Evaluation of the Proportion of Hormonal and Progenotoxic Effects of Estrogens and Glucose in Cancer Patients

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The progenotoxic (G, generation of reactive oxygen forms in mononuclears) and hormonal (H, reactive insulinemia) effects of oral glucose, on the one hand, and the same effects of estradiol (10^{-8} and 10^{-5} M) *in vitro* on blood mononuclears (G: by comet tail length; H: by expression of AMP kinase and TNF and IL-6 secretion), on the other, were compared with consideration for the concepts on endocrine genotoxic switch-over in patients with breast cancer and endometrial cancer in remission. Coculturing of mononuclears with estradiol in general led to an increase in comet tail and was associated with a trend to more intense expression of AMP kinase and IL-6 secretion. The reaction to estradiol (primarily in a concentration of 10^{-8} M) evaluated by the expression of AMP kinase and TNF secretion was more intensive than the reaction evaluated by comet tail lengths or by percentage of cells with comets in women with predominating progenotoxic effect of glucose *vs.* hormonal effect. This fact can be used as a landmark in search for means for optimization of the status and proportion of effects in the estrogen and glucose systems.

Key Words: *estrogens; glucose; hormonal and genotoxic effect*

Virtually universal acknowledgement of the fact that excessive estrogen stimulation and carbohydrate metabolism disorders occupy an important place among malignant tumor risk factors [1,4,10] does not preclude variability in the development these tumors. We analyzed the causes of this heterogeneity with respect to two main mechanisms of hormonal carcinogenesis and agree with the significance of hormone-induced DNA injury [7,15]; during the recent years we tried to understand which conditions promote the shift from a promotor to a less favorable genotoxic type of hormonal carcinogenesis and, presumably, from a less to a more aggressive variant of not only cancer but of some other main non-infectious human diseases. By the results of our studies we characterized three processes: the phenomenon of estrogen effect transition, dual function of glucose, and adipogenotoxicosis, constituting the so-called basal triad and involving the effects of estrogens, glucose, and factors produced by fatty tissue, respectively [1,2].

These effects, in turn, can be reduced to the two major effects: hormonal/endocrine and DNA impairing/genotoxic. The individual proportion of these effects varies greatly in humans even of the same gender and age. Transition towards more intense DNA impairing effect (its predominance over hormonal effect) is regarded as endocrine genotoxic transition (EGT) [2].

We tried to detect parallelism (or its absence) in the development of EGT in the glucose and estrogen "systems" and find the potential landmarks in order to attain the optimal balance of the genotoxic and hormonal effects within the frames of these systems.

MATERIALS AND METHODS

The study was carried out in 24 menopausal women (mean age 57.4±1.7 years), who had received no therapy because no signs of the disease had manifested and

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who had been operated on for breast cancer (8 women) or endometrial cancer (16 women) 2.7 years before the study. The glucose system status was evaluated by glucose (40 g/m^2 body surface) tolerance test after overnight fasting. The blood was collected at the beginning (0 min) of the test and 120 min later and basal and post-loading/reactive insulinemia, glucose-induced generation of reactive oxygen species (ROS), and the ratio of these parameters were evaluated. Serum insulin concentrations were measured by enzyme immunoassay with DSL kits, basal and glucose-stimulated production of ROS by luminol-induced chemiluminescent method as described previously [8]. Mononuclears were isolated in Ficoll-verograffin gradient. The ROS (120/0)/insulinemia (120/0) ratio was estimated as described previously [2,3,6]. In order to evaluate the estrogen system status, mononuclear aliquots were kept at -20°C in a buffer with fetal serum, DMSO, and 2-fold diluted RPMI. After defrosting, the cells were divided into three parts (comets, AMP kinase, cytokines) consisting of 3 portions each (130-150×10^3 cells per portion) which were denoted as control, estradiol 10^{-8} M, and estradiol 10^{-5} M. The samples were incubated with estradiol for 60 min at 37°C. Analysis of comets (as a DNA injury marker, including the estrogen-induced injury [13]) and their tails was carried out as described previously [12]. The expression of AMP kinase (enzyme sensitive to glucose and estrogens [9]) in mononuclears was evaluated by Western blot with polyclonal antibodies to phosphorylated AMP kinase (Phospho-AMPK, Thr172, Cell Signaling) in 1:1000 dilution according to the instruction [11] (Fig. 1). Cytokines (TNF-α and IL-6), whose production by mononuclears can change in oral estrogen therapy [5], were measured by enzyme immunoassay with Vector-Best and BioSource International kits after transfer of mononuclears into serum-free medium and their incubation with estradiol. The data were statistically processed by parametric and nonparametric methods using SigmaPlot and Statistica 6.0 software.

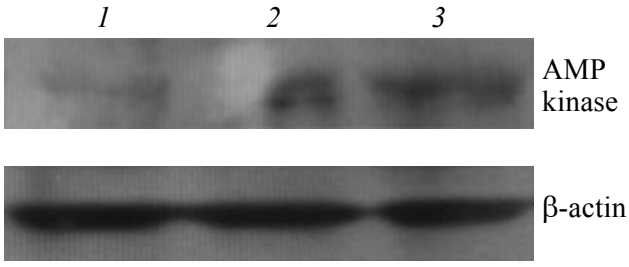

Fig. 1. Western blot analysis of AMP kinase expression in mononuclears before and after incubation with estradiol (patient N. M. S., 50 years). Here and in Fig. 2: *1*) control (no estradiol); *2*) estradiol, 10^{-8} M; *3*) estradiol, 10^{-5} M.

RESULTS

Incubation of blood mononuclears with estradiol *in vitro* led to a nonlinear elongation of comet tails (p=0.06 by Student's test) in the entire group of women and was paralleled by a trend to more intense expression of AMP kinase and secretion of IL-6 (p=0.23 and p=0.21, respectively, by Wilcoxon's test; Fig. 2). The probands were subdivided into 2 groups by the previously developed criteria [2,3,6]: 1) with predominating progenotoxic effect of glucose over hormonal effect (60% of examined women) and 2) without predominance of

TABLE 1. Mononuclear Reaction to Estradiol in Groups with Different Proportion of Progenotoxic and Hormonal Effects of Oral Glucose ($M\pm m$)

Parameter	Control	Estradiol (10^{-8} M)	Estradiol (10^{-5} M)
Group with predominating progenotoxic effect of glucose			
cells with comets, %	3.8±2.0	10.0±3.2	17.7±9.0
tail length, arb. units	1.42±0.25	1.89±0.34	2.60±1.17
AMP expression, arb. units	0.48±0.17	0.57±0.14	0.55±0.12
TNF secretion, %	100	112.2±15.9	104.0±7.8
IL-6 secretion, %	100	96.3±12.2	112.5±11.9
Group without predominating progenotoxic effect of glucose			
cells with comets, %	0.5±0.5	14.7±12.3	23.6±13.8
comet tail length, arb. units	1.09±0.25	3.52±1.63	2.43±0.73
AMP expression, arb. units	0.63±0.23	0.39±0.14	0.50±0.15
TNF secretion, %	100	92.6±9.7	90.1±9.0
IL-6 secretion, %	100	106.3±16.5	108.5±15.7

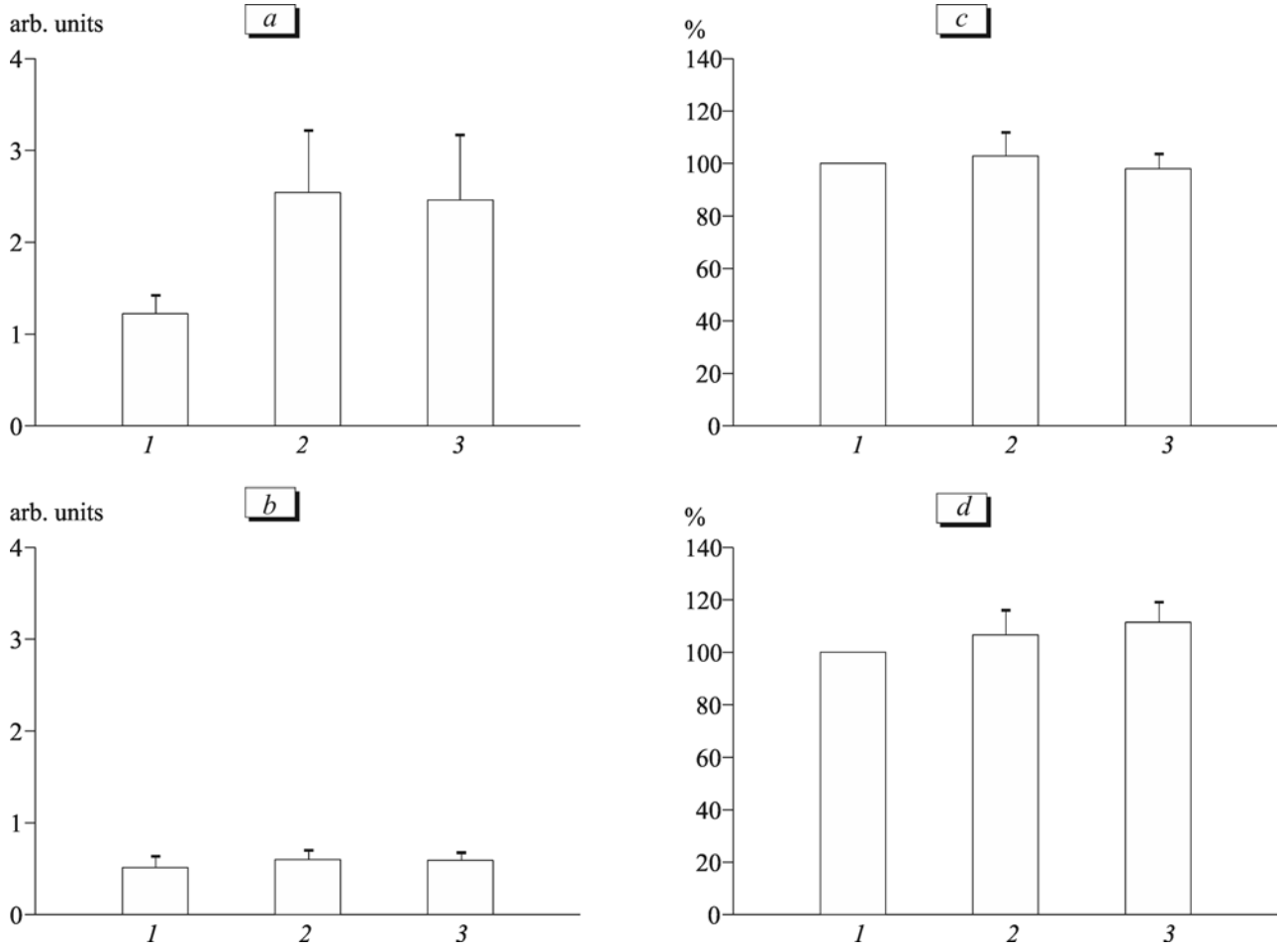

Fig. 2. Dynamics of comet tail length (*a*), AMP kinase expression (*b*), and secretion of TNF-α (*c*) and IL-6 (*d*) after 60-min incubation of blood mononuclears with estradiol.

this kind (40%). The reaction of mononuclears to estradiol (primarily in the physiological concentration of 10^{-8} M) evaluated by the expression of AMP kinase and TNF secretion (but not by other studied parameters, including the percentage of cells with comets and the comet tail lengths), was more manifest in group 1 *vs.* group 2 women (Table 1).

Hence, we conclude that the progenotoxic shifts in the glucose system are just partially coordinated (evaluated by the methods used in the study) with the respective shifts in the estrogen system; stimulation of AMP kinase expression, which was initially planned to be regarded as an indicator of hormonal effect of estradiol, could be also associated with the genotoxic effect of estradiol or, which is also probable, reflect one of the variants of insulin sensitivity reduction [15]. Judging from pilot data, comparison of the available data with the levels of urinary secretion of classical estrogens and their more and less progenotoxic catecholestrogenic metabolites will presumably help us decide which of the already tested or other parameters can be useful as the landmark in search for means to optimize the status and proportion of the effects in the estrogen and glucose systems.

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