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REGULATION OF THE EXCISION REPAIR SYSTEM IN HUMAN CELLS CULTURED IN VITRO BY MEASLES VIRUSES, DEPENDING ON THEIR ATTENUATION

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UDC 578.831.21:578.242.46

KEY WORDS: excision repair; UV radiation; attenuated and wild-type strains of measles virus.

Viruses may have a modifying effect on the excision repair system, either suppressing it [1, 2] or stimulating individual stages of this process. The effect of attenuated measles virus on the repair process is of practical as well as theoretical interest, because vaccination with attenuated virus is carried out annually on tens of thousands of children.

Accordingly, this paper describes the results of a comparative study of the effect of "wild-type" and attenuated strains of measles virus on different stages of excision repair induced by UV irradiation. Determination of activity of the system repairing DNA injuries produced by this mutagen is important because UV radiation is an ecologic factor to whose action vaccinated children are constantly exposed. Moreover, most chemical mutagens have an action of UV type, i.e., DNA repair requires involvement of basically the same enzyme systems. Consequently, information obtained with the use of UV radiation may be correct to some degree also for chemical substances of UV type which are widespread in the external environment.

EXPERIMENTAL METHOD

Experiments were carried out on a transplantable culture of human cells of line L-41. Two strains of measles virus were used: vaccine strain L-16 and the "wild-type" Edmonston strain. Cells were infected in suspension and the multiplicity of infection was 0.01-0.1 TCD_{50} per cell. As the source of UV radiation, two BUF-15 lamps (254 nm) were used. Activity of the initial stage of excision repair was investigated by a radiochromatographic method [5], whereby the number of thymine dimers formed during UV-radiation and their "excision" could be determined under postradiation conditions of incubation. With this aim, cells growing in a monolayer were irradiated 24 h after infection in a dose of 20 J/m^2. The content of thymine dimers in the cell DNA was determined immediately after irradiation and after 12 and 24 h of postradiation incubation. The number of thymine dimers was calculated by the equation:

$$\text{Number of dimers} = \frac{\text{Count in region of thymine dimers (in cpm)}}{\text{Count in region of thymine (in cpm)}} \times 100\%.$$

Reparative DNA synthesis activated by UV radiation was studied by a liquid scintillation method based on incorporation of [^{3}H]thymidine into the total mass of cells, with suppression of replicative DNA synthesis by hydroxyurea. The intensity of reparative synthesis was judged from the value of the stimulation index (SI), which is the ratio between the radioactivity counts (in cpm) in the irradiated cells to the radioactivity count in unirradiated cells.

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TABLE 1. Effect of Measles Infection on Repair of UV-Induced Injuries to DNA in Human L-41 Cells

Test object	Reparative DNA synthesis			Repair of DNA breaks			
	total radioactivity of 10^6 cells, cpm		SI	number of DNA breaks per dalton × 10^{-9}		efficiency of repair, %	coefficient of repair, %
	before UV-irradiation	after UV-irradiation		8 h after UV-irradiation	24 h after UV-irradiation		
Uninfected cells	7930±957	11102±1140*	1,39	2,5	0,3	76	88
Cells infected with measles vaccine virus (0.01 TCD_{50} per cell)	5955±991	11910±2040*	1,98	2,8	0,7	65	75
Cells infected with measles vaccine virus (0.1 TCD_{50} per cell)	5276±870	7389±963	1,45	2,8	1,0	57	68
Cells infected with measles vaccine virus (0.01 TCD_{50} per cell)	4173±710	4590±630	1,12	0,3	2,3	0	0

Legend. *$P < 0.05$.

To study recombination of DNA breaks formed as a result of removal of injuries in the course of excision repair the molecular weight of DNA was determined in the control and infected cells after 8 and 24 h of postradiation incubation, by ultracentrifugation of cell lysates in alkaline sucrose gradients [4]. The molecular weight of the treated and untreated cells was used to calculate the number of DNA breaks (per dalton), the efficiency of repair, and the fraction of breaks repaired (the repair coefficient). The experimental data were subjected to statistical analysis by the Fisher–Student method [3].

EXPERIMENTAL RESULTS

The study of the effect of measles infection on excision of UV-induced thymine dimers showed that the number of dimers in cells infected with measles vaccine virus during 12 h of postradiation incubation was reduced by 54% (from 1.09 ± 0.03 to 0.49 ± 0.04%) and after 24 h, it was reduced by 70% (to 0.34 ± 0.01%), whereas in uninfected cultures during 12 h after irradiation the number of dimers fell by 35% (from 1.20 ± 0.09 to 0.76 ± 0.01%), and after 24 h by 54% (to 0.56 ± 0.14%). Attenuated measles virus thus stimulated "excision" of induced DNA injuries, as a result of which, toward the end of the period of observation the number of unexcised injuries still remaining in the infected cells was 20% less than in the control (uninfected) cells. By contrast with attenuated virus, the "wild-type" strain, in the same infecting dose, caused inhibition of the excision stage of repair. During 12 h after UV-irradiation the number of thymine dimers in cells infected with this virus was reduced by only 20% (from 0.74 ± 0.05 to 0.59 ± 0.03%), whereas after 24 h only 32% of induced injuries to DNA were "excised."

The inhibitory effect of the "wild-type" strain on the reparative ability of the cells, obtained in this series of experiments, may perhaps be connected with its higher cytopathic activity compared with that of attenuated virus, observed with the same infecting dose of the cultures. In the next series of experiments, yet another dose of vaccine virus was therefore used, namely 0.1 TCD_{50} per cell, which gave a cytopathic effect similar to that observed in cultures infected with "wild type" virus in a dose of 0.01 TCD_{50} per cell.

The results obtained during a study of the effect of different doses of measles vaccine virus on activity of reparative DNA synthesis induced by UV-irradiation demonstrated the stimulating effect of this virus in a multiplicity of infection of 0.01 TCD_{50} per cell (Table 1). The stimulation index, reflecting the intensity of reparative DNA synthesis, was significantly higher in these cultures than the infected cultures ($P < 0.05$). On infection of the cells with vaccine virus in a higher dose (0.1 TCD_{50} per cell) no significant differences were found in the level of reparative synthesis between infected and control cultures. Infection of cells with the "wild type" strain of virus had an inhibitory effect on activity of reparative DNA synthesis induced by UV-irradiation. The results of these experiments thus correlated with those of a study of the activity of "excision" of thymine dimers from DNA of irradiated cells.

Restoration of the structural integrity of DNA (results of sedimentation analysis) in uninfected cells and cells infected with measles vaccine virus in a dose of 0.01 TCD_{50} per cell took place with equal efficiency. As Table 1 shows, the coefficient of repair 24 h after irradiation of the infected cells was 77% compared with 88% in the control ($P > 0.05$). On infection of the cells with measles vaccine virus in a dose of 0.1 TCD_{50} per cell, inhibition of repair of DNA breaks during 24 h after irradiation was observed. Meanwhile incision breaks in DNA

in these cells appeared at the same or a higher rate than in uninfected cells, evidence of the absence of an inhibitory effect of the vaccine virus on the initial stages of repair. In a study of cells infected with the "wild-type" strain of virus the rate of appearance of incision breaks in DNA after irradiation was found to be sharply reduced, and repair of the breaks did not take place during 24 h of postradiation incubation.

The results provide a complete picture of the nature of excision repair of DNA injuries induced by UV-irradiation in human cells infected with different strains of measles virus. Although the cytopathic effect was similar in this case, unlike the "wild-type" strain, measles vaccine virus had no effect on activity of reparative DNA synthesis.

On the one hand, therefore, confirmation of the hypothesis that the effect of the virus on reparative activity of the cell depends on multiplicity of infection was obtained. On the other hand, the fact that, despite the similar cytopathic action of the vaccine and "wild-type" strains, differences in their effect on repair processes in the cell still persisted, may confirm the role of genetic differences between viruses in the effects produced. The study of the effect of attenuated virus on function of an older cell mechanism on the evolutionary scale, namely repair of UV-injuries in DNA, can serve as a criterion for evaluation of the safety of vaccine preparations used to immunize children. If the vaccine strain was able to inhibit the repair system, its use in children in contact with natural forces (UV radiation), with possible medical procedures (x-ray irradiation, medication), and with definite pressure from environmental pollutants, may be a hazard from the point of view of increasing the frequency of virus-induced chromosomal aberrations in human cells. Under the conditions of stimulation of repair by attenuated measles virus, which we found, lowering of the level of virus-induced chromosomal aberrations can be postulated.

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EFFECT OF 1,2:5,6-DIANHYDROGALACTITOL AND 1,2:5,6-DIANHYDRO-3,4-DIACETYLDIANHYDROGALACTITOL ON DNA SYNTHESIS BY CELLS OF MOUSE MELANOMA B16, BONE MARROW, GASTROINTESTINAL MUCOSA, SPLEEN, AND LIVER

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UDC 547.963.32

KEY WORDS: 1,2:5,6-dianhydrogalactitol; 1,2;5,6-dianhydro-3,4-diacetyldianhydrogalactitol; DNA synthesis; melanoma B16.

The antitumor preparation dianhydrogalactitol, a bifunctional alkylating agent, is now used on a fairly wide scale in combination chemotherapy of human solid tumors [7].

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(Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 301-303, September, 1984. Original article submitted September 5, 1983.

0007-4888/84/9809-1203$08.50