EFFECT OF RIMANTIDINE AND DEUTIOPHORIN ON PATHOLOGY OF CELL REPRODUCTION

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As obligate intracellular paracytes, viruses utilize the metabolic pathways and synthetic material of the cell for their own reproduction. It is thus quite clear that chemotherapeutic agents directed against viruses must have some effect on the cells and on the cell population as a whole. The study of the effects of antiviral agents on cell reproductive processes has at least two applied aspects. One is connected with the need to develop an improved system of assessing the cytotoxicity of chemical compounds capable of allowing for disturbances of cellular reproduction in its early stages. The second and no less important aspect is the possibility of using information obtained by the study of the precise mechanisms of the antiviral action of chemotherapeutic agents (action on the virus "through the cell") [4].

The most demonstrative phase of the cell cycle, capable of revealing disturbances of synthetic processes arising in other phases, is that of mitosis [7]. Accordingly, when studying the action of the known antiviral chemotherapeutic agents rimantidine and deutiphorin on a cell culture we used as the test object pathology of the reproductive apparatus of the cells. Experiments were carried out on a model of a transplantable culture of Madin–Darby canine kidney (MDCK) cells.

EXPERIMENTAL METHOD

MDCK cells were grown on coverslips kept in flasks under penicillin. Rimantidine was obtained from the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, deutiphorin from the Institute of Organic Chemistry, Academy of Sciences of the Ukrainian SSR. The chemotherapeutic agents, in doses of 5, 10, 25, 50, and 100 μg/ml (rimantidine) and 5, 10, 25, 50, 100, and 500 μg/ml (deutiphorin) were added in complete growth medium to a monolayer culture in the stationary phase of growth. Morphological preparations were stained after 2, 4, 6, 8, 24, 48, 72, and 96 h with Heidenhain's hematoxylin. The control and experimental cultures were studied on an "Orthoplan" microscope (West Germany) equipped for stages for scanning and coupled to a "Leitz-TAS" television analyzing system (West Germany). The mitotic index (MI, in promille) and the ratio of the phases of mitosis were calculated, and pathological mitoses were counted. All procedures of determining the parameters and scanning the preparations were undertaken in accordance with an algorithm and programs specially worked out for this purpose in the Institute of Physiology, Academy of Sciences of the Belorussian SSR. The image of the preparation mounted on the scanning stage of the microscope was led to the television monitor, where the basic parameters were recorded with the aid of a light pen. If a mitotic figure appeared on the screen of the monitor the coordinates of this field were memorized for subsequent presentation to the operator. After choice of an assigned number of mitotic figures each field containing a mitotic figure was presented to the investigator for spreading into a table generated on the screen of the monitor, in which the corresponding zones were activated by the light pen. The results were presented in the form of data blocks and recorded on magnetic tape. The printouts and records of measurement of each preparation were printed on a digital printer after appropriate statistical analysis. The time course of the changes was analyzed on an "HP-85" computer, using the method of regression analysis and approximation of the data by analytical functions.

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TABLE 1. Percentage of Pathological Mitoses in MDCK Culture under the Influence of Rimantidine and Deutiphorin

Chemotherapeutic agent	Dose, μg/ml	Time of incubation of cells with chemotherapeutic agents, h			
		2	4	6	8
K[1]		1,6	2,3	1,5	0,9
Rimantidine	5	1,2	2,0	1,9	1,3
	10	1,5	1,8	1,9	2,1
	25	2,3	3,2	3,6	2,8
	50	1,7	2,9	3,8	6,2
	100	1,8	5,3	13,8	15,0
Deutiphorin	5	1,5	2,0	2,0	2,3
	10	1,7	2,3	1,7	2,1
	25	1,7	2,4	2,3	2,3
	50	1,8	2,2	3,1	3,6
	100	2,0	5,6	5,0	4,8
	500	2,4	17,0	28,0	17,0

Legend. K[1]) Intact MDCK culture.

EXPERIMENTAL METHOD

As the writers showed previously, deutiphorin and rimantidine had a triphasic inhibitory action on proliferation, the degree of depression of mitotic activity of the cells depending on the dose of the chemotherapeutic agents and the duration of their combined culture. In an intact MDCK culture the total percentage of pathological mitoses at the beginning of the stationary phase varied from 1.5 to 3, toward the end of passage as a rule it did not exceed 5-7, and only in one or two series of experiments did it reach 12.5. This increase in the number of pathological mitoses in intact cultures, as we know, may be connected with the conditions of culture, and the number of passages after thawing [1]. The following main types of pathological mitoses were observed: holding up of the cells in metakinesis, scattering of the chromosomes in metakinesis, asymmetrical mitoses, swelling and fusion of chromosomes, delay or absence of cytotomy, and also a very small number of degenerating mitoses, not more than 0.8-1.1% of the total number of pathological mitoses. In one series of experiments, incidentally, the number of degenerating mitoses in the intact culture increased to 15-17% of the total number of pathological mitoses, possibly due to the duration of passage or the unstandardized conditions of cell culture [5, 6].

Treatment with the chemotherapeutic agents caused an increase in the number of pathological mitoses in the cell culture. This increase was directly proportional to the doses of the chemotherapeutic agents used, and also to the duration of their coculture with the cells (Table 1, Fig. 1a). The dose-dependent increase in the total number of pathological mitoses took place simultaneously with redistribution of the mitoses among the groups. When the agents were used even in the highest concentrations (rimantidine 50 and 100 μg/ml, deutiphorin 100 and 500 μg/ml) no significant increase in the percentage of pathological mitoses connected with damage to chromosomes or the mitotic apparatus could be observed (Fig. 1b, c). Meanwhile there was a relative decrease in the number of pathological mitoses connected with damage to the chromosome and an increase in the number of mitoses with disturbances of cytotomy and the number of degenerating mitoses (Fig. 1d).

The study of the mitotic regimes showed that rimantidine and deutiphorin had no significant effect on the ratio between the phases of mitosis for 24 h after addition to the culture in all concentrations studied. With a further increase in the duration of exposure of the cells to the chemotherapeutic agents for 24-96 h there was a progressive increase in the number of metaphase mitotic figures (Fig. 2). Regression analysis of the delay of mitoses in metaphase, undertaken separately for times of observation of 24, 48, 72, and 96 h, revealed a close linear relationship between the number of metaphase mitotic figures and the dose of the agents. High coefficients of correlation, characterizing close correlation between the dose of the agent and its effect on the cell, were discovered under these circumstances, namely 0.989 for a duration of exposure to deutiphorin of 24 h, 0.968 for one of 48 h, 1.00 for 72 h, and 0.999 for 96 h. Meanwhile regression analysis of dependence of delay of mitosis in metaphase on the duration of action of the agents (rimantidine in doses of 50 and 100 μg/ml and deutiphorin in doses of 100 and 500 μg/ml) revealed an exponential type of relationship, and it was linear only for lower doses. Incidentally, in a cell culture incubated with the test agents, no new types of pathology of mitosis not typically found in the intact culture were discovered, with the exception of a group of aberrations connected with delay of mitosis in metaphase, and this may perhaps be indirect evidence of the stathmokinetic action of rimantidine and deutiphorin [2].

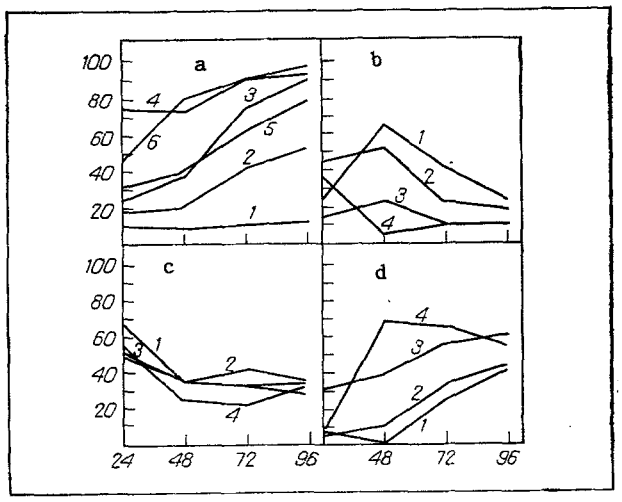

Fig. 1. Changes in number of pathological mitoses in MDCK cells and their redistribution among basic groups of pathological mitoses under the influence of rimantidine and deutiphorin. a) Total number of pathological mitoses in intact cell culture (1), in cell culture treated with deutiphorin in doses of 50 (2), 100 (3), and 500 μg/ml (4), and in cell culture treated with rimantidine in doses of 50 (5) and 100 μg/ml (6); b) number of pathological mitoses connected with damage to chromosomes in intact culture (1), in cell culture treated with deutiphorin in doses of 100 (2) and 500 μg/ml (3), and in cell culture treated with rimantidine in a dose of 100 μg/ml (4); c) number of pathological mitoses connected with damage to mitotic apparatus in intact cell culture (1), in cell culture treated with deutiphorin in doses of 100 (2) and 500 μg/ml (3), and in cell culture treated with rimantidine in a dose of 100 μg/ml (4); d) number of pathologically changed mitoses connected with disturbances of cytotomy and degenerating mitoses in intact cell culture (1), in cell culture treated with deutiphorin in doses of 100 (2) and 500 μg/ml (3), and in cell culture treated with rimantidine in a dose of 100 μg/ml (4). During calculation of total number of pathological mitoses, the total number of cells in the culture was taken as 100%. When redistribution of mitoses among groups was calculated, the total number of pathological mitoses was taken as 100%. Abscissa, time of coculture of cells with agents (in h); ordinate, number of mitoses (in %).

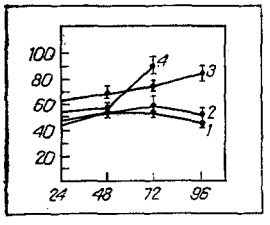

Fig. 2. Increase in number of metaphase mitotic figures under the influence of rimantidine and deutiphorin during their long-term culture (up to 4 days) with MDCK cells. 1) Number of metaphase mitotic figures in intact cell culture; 2, 3, 4) number of metaphase mitotic figures in cell culture treated with deutiphorin in doses of 100 μg/ml and 500 μg/ml and rimantidine in a dose of 100 μg/ml respectively. Abscissa, duration of coculture of cells with agents (in h); ordinate, number of metaphase mitotic figures (in % of total number of all phases of mitosis).

The ability of rimantidine and deutiphorin to distort reproductive processes in cells and thus to make perfect reproduction of viruses impossible suggests a probable action of these chemotherapeutic agents by a mechanism of "chemical preparation for virus-induced cytolysis," which can be represented as follows. Acting on the cell, chemotherapeutic agents modify its metabolism and convert it into an unstable state; additional action of the virus on these "chemically sensitized" cells leads to their death and elimination from the cell population. It can be tentatively suggested that such a mechanism also takes place in cases when infection precedes exposure to chemotherapy. From this point of view it is possible to understand the increased sensitivity of infected cells to the cytotoxic and cytodestructive action of several chemotherapeutic agents [3]. It is important to note that "chemical treatment" of cells with the aid of triggering the mechanism of the antiviral action of the agents can take place within a zone of concentrations nontoxic for the cell population.

The results obtained in this study on the ability of rimantidine and deutiphorin to induce pathology of the reproductive apparatus in a culture of MDCK cells may, in our view, be interesting in connection with a study of the toxic properties of chemotherapeutic agents, and it may also broaden existing knowledge of the possible mechanisms of their antiviral action.

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