol nitrate	1.8±0.1

Therefore, introduction of a nitro group into estrogen molecules does not reduce its antiradical activity. Presumably, this modification imparts an estrogen molecule with additional properties such as antiischemic effect characteristic of antianginal drugs nitroglycerin, nifedipine, *etc.* To test this hypothesis, we studied the effect of estrogens and their nitro derivatives on the size of experimental MI.

The data obtained on rats with experimental MI suggest that estrogens and their nitro derivatives restrict the area of MI due to antiischemic and/or antinecrotic effect (Fig. 1).

The estrogens (17β-estradiol and ethynyl estradiol) decreased the ischemic area to a greater degree than nitro estrogens such as nystranol and estradiol nitrate. Marked restriction of necrotic area and a decrease of the necrosis to ischemia ratio caused by nystranol attest to its greatest efficiency in restricting the size of experimental MI among the examined estrogens. These data open new prospects in the study of cardioprotective and antioxidant properties of nitro derivatives of natural estrogens.

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