Sensitivity of Peripheral Blood Lymphocytes from Breast Cancer Patients to Glucocorticoids

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We measured DNA-dependent RNA polymerase activity, calculated hormone-binding sites and intracellular association constant under conditions of phytohemagglutinin stimulation and dexamethasone treatment, and studied the interaction of cells with the glucocorticoid covalently bound to an inert carrier not penetrating into cells. Reduced sensitivity of peripheral blood lymphocytes from breast cancer patients to glucocorticoids was demonstrated. The biological mechanisms for hormonal regulation are reviewed here.

Key Words: *glucocorticoids; lymphocytes; breast cancer*

Glucocorticoids play a role in the maintenance of homeostasis, affect nearly all tissues in the organism, and act as potent multiparametric bioregulators in cells, tissues, and organs. The sensitivity of cells, tissues, and organs to glucocorticoids and levels of hormonal influences are the least studied aspects of the mechanism of their action. At the present time, there are no reliable criteria for these studies [8].

Reaction of tissues to glucocorticoids are different, but the molecular mechanism for their action usually involves the interaction of hormones with intracellular receptors modulating transcription activity of the target genes. These signal pathways determine a variety of hormonal effects [6]. However, rapid response of cells or reaction to short-term hormonal treatment cannot be explained from the viewpoint of classical genomic model and suggest the existence of membrane glucocorticoid receptors and realization of the nongenomic mechanism for hormonal influences [4]. Membrane glucocorticoid receptors were identified in amphibian neurons [3] and leukemic lymphoid cells [5]. It remains unknown whether these receptors are present in humans under physiological or pathophysiological conditions.

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To evaluate the biological mechanisms of hormonal regulation, we studied the sensitivity of peripheral blood lymphocytes from patients with non-lymphoid tumors (*e.g.*, breast cancer, BC).

MATERIALS AND METHODS

The age of patients was 25-45 years. We examined 30 patients with BC and 10 patients with benign breast tumor. Some patients with BC (n=15) received standard glucocorticoid drugs for 1-24 months. The remaining patients with BC were examined 6, 12, or more months after the end of treatment. The control group included 32 healthy donors (16 men and 16 women). The tests were performed with lymphocytes (separation by sedimentation on a nylon column), mononuclear cells and polymorphonuclear leukocytes (fractionation in a Ficoll-Verografin density gradient), and total pool of peripheral blood leukocytes. Immune reactivity was estimated by the ability of cells to form rosettes with heterologous lymphocytes. The hormonal effect on lymphoid tissue is related to variations in RNA synthesis. It should be emphasized that changes in activity of DNA-dependent RNA polymerase occurs most rapidly and is associated with the state of glucocorticoid receptors on cultured lymphocytes from human peripheral blood stimulated with phytohemagglutinin (PHA). Experimental tests [6]

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reflect the genomic model of hormonal influences. Activity of DNA-dependent RNA polymerase (EC 2.7.7.6) was estimated in the reconstruction system containing 4 nucleotide triphosphates. We measured activities of nuclear form A (synthesis of ribosomal RNA) and nucleoplasmic form B (synthesis of informational DNA-like RNA). Hormone-binding sites and intracellular association constant were calculated under conditions of PHA-dexamethasone stimulation. Specific binding was determined by the difference in ^{3}H-dexamethasone incorporation into the cytosol in the presence and absence of a 1000-fold excess of cold steroid. Binding of glucocorticoids to the cell surface was evaluated using conjugates of Sepharose 6B and covalently bound dexamethasone or triamcinolone (in experiments proving the existence of membrane steroid receptors and their role in the mechanism of nongenomic hormonal influences sepharose 6B was used as an inert carrier [2,7]). The number of cells fixed to the conjugate was estimated under a phase contrast microscope and calculated per 1000 nucleated cells (‰). Binding of the ligand was determined by the amount of radioactive hormone and infrared spectra. The results were analyzed by pairwise Student's *t* test. The data are expressed as $M\pm\sigma$. The differences were significant at $p<0.05$.

RESULTS

Both functional forms of RNA polymerase were found in lymphocyte nuclei from examined patients (Table 1). The total activity of DNA-dependent RNA polymerase in BC patients increased by 4 times compared to enzyme activity in healthy donors and patients with benign breast tumors. The number of hormone-binding sites and intracellular association constant in BC patients were 2 times higher than in healthy donors and patients with benign breast tumors. However, peripheral blood lymphocytes from BC patients were practically hormone-insensitive. Lymphocytes from BC patients did not differ from cells obtained from healthy donors and patients with benign breast tumors and treated with PHA for at least 20 h. The number of cells sorbed to the glucocorticoid conjugate decreased by 3 times in BC patients. The study of binding of purified lymphocytes to hormone-Sepharose conjugates produced a negative result. Peripheral blood lymphocytes isolated from the total pool of leukocytes by sedimentation on a nylon membrane, as well as mononuclear cells, were not sorbed to the hormone-Sepharose conjugates. However, the fraction of polymorphonuclear leukocytes containing 98% leukocytes exhibited binding capacity. To evaluate the specificity of this reaction, we examined the total pool of leukocytes obtained from the peripheral blood and characterized by binding capacity. The cells preincubated with this hormone lost this ability.

To evaluate the type of sorbed cells, they were removed from conjugates and subjected to a morphological study. These cells were identified as lymphocytes (60%), neutrophils (30%), and neutrophils losing nucleus segmentation (10%). Examination of healthy donors showed that physiological binding of peripheral blood cells in men is higher than in women (Table 2). The test parameters in women depended on menstrual cycle. The intensity of binding was low on days 5-9 of the cycle, which corresponded to the follicular phase. However, binding intensity in patients with benign tumor reached maximum during this period. These indexes in men underwent variations over 1 month, but were higher than in women. The number of hormone-binding sites and intracellular association constant for lymphocytes separated from the pool of peripheral blood leukocytes by sedimentation on the nylon column remained practically unchanged over 1 month (Table 2). The degree of cell binding in patients receiving hormone therapy was lower than in healthy donors. However, glucocorticoid treatment was followed by a transient decrease in the number of intracellular binding sites for glucocorticoids and reduction of the association constant (as compared to pretreatment values). The degree of cell binding increased 1 year after withdrawal of hormone therapy. The patients were divided into 2 groups depending on the type of binding. Group 1 patients were characterized by low-intensity binding. The degree of binding in group 2 patients was much higher than in healthy donors. The test parameters negatively correlated with the number of hormone-binding sites and intracellular association constant for lymphocytes separated from the total pool of peripheral blood leukocytes by sedimentation on a nylon column (Table 2). This attests to not only different functions, but also different mechanisms of regulation of the expression of membrane and cytosolic binding sites for glucocorticoids in lymphocytes. Our results provide indirect evidence that membranes of human peripheral blood lymphocytes and neutrophils carry specific binding sites for glucocorticoids. They probably play a role in the membrane mechanism of hormonal regulation, which is impaired during neoplastic transformation.

Our results are consistent with published data on the expression of membrane glucocorticoid receptors in mononuclear cells of peripheral blood from patients with rheumatoid arthritis [1]. These

TABLE 1. Sensitivity of Peripheral Blood Lymphocytes from Healthy Donors and Patients with Benign (Mastopathy) and Malignant Breast Tumors (BC) to Glucocorticoids ($M \pm \sigma$)

Conditions, groups	Time	DNA-dependent RNA polymerase activity (nucleus), cpm/mg DNA		Intracellular binding of dexamethasone (cytosol)		Number of cells bound to hormone-Sepharose 6B (pool of leukocytes), ‰	
		form A	form B	association, $K_{as} \times 10^9$ M^{-1}	number of binding sites, $n \times 10^{-13}$ M/mg protein	dexamethasone—Sepharose	triamcinolone—Sepharose
Baseline level							
healthy donors (n=16)	—	4720±1115	11 320±2950	2.66±1.10	0.9±0.2	6.96±5.47	7.76±6.24
mastopathy (n=10)	—	4670±1233	11 580±2300	2.95±0.90	0.85±0.20	6.08±7.38	8.16±3.12
BC (n=15)	—	25 390±3230	38 570±4320	4.75±1.30	2.98±0.30	—	2.09±1.15
PHA stimulation							
healthy donors	30 min	4490±1340	11 770±900	2.66±1.10	0.9±0.2	—	—
	3 h	4810±1045	11 190±2120	2.66±0.50	0.9±0.2	—	—
	6 h	5120±2137	12 970±1850	3.95±0.70	2.85±0.10	—	—
	20 h	9540±520	35 700±1126	4.8±1.4	2.92±0.10	—	—
	24 h	10 230±415	33 480±2950	4.25±1.70	3.87±0.20	—	—
mastopathy	30 min	4570±1700	12 300±1150	2.95±0.90	0.85±0.20	—	—
	3 h	4260±1270	11 410±1700	2.9±0.7	0.9±0.1	—	—
	6 h	5087±1639	12 000±1450	3.7±1.1	0.9±0.3	—	—
	20 h	9230±1048	29 430±1095	4.9±0.2	2.9±0.3	—	—
	24 h	11 435±643	30 720±1137	4.25±0.40	3.7±0.2	—	—
BC	30 min	25 780±2750	37 940±3970	4.75±1.30	2.98±0.10	—	—
	3 h	25 390±750	38 100±3700	4.7±0.5	2.96±0.20	—	—
	6 h	26 000±786	39 740±1820	4.8±1.2	2.98±0.30	—	—
	20 h	27 230±1230	40 340±968	4.75±1.10	3.07±0.10	—	—
	24 h	26 430±1730	37 980±645	4.25±0.20	2.94±0.30	—	—
PHA stimulation and dexamethasone treatment							
healthy donors	30 min	4700±340	10 780±2630	2.66±1.10	0.9±0.2	—	—
	3 h	4620±1430	11 700±1950	2.66±0.70	0.94±0.20	—	—
	6 h	5120±2137	12 970±1850	2.95±1.10	0.89±0.10	—	—
	20 h	5540±520	12 680±1126	2.8±1.3	0.95±0.10	—	—
	24 h	4970±1370	11 700±750	2.25±1.10	2.25±1.10	0.87±0.20	—
mastopathy	30 min	4590±1870	15 240±3900	2.95±0.90	0.85±0.20	—	—
	3 h	4610±980	11 500±900	2.66±0.50	0.9±0.2	—	—
	6 h	4987±1630	10 000±950	2.7±1.1	0.92±0.20	—	—
	20 h	5540±1740	13 780±1420	2.8±0.2	0.9±0.3	—	—
	24 h	3435±643	15 130±1780	2.25±1.10	0.85±0.20	—	—
BC	30 min	25 390±1970	39 570±4500	4.75±1.30	2.98±0.1	—	—
	3 h	23 700±3900	38 000±5700	4.67±1.10	3.7±0.2	—	—
	6 h	26 320±1130	37 190±2490	4.85±0.20	2.85±0.1	—	—
	20 h	25 470±230	42 570±1934	4.75±1.10	2.9±0.1	—	—
	24 h	25 980±2980	40 570±1775	4.6±1.2	2.9±0.3	—	—

Note. Here and in Table 2: n, number of patients.

TABLE 2. Glucocorticoid Binding Capacity of Peripheral Blood Lymphocytes from Healthy Donors and Patients with Benign (Masto-pathy) and Malignant Breast Tumors (BC; *M*±s)

Conditions, groups		Intracellular binding of dexamethasone (lymphocyte cytosol)		Number of cells bound to hormone-Sepharose 6B (pool of leukocytes), ‰	
		association, $K_{as}\times10^{9}$ M^{-1}	number of binding sites, $n\times10^{-13}$ M/mg protein	dexamethasone—Sepharose	triamcinolone—Sepharose
Male donors (n=16)	day 1	2.66±1.10	0.9±0.2	25.14±8.70	30.27±10.03
	day 15	2.7±0.9	0.85±0.10	33.21±10.11	59.40±16.27
	day 30	2.6±1.5	0.7±0.3	20.17±15.05	25.11±11.75
Female donors (n=16)	days 5-9	2.6±1.3	0.65±0.10	3.21±1.09	6.17±3.14
	days 12-15	2.47±1.20	0.9±0.4	5.70±3.14	14.31±2.27
	days 19-26	2.7±1.1	0.7±0.8	5.32±0.95	5.63±1.35
Mastopathy (n=10)	days 5-9	2.55±1.10	0.8±0.2	10.53±2.19	25.69±12.07
	days 12-15	2.66±0.30	0.75±0.70	3.29±0.75	6.08±2.84
	days 19-26	2.1±0.9	0.9±0.1	4.24±1.15	6.10±1.53
BC patients (n=15), glucocorticoid therapy	before therapy	4.75±1.50	2.85±0.30	—	2.09±0.57
	1 month	3.04±0.70	1.9±0.1	—	3.53±2.15
	6 months	2.9±1.2	0.9±0.2	—	3.80±1.07
	12 months	1.95±1.10	0.7±0.2	—	4.43±3.92
	24 months	1.15±0.90	0.75±0.30	—	3.72±1.85
BC patients (n=15), previous glucocorticoid therapy	6 months	2.5±0.9	0.9±0.3	—	5.08±3.20
	12 months	2.75±1.10	0.9±0.1	—	3.41±3.09
	18 months	7.3±1.7	1.8±0.3	—	24.36±7.67
BC patients (8 of 15 patients), previous glucocorticoid therapy without recovery		4.6±1.5	2.85±0.30	—	3.97±0.22

receptors or at least specific binding sites for glucocorticoids act as reporter regulators of the immune reaction to hormonal treatment. They are present on blood cells from healthy donors, but cannot be identified by standard tests due to low mean number and high lability and specificity for certain cell population or subpopulation. The use of covalently bound steroids and inert carriers not penetrating into cells allow identifying the subpopulation of glucocorticoid-sensitive and glucocorticoid-resistant cells that specifically interact with hormones at the membrane level. The ratio of these cells in the total population of cells and specificity of the membrane interaction require further investigations.

Our findings show that the sensitivity of peripheral blood lymphocytes from BC patients to glucocorticoids decreases at the genomic and membrane level. These changes probably serve as a criterion for immune cell dysfunction and reflect the systemic effect of malignant tumor in the organism.

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