ACTION OF SOME PYRAZOLONE DERIVATIVES ON PARAMETERS OF THE MITOTIC CYCLE IN CELL CULTURE

A. S. Saratikov, V. E. Yavorovskaya, T. P. Pishchep, S. K. Blagerman, V. N. Kiseleva, and V. I. Teterina

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The kinetics of cell populations of human embryonic fibroblasts (HEF) under the influence of pyrazolone derivatives before and after infection of the tissue with Coxsackie A13 virus was studied by autoradiography. This virus induces an increase in mitotic activity of cultures of HEF cells, which is followed by inhibition. Pyrazolone derivatives considerably reduce all the parameters of the mitotic cycle of cells of HEF cultures infected or not infected with Coxsackie A13 virus. This effect is evidently the reason why pyrazolone derivatives can induce an antiviral inhibitor.

Previous work has shown that pyrazolone derivatives significantly increase the functional activity of cells in cultures of human embryonic fibroblasts (HEF) and induce the production of cell inhibitors which inhibit the cytopathic effect of Coxsackie A13 virus.

To study the dynamics of proliferative processes and the duration of the mitotic cycle of cells under the influence of pyrazolone derivatives in tissues infected with Coxsackie A13 virus and in uninfected tissues the autoradiographic method was used in conjunction with continuous incubation of the cells with thymidine-H^3 in a dose of 0.26 μCi/ml [1].

EXPERIMENTAL METHOD

Material for the experiments and control (series I) consisted of a monolayer culture of HEF obtained by the standard method of trypsinization and growth on cover slips. In the experiments of series II the preparations for testing were added to the tissue culture: stearic acid antipyrylamide, p-aminobenzoic acid methylantipyryl-amide, and butadione in concentrations of 2 mg% (contact for 48 h). In the experiments of series III the culture of HEF cells was infected with a reference strain of Coxsackie A13 virus in a dose of 100 TCD_{50}/ml (contact for 48 h). In the experiments of series IV, after contact between the test preparations and HEF cells for 48 h Coxsackie A13 virus was added in the above dose (contact for 48 h).

Treatment of the cover slips with the culture was carried out 1, 2, 3, 6, 9, 12, 15, 18, 21, 24, 36, and 48 h after contact with thymidine-H^3 (two cover slips were tested each time). To remove unincorporated isotope from the cells the cover slips were washed three times with Hanks' solution and then fixed in a mixture of methyl alcohol and acetic acid (3:1). Autoradiograms were obtained in type M liquid emulsion with an exposure of 5 days. After development the preparations were stained with azure-eosin and the percentage of labeled mitoses was determined by examination of 100 mitoses, the percentage of labeled nuclei was determined in 200 cells, and the number of grains of silver per labeled nucleus was obtained in 50 cells. The cell nuclei were regarded as labeled if there were at least six grains of silver above them.

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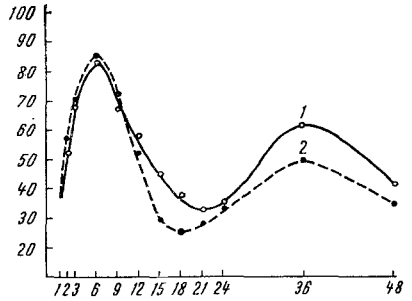

Fig. 1. Percentage of labeled mitoses in intact (1) and infected tissue (2) at various times after treatment with thymidine-H^3. 1) G_2 = 2h, S = 12 h, T = 30 h G_1 + m = 16 h; 2) G_2 = 1.5 h, S = 10.5 h, T = 30 h, G_1 + m = 18 h. Here and in Fig. 2: abscissa, time after contact of tissue with thymidine-H^3 (in h); ordinate, percentage of labeled mitoses.

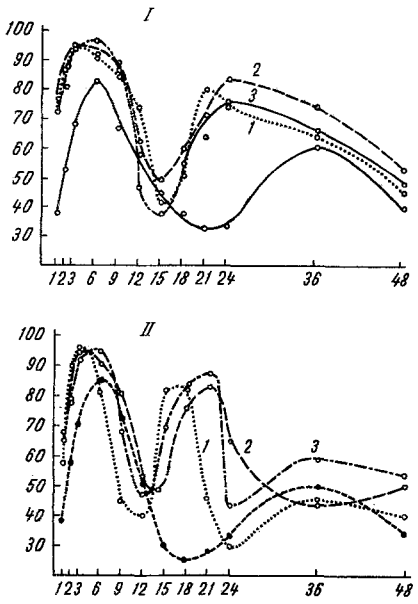

Fig. 2. Percentage of labeled mitoses in intact (I) and infected (II) tissue after treatment with pyrazolone derivatives: 1) stearic acid antipyrylamide: I) G_2 < 1h, S = 13 h, T = 18 h, G_1 + m = 4 h, II) G_2 < 1 h, S = 8 h, T = 13.5 h, G_1 + m = 4.5 h; 2) p-aminobenzoic acid methylantipyrylamide: I) G_2 < 1 h, S = 14 h, T = 21 h, G_1 + m = 6 h; II) G_2 < 1 h, S = 11 h, T = 18 h, G_1 + m = 6 h; II) G_2 < 1 h, S = 10 h, T = 15 h, G_1 + m = 4 h.

EXPERIMENTAL RESULTS

Determination of the durations of individual periods of the mitotic cycle in cells of the HEF culture (Fig. 1) in the usual way [5] gave values of 2 h for the postsynthetic (G_2) period, 12 h for the synthetic (S) period, 30 h for the total mitotic cycle (T), and 16 h for the combined presynthetic period and mitosis proper (G_1 + m). These results are in agreement with those in the literature [4].

In the infected culture, while the total duration of the mitotic cycle remained unchanged, the value of G_2 (1.5 h) and S (10.5) was reduced (fig. 1). The drawnout character of the curve in the second half and relatively low level of the second maximum indicate an increase in duration of the (G_1 + m) period (18 h).

The effect of pyrazolone derivatives on the mitotic cell cycle in the HEF culture and on its individual periods is basically similar (Fig. 2, I). The percentage of labeled mitoses 1 h after addition of the isotope reached 73 (stearic acid antipyrylamide), 74 (p-aminobenzoic acid methylantipyrylamide), and 77 (butadione). The minimal duration of the G_2 period was thus shorter than 1 h. The total duration of the cycle in cells treated with stearic acid antipyrylamide and butadione was 18 h, and in cells treated with p-aminobenzoic acid methylantipyrylamide 21 h. Consequently, pyrazolone derivatives lead to considerable shortening of the duration of the cycle, principally on account of the presynthetic period and of mitoses proper.

Infection with Coxsackie A13 virus leads to an increase in mitotic activity of the HEF cell culture, followed by its inhibition (Fig. 2, II), in agreement with data in the literature [2, 3] regarding the biphasic effect of different viruses (vaccinia, cowpox, St. Louis encephalitis) on mitotic activity of cell cultures.

Pyrazolone derivatives are similar in their effect on the cell cycle of HEF infected with virus, but the compound stearic acid antipyrylamide, which also exhibits more marked protective properties against Coxsackie A13 virus, gives rise to a more considerable degree of shortening of the cell cycle.

Antipyrylamides and butadione cause considerable shortening of all periods of the mitotic cycle, the greatest changes being observed in the presynthetic period and in mitosis. The marked decrease in the duration of all periods of the cell cycle reflects the state of proliferative processes in the tissues and the greater intensity of metabolic processes in the cell. One result of the faster entry into mitosis of cells which have completed their DNA synthesis is a high mitotic index.

The values of the duration of the mitotic cycle and its periods obtained graphically are confirmed by calculation of the percentage of labeled cells and the number of grains of silver per labeled nucleus.

Under the influence of butadione and antipyrylamides active growth of the cells of HEF cultures, whether uninfected or infected with Coxsackie A13 virus, is thus obtained. The biphasic character of the effect of viruses on the cell cycle (an increase, followed by a decrease, in mitotic activity) is evidently attributable to reproduction of the viruses [2, 3]. The increase in functional activity of cells of HEF cultures infected with Coxsackie A13 virus, observed under the influence of the tested compounds, may be responsible for the induction of an antiviral inhibitor by these agents which the writers describe previously.

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