PHARMACOLOGY AND TOXICOLOGY

Combined Action of Doxorubicin and Gestagens on Doxorubicin-Sensitive and Doxorubicin-Resistant MCF-7 Cells

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The combined cytostatic effect of doxorubicin and gestagens progesterone, medroxyprogesterone acetate, mecigestone, and butagest on doxorubicin-resistant and doxorubicin-sensitive human breast cancer MCF-7 cells was studied by the MTT assay. On the 6th day of incubation progesterone, medroxyprogesterone acetate, mecigestone, and butagest in high concentrations (10^{-5} M) potentiated the cytostatic action of doxorubicin in sensitive and resistant cells by 30-50%. Potentiation of the cytostatic effect produced by doxorubicin in sensitive cells is related to intrinsic cytotoxic activity of gestagens. In resistant cells these changes are associated with potentiation of the effect of doxorubicin.

Key Words: *multidrug resistance; P-glycoprotein; gestagens; doxorubicin; MCF-7*

Highly efficient and nontoxic chemosensitizers increasing sensitivity of tumor cells to chemotherapy are required for preventing the development of multidrug resistance (MDR). Published data show that gestagens hold much promise in this respect [1]. Steroid hormones (gestagens) are physiological substrates for P-glycoprotein. This major MDR protein pumps cytostatics from cells against the concentration gradient [10]. Gestagens in high concentrations inactivate P-glycoprotein and inhibit its expression of [5,7], which probably contributes to their chemosensitizing effect on resistant tumor cells [1].

Here we studied the effects of gestagens progesterone, medroxyprogesterone acetate (MPA), mecigestone, and butagest administered alone or in combination with doxorubicin on doxorubicin-resistant and doxorubicin-sensitive MCF-7 cells.

MATERIALS AND METHODS

MCF-7 cells sensitive (MCF-7/WT) and resistant to doxorubicin (MCF-7/R) were obtained from the collection of cells of the All-Russia Research Center for Molecular Diagnostics and Therapy. The cells were cultured in a LB-V laminar box on DMSI medium (Gibko) containing 10% inactivated fetal bovine serum and antibiotics gentamicin sulfate and streptomycin sulfate in a concentration of 40 mg/ml at 37°C and 5% CO_2 under sterile conditions [6].

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Cell viability was determined by MTT test [9]. The sensitivity of cells to the cytotoxic effect of doxorubicin was estimated by IC_{50} (doxorubicin concentration causing death of 50% cells after 4-6-day incubation). Transport function of P-glycoprotein was evaluated by the release of fluorescent dye rhodamine 123 from cells (P-glycoprotein substrate) [11].

The following gestagens were used: analytic mecigestone (6α-methyl-16α,17α-cyclohexanoprogesterone, Laboratory for Chemistry of Steroids and Oxylipins, N. D. Zelinskii Institute of Organic Chemistry); butagest (Center for Chemistry of Medicinal Preparations, All-Russia Chemical and Pharmaceutical Institute), MPA (Sigma), and progesterone (Sigma) in DMSO; and doxorubicin (Lens-farma) in DMSI medium.

The results were analyzed by Student's *t* test.

RESULTS

Chemosensitizing activity of gestagens was determined after incubation of MCF-7/R and MCF-7/WT cells with doxorubicin for 1, 2, 3, 4, and 6 days. We compared the cytostatic effects observed after culturing with doxorubicin alone or in combination with gestagens. MCF-7/R and MCF-7/WT cells were incubated with doxorubicin (5×10^{-6} and 10^{-7} M, respectively), progesterone, MPA, mecigestone, and butagest (10^{-5} and 10^{-6} M).

On days 1 and 2 of incubation gestagens did not modulate the cytostatic effect of doxorubicin on MCF-7/R and MCF-7/WT cells. These results were obtained after simultaneous and consecutive addition of preparations (1-day preincubation with gestagens). On day 3 gestagens slightly (by 15%) potentiated the cytostatic effect of doxorubicin on MCF-7/R cells. After 4-day incubation gestagens significantly potentiated the cytostatic effect of doxorubicin. Mecigestone, butagest, progesterone, and MPA in a concentration of 10^{-5} M increased cytotoxic activity of doxorubicin by 40, 36, 36, and 32%, respectively ($p<0.01$). In a concentration of 10^{-5} M progesterone and mecigestone had no effect, while butagest and MPA potentiated the cytostatic effect of doxorubicin by 14.5 and 20%, respectively ($p<0.01$).

On day 6 of incubation mecigestone, butagest, and MPA more significantly potentiated the cytostatic effect of doxorubicin compared to day 4. However, activity of progesterone decreased in this period. In a concentration of 10^{-5} M mecigestone, butagest, progesterone, and MPA increased cytotoxic activity of doxorubicin by 54, 41, 24, and 52%, respectively (Fig. 1). The number of dead cells was similar to that observed after incubation with a 10-fold concentration of doxorubicin (5×10^{-5} M). In a concentration of 10^{-6} M butagest, progesterone, and mecigestone did not modulate, while MPA suppressed the cytotoxic effect of doxorubicin ($p<0.01$). The ability of gestagens in a concentration of 10^{-5} M to potentiate the cytotoxic effect of doxorubicin after 4-day incubation decreased in the following order: mecigestone>butagest=progesterone>MPA. On day 6 of incubation with gestagens in a concentration of 10^{-5} M this series looked as follows: mecigestone>MPA>butagest>progesterone.

Gestagens potentiated the cytotoxic effect of doxorubicin on MCF-7/WT cells on day 6 of incubation. Under these conditions gestagens produced a dose-depended effect (similarly to experiments with MCF-7/R cells). In a concentration of 10^{-5} M mecigestone, butagest, progesterone, and MPA increased cytotoxic activity of doxorubicin by 52, 30, 40, and 47%, respectively (Fig. 1). After incubation with gestagens in a concentration of 10^{-6} M the cytotoxic effect of doxorubicin increased by 29, 31, 24, and 43%, respectively (Fig. 2). The ability of gestagens in a concentration of 10^{-5} M to potentiate the cytotoxic effect of doxorubicin decreased in the following order: mecigestone>MPA>butagest>progesterone. After incubation with gestagens in a concentration of 10^{-6} M their efficiency decreased as follows: MPA>butagest>mecigestone>progesterone.

Gestagen-mediated potentiation of the cytotoxic effect of doxorubicin was different in resistant and sensitive MCF-7 cells. Progesterone in a concentration of 10^{-5} M was least efficient, while mecigestone most significantly potentiated the cytotoxic influence of doxorubicin on cells of both types.

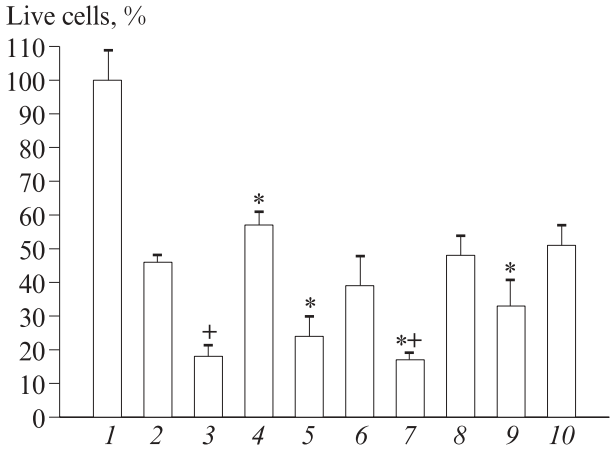

Fig. 1. Effect of combination treatment with gestagens and doxorubicin on viability of MCF-7/R cells during 6-day incubation: control (*1*); doxorubicin (5×10^{-6} M, *2*); medroxyprogesterone acetate (MPA, 10^{-5} M) and doxorubicin (*3*); MPA (10^{-6} M) and doxorubicin (*4*); butagest (10^{-5} M) and doxorubicin (*5*); butagest (10^{-6} M) and doxorubicin (*6*); mecigestone (10^{-5} M) and doxorubicin (*7*); mecigestone (10^{-6} M) and doxorubicin (*8*); progesterone (10^{-5} M) and doxorubicin (*9*); progesterone (10^{-6} M) and doxorubicin (*10*). $p<0.01$: *compared to doxorubicin (*2*); +compared to progesterone (*9*).

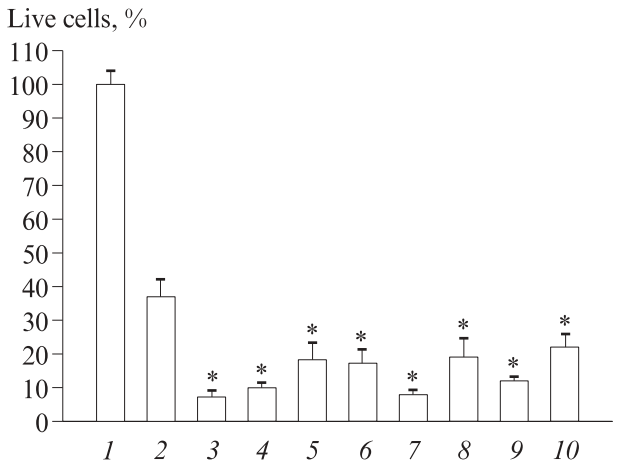

Fig. 2. Effect of combination treatment with gestagens and doxorubicin on viability of MCF-7/WT cells during 6-day incubation: control (*1*); doxorubicin (10^{-7} M, *2*); MPA (10^{-5} M) and doxorubicin (*3*); MPA (10^{-6} M) and doxorubicin (*4*); butagest (10^{-5} M) and doxorubicin (*5*); butagest (10^{-6} M) and doxorubicin (*6*); mecigestone (10^{-5} M) and doxorubicin (*7*); mecigestone (10^{-6} M) and doxorubicin (*8*); progesterone (10^{-5} M) and doxorubicin (*9*); progesterone (10^{-6} M) and doxorubicin (*10*). *$p<0.01$ compared to doxorubicin.

The cytotoxic effect of gestagens in MCF-7/WT cells was more pronounced than in MCF-7/R cells. Cytotoxic activity of doxorubicin in these cultures increased to a similar degree after incubation with gestagens in a concentration of 10^{-5} M (Table 1). The increase in cytotoxic activity of doxorubicin in MCF-7/WT cells was probably related to the cytotoxic effect of gestagens. Changes in these cells produced by gestagens were similar to those observed after combination treatment with gestagens and doxorubicin. It should be emphasized that gestagens potentiated the cytotoxic influence of doxorubicin on MCF-7/R cells by another mechanism.

Doxorubicin-resistant MCF-7 tumor cells are low differentiated and do not carry receptors for estrogens and gestagens [4]. Cytotoxic activity of gestagens and potentiation of the effect produced by doxorubicin in MCF-7/R cells are probably realized via the MDR system, but not via receptors [1]. Gestagens suppress expression of P-glycoprotein on day 3 of incubation, which is related to the presence of a progesterone-sensitive element in the MDR-1 gene [8]. It can be hypothesized that gestagens are responsible for the substrate inhibition of P-glycoprotein activity. Doxorubicin is not pumped from the cell and produces the cytotoxic effect. Gestagens and doxorubicin are similarly efficient in MCF-7/WT cells expressing low amounts of P-glycoprotein. We observed summation of the effects produced by gestagens and doxorubicin in various concentrations. In MCF-7/R cells gestagens themselves were low efficient, but potentiated the cytotoxic influence of doxorubicin.

The hypothesis that gestagens modulate the transport of doxorubicin in P-glycoprotein-expressing cells is confirmed by direct measurements of P-glycoprotein activity with P-glycoprotein-specific fluorescent dye rhodamine 123. Gestagens decreased P-glycoprotein activity by 28-50%.

Our results indicate that new gestagen preparations should be subjected to clinical trials as antitumor drugs and compounds potentiating the antitumor effect of cytostatics. Cytostatic activity of butagest compares well with MPA. Mecigestone is most effective in this respect. Clinical trials showed that chemosensitizers in effective concentrations cause various side effects. For example, cyclosporine and verapamil possess neurotoxicity and cardiotoxicity, respectively. Gestagens differ from these preparations in low toxicity (even in

TABLE 1. Cytostatic Effect of Gestagens Administered Alone or in Combination with Doxorubicin during 6-Day Incubation with MCF-7/WT and MCF-7/R Cells (Inhibition of Cell Growth, %)

Preparation		MCF-7/WT		MCF-7/R	
		10^{-5} M	10^{-6} M	10^{-5} M	10^{-6} M
Doxorubicin		100	100	100	20
Progesterone		90.1	39.6	33.0	4.0
	+doxorubicin	88*	78*	67*	49**
Butagest		83.6	77.0	49.0	39.0
	+doxorubicin	81.7*	82.8*	76.0**	61.0**
MPA		93.1	85.0	44.0	36.0
	+doxorubicin	92.8*	90.1*	82.0**	43.0**
Mecigestone		96.7	95.2	92.0	36.0
	+doxorubicin	96.1*	81.0*	83.0**	52.0**

Note. Combination treatment with doxorubicin in concentrations of 10^{-7} (*) and 5×10^{-6} M (**) inhibiting cell growth by 63 and 54%, respectively.

high concentrations, 10^{-3} M). They are used in oncological practice as palliative or additional medicines [2]. Moreover, gestagens possess antiproliferative activity [3]. The chemosensitizing effect of new gestagens mecigestone and butagest on cells sensitive and resistant to doxorubicin allows decreasing the dose of this cytostatic during the therapy of hormone-dependent and independent tumors.

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