Long-Term Psychoemotional Stress: Mutation Inductor and Mutagenesis Modifier in Mammals

F. I. Ingel', N. M. Gevorkyan, N. A. Ilyushina, B. I. Leitina, L. M. Prikhozhan, E. L. Pereverzeva, and Yu. A. Revazova

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Changes in the physiological status of the mammalian and human organism are known to be capable of inducing various mutational events [1-4]. However, the possible genetic consequences of long-term psychoemotional stress have not been sufficiently studied. The present research, including studies by cytogenetic methods of the comparative response of body cells and tissues, was undertaken to elucidate the injurious effect of stress alone and in combination with a known mutagen.

MATERIALS AND METHODS

Psychoemotional stress was induced in F_1 CBA× ×C57B16/j male mice weighing 20 g, several groups of five animals, by suspending them by the neck fold for 4 h daily for 20 days. Cyclophosphamide (a cytostatic) was injected i.m. in a dose of 10 mg/kg to animals exposed or not exposed to stress 24 h before sacrifice. The mice were sacrificed 1, 5, 10, 15, 20, and 25 days after the start of the experiment by cervical dislocation. To detect the presence of psychoemotional stress in the sacrificed animals, their body, thymus, and adrenal mass was measured, and visual and histological examination of the stomach and intestine was carried out. The genotoxic effects of psychoemotional stress and cyclophosphamide were assessed by changes in the level of DNA extra synthesis in peripheral blood lymphocytes [8], in chromosomal aberrations in bone marrow cells [6], and in micronuclei of bone marrow polychromatophilic red cells [7]. The results were sta-tistically processed using the χ^2 and Student t tests.

RESULTS

The fact of psychoemotional stress development and its depth were monitored after Selye [4]. On the whole our data indicate that stress developed between days 5 and 15 of the experiment. Starting from the first day of stress exposure and until the end of the experiment changes in thymic mass had a wavelike pattern; inflammatory and atrophic symptoms were found in the gastrointestinal wall. Erosions and ulcers were detected in the gastroduodenal mucosa on days 5, 10, and 15.

The results of assessment of the injurious effects of psychoemotional stress and cyclophosphamide are presented in Fig. 1, *a-h*. For convenience of comparison the values of the relevant parameters are juxtaposed with those in the controls. One can see a wave pattern of chromosomal aberration induction and DNA extra synthesis processes in animals exposed to psychoemotional stress alone, this pattern evidently reflecting the development of adaptative changes in these animals. The results of

Research Institute of Prophylactic Toxicology and Disinfection, Moscow. (Presented by N. P. Bochkov, Member of the Russian Academy of Medical Sciences)

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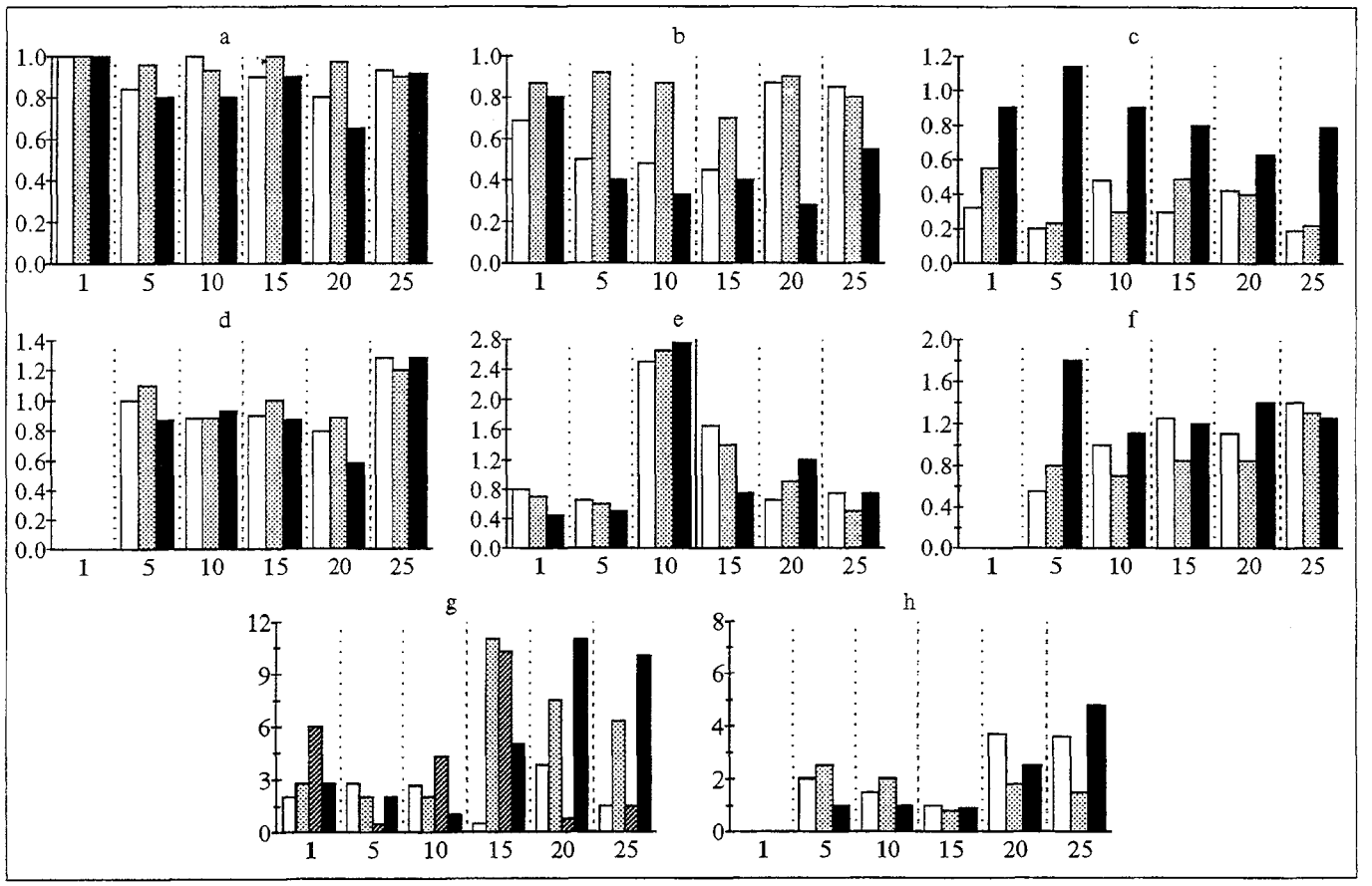

Fig. 1. Damaging effect of psychoemotional stress and cyclophosphamide (vis – a – vis control). *a*) murine body mass; *b*) thymic mass; *c*) adrenal mass; *d*) bone marrow erythropoietic activity; *e*) mitotic index; *f*) induction of micronuclei in bone marrow polychromatophilic red cells; *g*) induction of chromosomal aberrations in bone marrow lymphocytes; *h*) induction of repair synthesis. White bars denote stress (single fragments; light grey bars, stress (multiple fragments); Black bars, cyclophosphamide (single fragments); cross – hatched bars, cyclophosphamide (multiple fragments); diagonal graph bars, stress + cyclophosphamide (single fragments); white bars, stress + cyclophosphamide (multiple fragments).

assessing the rate of DNA extra synthesis in peripheral blood lymphocytes and the quantitative evaluation of chromosomal aberrations in bone marrow cells (Fig. 1, *g*) imply that psychoemotional stress is a mutagenic factor comparable to cyclophosphamide in a dose of 10 mg/kg. Thus, synchronous changes in the levels of chromosomal aberrations and DNA extra synthesis have been demonstrated for exposure to both psychoemotional stress and cyclophosphamide.

It should be mentioned that although psychoemotional stress did not influence the ratio of bone marrow polychromatophilic to normochromatophilic red cells (PCRC/NCRC), it induced micronuclei only on days 15 and 25, this corresponding, if judged by chromosomal aberrations and DNA extra synthesis levels, to an increase in damaged lymphocyte counts.

Combined subjection of animals to psychoemotional stress and cyclophosphamide resulted in more pronounced changes of all the tested physiological parameters, which normalized much more slowly than after alternative exposures (Fig. 1, *a-d*). Moreover, analysis of the cyclophosphamide level showed the appearance of numerous multiple chromosomal fragments. In the micronuclei test (Fig. 1, *f*), after combined exposure to psychoemotional stress and cyclophosphamide, an effect was observed only on day 5.

The negligible fluctuations of erythropoietic activity indicated by the changes in the PCRC/NCRC ratio most likely prove that combined exposure to psychoemotional stress and cyclophosphamide has no noticeable effect on erythropoiesis.

The results are evidence, first, of a mutagenic effect of long-term psychoemotional stress and, second, enhancement of the genotoxic effect of the standard mutagen cyclophosphamide during exposure to psychoemotional stress. The detected correlations between damage to the genetic apparatus of the lymphocytes and the status of the body systems suggest a contribution of the thymico-lymphocyte system along with the hormonal system to mutation development.

The reported data are preliminary results of ongoing research into the mechanisms of the mutagenic effects of long-term psychoemotional stress connected with the participation of immunocompetent cells, as well as an analysis of the possibilities of suppressing mutagenic effects by immunocorrection.

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ONCOLOGY

Cross Resistance to Cytostatics of P388 Leukemia Cells with Induced Resistance to Doxorubicin

F. V. Donenko, S. M. Sitdikova, A. O. Kabieva, and L. V. Moroz

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Studies of the mechanisms by which tumor cells develop resistance to cytostatics and investigation of possible ways of overcoming this resistance are of key importance for the chemotherapy of malignant neoplasms. One of the commonest and best studied forms of resistance is multiple drug resistance (MDR), which is characterized by the ability of tumor cells to withstand the effects of a broad class of antineoplastic drugs of natural origin and their synthetic analogs [1]. A remarkable feature of MDR is that treatment of tumor cells with only one cytostatic is in most instances sufficient for these cells to become resistant to a range of cytostatics [1]. Accordingly, if an effective strategy for the chemotherapy of tumors with an MDR phenotype is to be developed, their cross resistance and collateral sensitivity to cytostatics of various groups must be known. The purpose of this study was to examine the cross resistance to several cytostatics of murine P388 leukemia cells with induced resistance to doxorubicin. This cell sub-

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