Effects of the Antiandrogens Cyproterone Acetate and Niphtholid on Free Radical Oxidation in Modeled Biological Systems *In Vitro*

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The antioxidant activity of niphtholid is confirmed by inhibition of the slow flash of Fe^{2+}-induced chemiluminescence, reduced concentration of TBA-reactive products in egg yolk liposome suspension, and low amplitude of the fast flash in the samples containing microquantities of blood plasma in the presence of the drug. Cyproterone acetate has no effect on free-radical oxidation.

Key Words: *antiandrogens; antioxidant activity; chemiluminescence*

Antiandrogen drugs developed during the last two decades have been successfully used in the therapy of prostate cancer and hyperandrogynism in women and children (precocious puberty). It has been generally accepted that the therapeutic effect of these drugs is linked with competitive blockade of cytosolic androgen receptors [5]. Meanwhile, membranotropic effects of antiandrogens are poorly understood. Recent findings suggest that biological activity of other antihormone preparations, for example, antiestrogens, strongly depends on their antioxidant properties [6]. Experimental studies of the effect of antiandrogens on free radical oxidation (FRO) provide more insight into the molecular mechanisms underlying therapeutic effects of these agents, which is important for their clinical application.

Our objective was to evaluate antioxidant activity of cyproterone acetate and niphtholid *in vitro*.

MATERIALS AND METHODS

The steroid drug cyproterone acetate (Sigma) and non-steroid antiandrogen niphtholid (Institute of Endocrinology and Metabolism, Ukrainian Academy of Sciences, Kiev) were tested.

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Free radical oxidation was studied in a model biological system, containing a suspension of multilayer liposomes prepared from egg yolk lipoproteins as described elsewhere [2]. The intensity of free radical reactions was assayed by recording the kinetics of Fe^{2+}-induced chemiluminescence (CL) and by measuring the concentration of 2-thiobarbituric acid (TBA)-reactive products. A principal scheme for CL recording was described previously [4]. The following solutions were successively added to the measuring cell: 7.6 ml phosphate buffer (20 mM KH_2PO_4, 105 mM KCl, pH 7.4), 1 ml eosin (2.7 mM), 0.3 ml egg yolk lipoprotein suspension, and 0.1 ml ethanol solution of cyproterone acetate or niphtholid (final concentration 10^{-3}-10^{-6} M). After a 2-min incubation with constant stirring, 1 ml $FeSO_4 \times 7H_2O$ (20 mM) was added, and the kinetics of CL was recorded. In order to evaluate antioxidant activities of the agents, the amplitude of the fast CL flash and tangent of the slope of the exponential stage of the slow flash were calculated.

The concentration of TBA-reactive products was measured, and antioxidant activity in the model system containing multilayer yolk liposomes was calculated as described previously [2].

In parallel, the effect of antiandrogens on the intensity of rapid flash was studied in a model system

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TABLE 1. Effects of Niphtholid and Cyproterone Acetate on Free Radical Oxidation in Multilayer Liposomes from Egg Yolk Lipoproteins

Concentration, M	Amplitude of fast flash, % of control	Rate of slow flash rise, % of control	Concentration of TBA-reactive products, % of control
Niphtholid			
10^{-6}	93.8±6.3	114.4±13.9	96.1±3.8
10^{-5}	103.3±7.8	113.6±13.5	93.3±2.9
10^{-4}	108.4±9.5	73.6±6.0*	84.5±5.7*
5×10^{-4}	90.6±15.1	35.9±7.2*	46.6±3.9*
10^{-3}	87.8±10.6	12.6±2.2*	37.8±2.1*
Cyproterone acetate			
10^{-6}	94.4±5.4	87.4±8.7	106.4±1.7
10^{-5}	96.7±5.1	91.2±7.9	97.7±4.5
10^{-4}	98.1±8.3	102.9±3.9	89.8±6.5*
10^{-3}	103.2±6.1	96.6±6.6	79.3±2.4*

Note. Here and in Table 2: *$p<0.05$ compared with control.

containing microquantities (50 μl) of plasma from male albino rats. The procedure for recording this parameter was described in detail [4].

Standard statistical tests were employed. The significance of differences was evaluated using the non-parametric Wilcoxon—Mann—Whitney *U* test.

RESULTS

Niphtholid (10^{-5}-10^{-6} M) had no effect on FRO in a model biological system containing multilayer egg yolk liposomes. At higher concentrations it exhibited antioxidant activity, as evidenced by a decrease in the content of TBA-reactive products and in the rate of the slow flash increase (Table 1). Niphtholid produced the maximum effect at millimolar concentrations: the content of TBA-reactive products dropped 2.6-fold, and the slope tangent 7.9-fold, compared with the control. Antioxidant activity, calculated by the standard method ($1.71\pm0.22\times10^3$ M^{-1}), indicates that this drug has a moderate antioxidant activity. At the same time, none of the tested concentrations of niphtholid (10^{-3}-10^{-6} M) affected the intensity of fast flash of Fe^{2+}-induced CL, which depended on the content of lipoperoxides in the sample.

In contrast to niphtholid, cyproterone acetate did not change FRO in the model system. This agent slightly inhibited generation of TBA-reactive products (by 10-20% in the concentration range of 10^{-3}-10^{-4} M, Table 1), but this effect was not sufficient for quantitative evaluation of antioxidant activity. The intensity of rapid flash and the slope tangent did not change in comparison with the control throughout the entire range of concentrations.

The effects of antiandrogens on the intensity of the fast flash of Fe^{2+}-induced CL in a model system containing microquantities of plasma are consistent with the above-mentioned data, suggesting that FRO can be inhibited only by high concentrations of niphtholid (5×10^{-4}-10^{-3} M). The intensity of fast flash decreases by approximately 15% compared with the control (Table 2). It should be noted that niphtholid was inferior to the conventional inhibitors of FRO (mexidol, probucol, etc.), which under the same conditions reduced the amplitude of fast flash by 20-25% [4].

As expected, cyproterone acetate had no effect on the intensity of fast flash in the studied concentration range, implying that this drug has no antioxidant activity.

Inhibition of the slow flash of Fe^{2+}-induced CL at a constant intensity of the fast flash in a model system containing multilayer liposomes prepared from egg yolk lipoproteins indicates that this agent can scavenge free radicals. This may be due to the presence of the anilide group in the benzene ring. As follows from the theory of CL technique and pre-

TABLE 2. Effect of Niphtholid and Cyproterone Acetate on the Intensity of Fast Flash of Fe^{2+}-Induced Chemiluminescence of Blood Plasma

Concentration, M	Intensity of fast flash, % of control	
	niphtholid	cyproterone acetate
10^{-6}	99.9±5.5	104.2±5.8
10^{-5}	95.7±5.9	91.7±5.3
10^{-4}	105.6±4.5	95.9±9.1
5×10^{-4}	86.8±4.2*	-
10^{-3}	84.7±5.0*	104.3±4.6

vious experiments, the intensity of fast flash in a plasma-containing model system is an integral characteristic strongly dependent on the presence of antioxidants: scavengers of free radicals and active oxygen species, complexons for transition metals, etc. Since niphtholid in millimolar concentrations lowers the amplitude of fast flash in this model, it can be hypothesized that its antioxidant activity is mediated by another mechanism.

The finding that these antiandrogens differ considerably in antioxidant activity is important for their use in clinical practice. Recent investigations showed that antiandrogens are useful in the treatment of oncologic diseases accompanied by hormonal disturbances, for example, bone sarcoma [3]. In this case, antiandrogens should be tested for the compatibility with conventional chemotherapeutic agents, since some of them act via the initiation of FRO [1].

It has been generally recognized that antioxidant preparations increase the organism's resistance to various damaging factors; therefore, niphtholid may have a wide therapeutic application. However, further experimental and clinical studies are necessary to clarify this issue.

REFERENCES

1. E. B. Burlakova and N. P. Pal'mina, *Vopr. Onkol.*, **36**, No. 10, 1155-1162 (1990).
2. G. I. Klebanov, I. V. Babenkova, Yu. O. Teselkin, *et al.*, *Lab. Delo.*, No. 5, 59-62 (1988).
3. N. E. Kushlinskii and V. N. Mitin, *Vopr. Onkol.*, **38**, No. 5, 571-576 (1992).
4. P. V. Sergeev, A. G. Belykh, S. A. Chukaev, and B. M. Gukasov, *Eksp. Klin. Farmakol.*, **55**, No. 2, 60-62 (1992).
5. M. Breckwoldt (Ed.), *Diagnosis and Therapy of Androgenisation*, Berlin (1992).
6. H. Wiseman, *Trends Pharmacol. Sci.*, **15**, No. 3, 83-89 (1994).

Analgesic Action of the New Drug Semax

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Intranasal administration of the new regulatory peptide Semax (0.5 mg/kg) relieves migraine headache and pain caused by dental plexalgia. The analgesic effect of Semax is due to spasmolytic and general regulatory activities but not to its influence on pain sensitivity system.

Key Words: *pain; rheoencephalogram; adrenocorticotropic hormone fragments*

Considerable investigative effort has been focused on the development of drugs based on endogenous regulatory peptides or their synthetic analogs. The interest in peptide drugs is motivated by the following reasons. First, peptides are effective in very low doses. Second, enzyme systems that can rapidly inactivate peptides are present in the body. Third, peptides produce no significant side effects. There are a number of immunostimulating, nootropic, analgesic, and other drugs based on regulatory peptides. Dalargin, oxytocin, vasopressin, and thymogen have been successfully used in this country. Clinical trials of the analog of the adrenocorticotropic hormone fragment 4-10 have been started. This heptapeptide (O_2 MEHF-PGP, Semax) was developed at the Institute of Molecular Genetics (Russian Academy of Sciences). Semax exerts prolonged (up to 24 h) beneficial effects on the function of the forebrain: it improves attention and accelerates learning [1,4].

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