

SHORT COMMUNICATIONS

EFFECT OF PROGESTERONE AND 17 β -ESTRADIOL ON THE CYTOTOXICITY MEDIATED BY TUMOR NECROSIS FACTOR

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ВЛИЯНИЕ ПРОГЕСТЕРОНА И 17 β -ЭСТРАДИОЛА НА ЦИТОТОКСИЧНОСТЬ, ОПОСРЕДОВАННУЮ ФАКТОРОМ НЕКРОЗА ОПУХОЛЕЙ

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The female steroid hormones are known to modulate release and activity of cytokines. The goal of this study was to investigate the effect of progesterone and 17 β -estradiol on the TNF-mediated cytotoxic activity in L929 cells. Our data have indicated that progesterone and 17 β -estradiol protected L929 cells from TNF-mediated cytotoxicity independently from receptor-mediated mechanism and in dose-dependent manner. Irrespective to the duration of treatment progesterone had stronger protective effect compared to 17 β -estradiol. Also, it was shown that progesterone or 17 β -estradiol inhibited the survival of L929 cells.

Key Words: tumor necrosis factor, cytotoxicity, progesterone, 17 β -estradiol, L929 cells.

Известно, что прогестерон и 17 β -эстрадиол могут модулировать продукцию и активность цитокинов. Нами было изучено влияние прогестерона и 17 β -эстрадиола на цитотоксическую активность фактора некроза опухолей (ФНО) против клеток L929. Показано, что указанные стероидные гормоны в дозозависимом режиме ингибировали цитотоксическую активность ФНО; при этом прогестерон оказывал более сильный ингибирующий эффект, чем 17 β -эстрадиол. В то же время прогестерон и 17 β -эстрадиол снижали жизнеспособность L929 клеток.

Ключевые слова: фактор некроза опухолей, цитотоксичность, прогестерон, 17 β -эстрадиол, клетки L929.

TNF- α is a multifunctional cytokine that has been implicated in diverse physiologic and pathophysiologic events including inflammation, cellular survival, growth, differentiation, induction of apoptosis and necrosis of multiple cell lines and tumor types [1, 7, 11]. It has been shown that TNF may have both tumor-necrotic and tumor-promoting activity [10]. This cytokine have been implicated in the growth and survival of certain tumors such as myeloma, B-cell lymphomas, cutaneous T-cell lymphomas [5], glioblastoma, ovarian sarcoma, melanoma, breast carcinoma, B-cell chronic lymphocytic leukemia [1] and other.

Both female sex steroids, progesterone and estrogen, are known to be involved in the proliferative changes in the reproductive organs that occur during the menstrual cycle, pregnancy and lactation. Many studies have shown direct effects of these hormones on the growth and metastasis of breast cancer and endometrium/miometrium tumors. Progesterone has suppressive effects on the proliferation and development of hormone-dependent tumors, while 17 β -estradiol stimulated growth of the same tumors [2, 8, 13].

It is known that progesterone and 17 β -estradiol can modulate activity of immune cells, including production of cytokines. Sex steroid hormones dose-dependently modulated release of TNF from LPS-treated macrophages [3, 9, 14], and influenced the function of these cells [4, 6].

The aim of this study was to investigate effect of progesterone and 17 β -estradiol on the TNF-mediated cytotoxic activity *in vitro*.

The mouse fibroblast cell line L929 was obtained from cell culture collection of Institute of Cytology, Academy of Sciences of Russian Federation (St.-Peterburg, Russia). The cells were grown in phenol red free RPMI 1640 medium (Sigma, USA) supplemented with 5% dialyzed fetal bovine serum (BioMark Inc, Ukraine) (levels of progesterone and estrogen were < 1 ng/ml) and 40 μ g/ml gentamycin at 37 °C under a humidified 5% CO₂/95% air atmosphere. The cells were maintained in the log phase by routine passaging every 3–4 days.

TNF cytotoxic activity was quantified using a colorimetric microassay for L929 cell.

L929 cells at a density 25 · 10³ cells/well were incubated in 96-well microculture plates (Falcon, BD Labware, USA) for 24 h in the absence or presence of water soluble progesterone (20 μ g/ml) or 17 β -estradiol (2 μ g/ml) (Sigma, USA). Then known amounts of recombinant TNF ("Vector", Russia) in the presence of 25 μ g/ml cycloheximide (Sigma, USA) were added and cells were incubated for 18 h at 37 °C under a humidified 5% CO₂/95% air atmosphere.

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Abbreviations used: IC50 — half-maximum inhibitory concentration; TNF — tumor necrosis factor.

In other variant of test L929 cells at a density $5 \cdot 10^3$ cells/well were seeded in 96-well plates. Different doses of progesterone, 17 β -estradiol, their combination and recombinant TNF without cycloheximide were added simultaneously. Cells were incubated for 72 h at 37 °C under a humidified 5% CO₂/95% air atmosphere.

After incubation viable adherent cells were stained with 0.2% crystal violet in 20% methanol for 30 min at room temperature. Excess crystal violet was removed by washing with phosphate-buffered saline. Crystal violet was dissolved in 1 M acetic acid for 10 min and the absorbance was determined using a Microplate reader MCC/340 (Lab-system Oy, Finland) at a wavelength of 540 nm (OD sample). L929 cells cultured in the absence of TNF standard were used to obtain baseline absorbance (OD 0) and cells cultured in the presence of 25 ng/ml TNF were used to determine absorbance at 100% cytotoxicity (OD 100). Percent of cytotoxicity was calculated as follows:

$$\% \text{ cytotoxicity} = (1 - [\text{OD sample} - \text{OD 100}] / [\text{OD 0} - \text{OD 100}]) \cdot 100.$$

To investigate rapid (within 24 h) effect of female steroid hormones on L929 cells survival an assay in presence of cycloheximide was conducted. Thus, the specific receptor-mediated mechanism of steroid hormones activity was blocked. Progesterone and 17 β -estradiol inhibited L929 cells viability by 25% ($p < 0.05$) and 6% ($p > 0.05$), respectively. The pretreatment with progesterone and 17 β -estradiol had protective effect on the L929 cells survival under influence of TNF. Treatment of 20 $\mu\text{g/ml}$ of progesterone and 2 $\mu\text{g/ml}$ of 17 β -estradiol led to an increase in the number of viable cells at the doses of TNF 12.5–100 pg/ml. Progesterone had stronger protective effect in comparison with 17 β -estradiol. Half-maximum inhibitory concentration (IC₅₀) for TNF was 6.2 pg/ml, for TNF in presence of 20 $\mu\text{g/ml}$ progesterone was 23.5 pg/ml, for TNF in presence of 2 $\mu\text{g/ml}$ 17 β -estradiol was 8.2 pg/ml. These results suggest that the female steroid hormones can upregulate survival of L929 cells and modulate the response of these cells to TNF (Fig. 1).

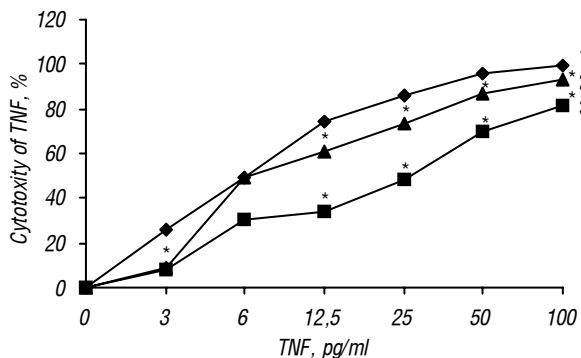


Fig. 1. Influence of female steroid hormones (preincubation for 24 h) on TNF-mediated cytotoxicity toward L929 cells in the presence of cycloheximide. 1 — TNF-mediated cytotoxicity, control; 2 — in presence of 17 β -estradiol (2 $\mu\text{g/ml}$); 3 — in presence of progesterone (20 $\mu\text{g/ml}$).

The results present the mean obtained in 4 independently performed experiments. Student's *t*-test was performed to evaluate the statistical significance of differences between experimental groups. Statistical significance was assigned to the level of $P < 0.05$.

* $P < 0.05$ Student's *t*-test as compared with control

To confirm that female steroid hormones had prolonged mechanism of influence on the TNF-mediated cytotoxicity toward L929 cells a cytotoxic assay without cycloheximide was performed. Hormones and TNF were added to the cultured cells simultaneously. All concentrations of female steroid hormones decreased survival of L929 cells after 72 h incubation, but only two points were statistically significant. Progesterone inhibited cell survival by 14% in the dose 2 $\mu\text{g/ml}$, and combination of progesterone (2 $\mu\text{g/ml}$) and 17 β -estradiol (0.2 $\mu\text{g/ml}$) inhibited cell survival by 11% (Fig. 2). Progesterone, 17 β -estradiol and their combinations had protective dose-dependent effects on the L929 cells under influence of TNF (Table) but for progesterone it was the most pronounced.

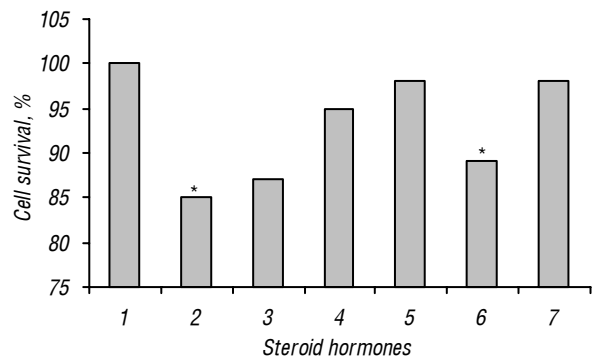


Fig. 2. L929 cell survival upon 72 h incubation with female steroid hormones: 1 — without hormones, control; 2 — progesterone, 2 $\mu\text{g/ml}$; 3 — progesterone, 20 ng/ml; 4 — 17 β -estradiol, 0.2 $\mu\text{g/ml}$; 5 — 17 β -estradiol, 2 ng/ml; 6 — progesterone, 2 $\mu\text{g/ml}$ + 17 β -estradiol, 0.2 $\mu\text{g/ml}$; 7 — progesterone, 20 ng/ml + 17 β -estradiol, 2 ng/ml.

The results present the mean obtained in 3 independently performed experiments. Student's *t*-test was performed to evaluate the statistical significance of differences between experimental groups. Statistical significance was assigned to the level of $P < 0.05$. * $P < 0.05$ — Student's *t*-test as compared with control

Table. Effect of female steroid hormones on the TNF-mediated cytotoxicity

Hormones	TNF-mediated cytotoxicity, (%)			IC ₅₀ , pg/ml
	TNF, 50 pg/ml	TNF, 100 pg/ml	TNF, 200 pg/ml	
Control, without hormones	48.48	70.64	75.59	51.6
Progesterone, 2 $\mu\text{g/ml}$	32.68	38.70*	47.15*	267.0
Progesterone, 20 ng/ml	12.30*	36.67*	58.49*	143.4
17 β -estradiol, 0.2 $\mu\text{g/ml}$	18.00*	43.00*	61.39*	122.9
17 β -estradiol, 2 ng/ml	19.51*	44.56*	64.01*	116.6
Progesterone, 2 $\mu\text{g/ml}$ + 17 β -estradiol, 0.2 $\mu\text{g/ml}$	14.44*	33.64*	50.91*	190.3
Progesterone, 20 ng/ml + 17 β -estradiol, 2 ng/ml	8.28*	41.51*	56.58*	126.0

The results present the mean obtained in 3 independently performed experiments. Student's *t*-test was performed to evaluate the statistical significance of differences between experimental groups. Statistical significance was assigned to the level of $P < 0.05$.

* $P < 0.05$ Student's *t*-test as compared with control.

These data indicate that progesterone and 17 β -estradiol protected L929 cells from TNF-mediated cytotoxicity independently from receptor-mediated mechanism. Both hormones inhibited TNF-mediated cytotoxic activity towards L929 cells in dose-dependent manner.

In conclusion, we demonstrated that sex steroid hormones played protective role in the manifestation of cytokine-mediated cytotoxicity. Our results correspond with the data that demonstrated receptor-mediated protection of TNF-treated premonocytic U937 cells by physio-

logical concentration of progesterone and estrogen [11]. However, it was shown that those hormones didn't influence cell survival rate [12]. Present experiments clearly show that progesterone and 17 β -estradiol have inhibitory effect on the survival of L929 cells.

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