6α-Methyl-16α,17α-Cyclohexane Progesterone and Progesterone Inhibit Growth of Doxorubicin-Sensitive MCF-7 and HeLa Tumor Cells

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Temporal and concentration dependencies of the effects of gestagens (6α-methylpentarane and progesterone) and cytostatic doxorubicin on proliferation of MCF-7 and HeLa tumor cells was studied using ^{3}H-thymidine test. Gestagens produced the maximum inhibitory effect of on cell proliferation in a concentration of 10^{-5} M; the effect developed on day 6 of incubation. 6α-Methylpentarane in a concentration of 10^{-8} inhibited proliferation of HeLa cells more effectively than progesterone ($p<0.05$). In experiments with combined treatment of doxorubicin-sensitive MCF-7 and HeLa cells, progesterone in a concentration of 10^{-7} M attenuated the cytostatic effect of doxorubicin ($p<0.05$), while 6α-methylpentarane in the studied concentrations did not modulate it.

Key Words: *proliferation; gestagens; doxorubicin; MCF-7; HeLa*

The search for more effective methods for drug therapy of hormone-dependent breast tumors is still a pressing problems of modern oncology. Cytostatic antibiotic doxorubicin is prescribed as the first-line drug for the therapy of breast cancer [3,11], but the desired results are not always attained because of resistance of tumor cells. Drugs improving the efficiency of cytostatic treatment are needed. Combinations of cytostatics with gestagens or antiestrogens seem to be promising in this respect, because the latter agents can prolong patient's life and improve its quality [4]. According to published reports, antitumor activity of hormonal drugs, specifically gestagens, in well-differentiated tumors can be due to suppression of estrogen receptors [8,13] and in poorly differentiated and resistant tumors due to chemical sensitization, *i.e.* sensitization of cells to cytostatics [5,6,10,14].

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6α-Methylpentarane is a representative of a new class of progesterone analogs pregna-D'-pentaranes; it is characterized by high progestagen activity *in vivo* [1], but its effects on hormone-dependent tumors cell, both in monotherapy and in combinations with cytostatics, remain not studied.

We investigated the effect of 6α-methylpentarane on the proliferation of doxorubicin-sensitive MCF-7 and HeLa cells expressing progesterone and estradiol receptors [6,9], elucidated whether 6α-methylpentarane and progesterone exhibit antiproliferative activity in breast cancer and cervical carcinoma cells (MCF-7 and HeLa, respectively), studied the concentration and temporal relationships of the effects of progesterone and 6α-methylpentarane on MCF-7 and HeLa cell cultures, and compared the antiproliferative effect of doxorubicin used as monotherapy and in combination with gestagens.

MATERIALS AND METHODS

Human tumor cell cultures HeLa (human cervical carcinoma) and MCF-7 (human breast cancer) were cul-

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tured under sterile conditions using a laminarbox (LB-B) in standard media 199 and RPMI-1640 supplemented with 10-20% heat-inactivated fetal calf serum, L-glutamine (100 μg/ml), gentamicin sulfate and streptomycin sulfate (40 μg/ml). The serum contained 0.6×10.0^{-10} M estradiol, which gave grounds to consider cell growth as estradiol-stimulated [13]. 6α-Methyl-16α,17α-cyclohexane progesterone (synthesized at Laboratory of Steroid and Oxylipin Chemistry, N. D. Zelinskii Institute of Organic Chemistry), progesterone (Sigma, in 96% ethanol), and doxorubicin (Lance-Pharm) were used.

Antiproliferative activity of the studied compounds was studied by the ^{3}H-thymidine incorporation [2]. Suspension of tumor cells (200 μl) was transferred to a 96-well flat-bottomed plate (Costar). Cell concentration (5×10^5/ml) was measured in a Goryaev chamber. Test compounds were dissolved in RPMI to concentrations of 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M (from 10^{-2} M stock solution in DMSO) and added to cells (10 μl/well). Control wells were incubated with 10 μl RPMI. Each test was repeated 3 times. Cell adhesion was controlled after 24 h under a light microscope. After 5 days ^{3}H-thymidine (10 μl, 1 μCi/ml) was added to each well for 24 h. After removal of ^{3}H-thymidine-containing medium the cells were treated with 100 μl 0.25% crude trypsin for 30 min in a thermostat. The suspension (50 μl) was transferred to 2×2 cm Whatman filters, the filters were washed in trichloroacetic acid, dried, put into vials with 5 ml scintillation fluid, and radioactivity was measured in an SL-30 liquid scintillation counter (Intertechnique).

The results are presented as $M\pm m$. The significance of differences was evaluated using Wilcoxon rank test for a significance level $\alpha=5\%$.

RESULTS

During the first stage of the study we investigated the concentration and themporal dependencies of the effects of progesterone and 6α-methylpentarane on proliferation of MCF-7 and HeLa cells (Fig. 1). Gestagens had no appreciable effects on cell proliferation within the first 4 days of incubation, while doxorubicin produced a pronounced antiproliferative effect as early as after 24-h incubation. Pronounced antiproliferative effect of the studied gestagens was observed only after 6-day incubation of cells with progesterone and 6α-methylpentarane in the maximum concentration (10^{-5} M) (Fig. 1). The effect of the new gestagen 6α-methylpentarane on MCF-7 cell culture was observed on day 4 of incubation. On day 6 of incubation 6α-methylpentarane inhibited cell growth in both cultures more effectively than progesterone.

The effects of progesterone and 6α-methylpentarane directly depended on their concentrations. The maximum inhibitory effects of 6α-methylpentarane and progesterone on proliferation of MCF-7 cells was attained at a concentration of 10^{-5} M (65 and 54% inhibition, respectively; Fig. 2, *a*). The drugs produced a similar inhibitory effect on HeLa cell culture, but 6α-methylpentarane in a concentration of 10^{-8} M was more potent inhibitor than progesterone ($p<0.05$; Fig. 2, *b*). The inhibitory effect of the test compounds on HeLa culture peaked at a concentration of 10^{-5} M for both progesterone (52% inhibition) and 6α-methylpentarane (77% inhibition). Progesterone in a concentration of 10^{-8} M stimulated proliferation of HeLa cells by 10% ($p<0.05$), while 6α-methylpentarane inhibited proliferation in all studied concentrations.

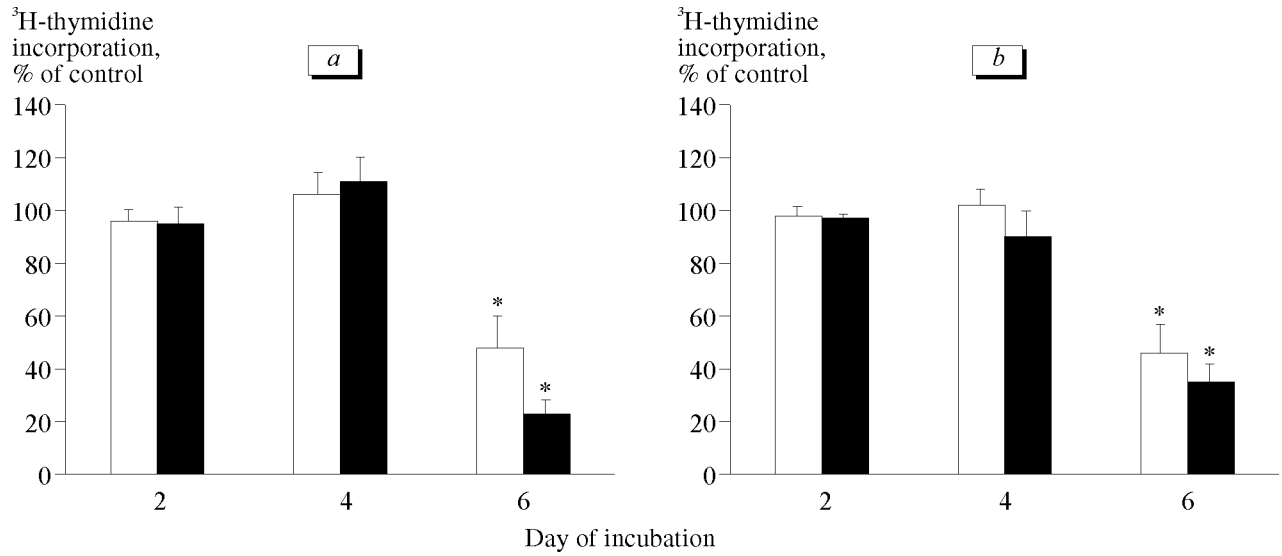

Fig. 1. Effects of progesterone (open bars) and 6α-methylpentarane (dark bars) in a concentration of 10^{-5} M on proliferation of HeLa (*a*) and MCF-7 (*b*) cells. Here and in Figs. 2 and 3: *$p<0.05$ compared to the control.

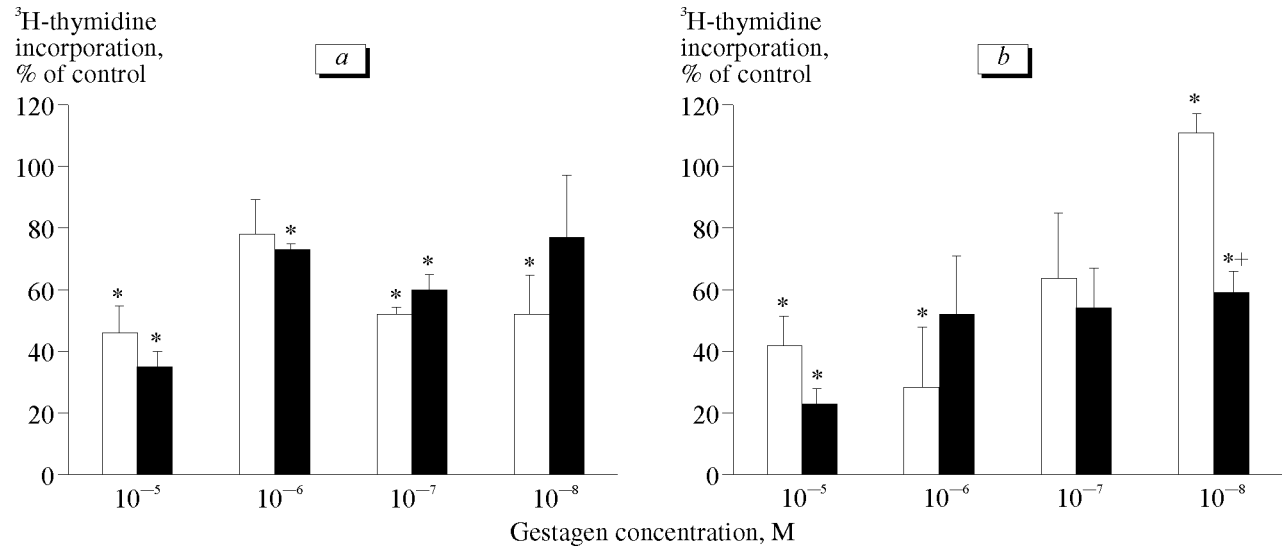

Fig. 2. Effects of progesterone (open bars) and 6α-methylpentarane (dark bars) on proliferation of MCF (*a*) and HeLa (*b*) cells after 6-day incubation.

These data can be explained if we regard proliferation of the studied cell as estradiol-stimulated growth. From this viewpoint antiproliferative effects of the test gestagens were presumably due to their antiestrogen activity. Antiestrogen activity of gestagens is determined by many factors; the most important mechanisms are stimulation of 17β-hydroxysteroid dehydrogenase and estrone sulfotransferase (enzymes oxidizing estradiol into estrone and then converting it into inert estrone-sulfate) and down-regulation of estrogen receptors. Another possible explanation is stimulation of apoptosis by gestagens [7].

The cytostatic effect of doxorubicin alone on MCF-7 cells reached the maximum in a concentration of 10^{-5} M (Fig. 3). In combination with progesterone the cytostatic effect of doxorubicin decreased by 24% (63% inhibition, 10^{-7} M), while in combination with 6α-methylpentarane the effect of doxorubicin did not decrease significantly ($p<0.05$, 10^{-7} M; Fig. 3). The results in experiments with HeLa culture were virtually the same as with MCF-7 culture (data not presented).

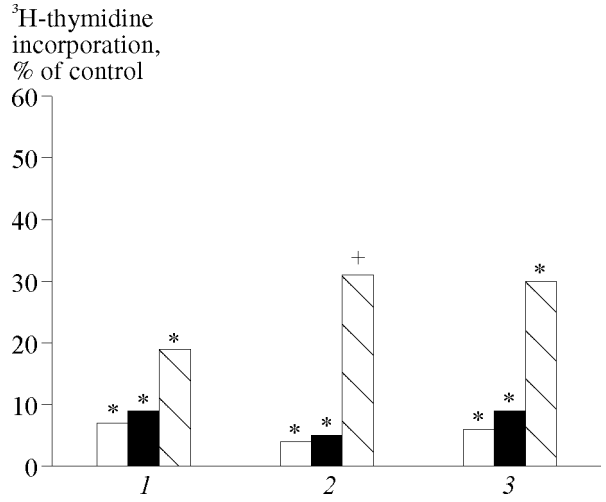

Fig. 3. Effects of gestagens in combination with doxorubicin (gestagen:doxorubicine ratio 1:1) on MCF-7 cell proliferation after 6-day incubation. *1*) doxorubicin; *2*) doxorubicin+progesterone; *3*) doxorubicin+6α-methylpentarane. Drug concentrations, M: open bars: 10^{-5}; dark bars: 10^{-6}; cross-hatched bars: 10^{-7}. *$p<0.05$ compared to the effect of doxorubicin.

Hence, progesterone and 6α-methylpentarane inhibited proliferation of MCF-7 and HeLa cells, but did not potentiate (and 6α-methylpentarane even decreased) the cytostatic effect of doxorubicin in combined treatment.

The absence of potentiation of the cytostatic effect of doxorubicin in the presence of gestagens can be explained by different mechanisms of their antiproliferative effects. Doxorubicin exhibits a direct genotropic effect via intercalation in DNA molecule and induction of breaks, while gestagens induce cell transition from proliferation to differentiation states by changing their sensitivity to growth factors. Inefficiency of doxorubicin combination with gestagens in our study can be due to the fact that both cultures are sensitive to doxorubicin. A negligible decrease of doxorubicin effect in the presence of progesterone is in line with the results of J. Claudio *et al.* [6] demonstrated on MCF-7 cells with medroxyprogesterone acetate for gestagen. This gestagen did not potentiate the cytostatic effect of doxorubicin in MCF-7 culture sensitive to doxorubicin, but synergetic effects of medroxyprogesterone acetate and doxorubicin was observed in MCF-7 culture resistant to doxorubicin. Similar data were obtained by J. Li *et al.* [10] also on MCF-7 culture with nomegestrol for gestagen.

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