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

Experiments in vivo on mice with Ehrlich's ascites carcinoma have shown that this preparation induces reversible accumulation of tumor cells in the S and $G_2 \rightarrow M$ phases of the cell cycle and potentiates the cytotoxic action of phase-specific agents such as 1-β-D-arabinofuranosylcytosine and bleomycin [3].

Dianhydrogalactitol, like most alkylating agents, causes death not only of tumor cells, but also of normal dividing cells (bone marrow, epithelium of the small intestine, spleen). Synthesis of new analogs of this compound with a more selective action and the study of their biochemical mechanisms of action are urgent matters.

In the investigation described below the effect of 1,2:5,6-dianhydrogalactitol (DAG) and of 1,2:5,6-dianhydro-3,4-diacetyldianhydrogalactitol (Diac-DAG) on DNA synthesis was studied in neoplastic and normal cells from the bone marrow, small intestinal epithelium, spleen, and liver of mice with melanoma B16 at different times after administration of the compound.

EXPERIMENTAL METHOD

(CBA × C57Bl/6)F_1 mice weighing 18-20 g, into which solid tumors (melanoma B16) were inoculated subcutaneously, were used.

The antitumor preparations DAG and Diac-DAG, whose synthesis was described previously [4, 10], were injected into the mice in therapeutic doses (5 and 12 mg/kg respectively) once, intraperitoneally, on the 12th day after inoculation, when the weight of the tumor was about 1 g (time 0). Immediately before the beginning of the experiment the compounds were dissolved in 0.9% NaCl solution. The investigations were carried out during the period of active tumor growth (on the 12th-16th day), when the weight of the melanoma B16 had doubled itself, but DNA synthesis remained virtually unchanged within this time interval [1].

DNA synthesis in cells of melanoma B16, bone marrow, small intestinal epithelium, spleen, and liver was studied by measuring incorporation of [2-^{14}C]thymidine, injected in a dose of 2 μCi per mouse (specific activity 54 μCi/mmole). It was shown previously that incorporation of the labeled precursor under these experimental conditions is blocked after injection of the replication inhibitor, hydroxyurea [2]. The mice were killed (five animals in each group) 1 h after injection of labeled thymidine and at definite time intervals after injection of the compounds (6, 12, 24, 48, 72, and 96 h). The organs and tissues of the animals in each group were pooled and homogenized in a glass homogenizer at 4°C in medium containing 0.25 M sucrose, 0.3 M Tris-HCl (pH 7.6), 0.025 M KCl, and 0.005 M $MgCl_2$. From each fraction samples of 0.1 ml homogenate were transferred into test tubes, 4 ml of cold 10% TCA was added to each tube, after which the tubes were placed in an ice bath and left for 30 min at 0°C. The samples were then transferred to Millipore filters with a pore diameter of 1.5 μm (Synpor, from Chemapol, Czechoslovakia), and the residues were washed with cold 5% TCA and dried at 50-60°C. Radioactivity in the samples was determined by means of Mark II scintillation counter (from Nuclear Chicago, USA). To determine radioactivity the Millipore filters were placed in toluene scintillator containing 5 g PPO and 0.3 g POPOP in 1 ml toluene. The DNA content in the tumor cells and each fraction of homogenate of the animal organs was determined by Schmidt and Thannhauser's method in the modification of Fleck and Monro.

EXPERIMENTAL RESULTS

Kinetic curves of incorporation of [2-^{14}C]thymidine into DNA of melanoma B16 and mouse organ cells after injection of DAG and Diac-DAG into the mice with tumors are shown in Fig. 1. Both preparations 24 h after injection caused maximal inhibition of DNA synthesis in melanoma B16 cells (70-90%), after which DNA synthesis was only partially restored, and during the next 3 days the inhibitory effect continued at the 60% level.

Disturbances in DNA synthesis may be due to injury to the DNA template or inactivation of DNA-polymerases. DAG, in experiments in vitro, alkylates DNA with the formation of N-7-monogalactitylguanine and 1,6-dideoxy-1,6-di(guanin-7-yl)galactitol [5, 9]. The formation of intramolecular cross-linkages during incubation of Yoshida sarcoma cells or preparations of isolated DNA with DAG has been demonstrated by physicochemical methods [5, 6]. It can be tentatively suggested that similar modifications to the DNA template were responsible for the changes observed in DNA synthesis.

DNA synthesis was inhibited maximally 24 h after injection of DAG and Diac-DAG in the organs of mice with tumors, just as in melanomia B16. However, the rate of recovery of DNA synthesis differed in the normal and tumor cells. The more rapid recovery of DNA synthesis in normal cells than in tumor cells will be noted (Fig. 1).

A similar differential effect was observed during a study of the injuring action of various antitumor preparations and, in particular, of N-nitrosourea, on DNA synthesis [2, 11], and it may perhaps be linked with the

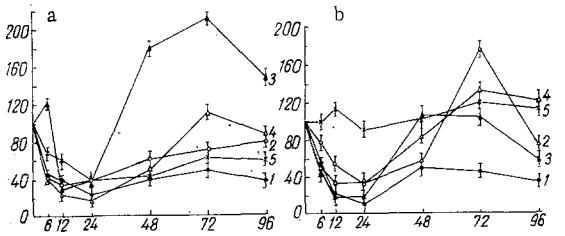

Fig. 1. Effect of DAG (a) and Diac-DAG (b) on incorporation of [2-^{14}C]thymidine into DNA of cells of mouse melanoma B16, bone marrow, small intestinal epithelium, spleen, and liver. Abscissa, time after injection of compound (in h); ordinate, specific radioactivity (in %). Incorporation of labeled thymidine into DNA of cells of mouse melanoma B16 (1), bone marrow (2), small intestinal epithelium (c), spleen (4), and liver (5) was 27,205, 105,490, 69,053, 39,929, and 15,000 cpm/mg DNA respectively.

formation of a greater number of intramolecular cross-linkages in the DNA molecule of tumor cells compared with normal cells, as has recently been demonstrated for the preparation mephalan [8].

It will be clear that the inhibitory effect of Diac-DAG on DNA synthesis in bone marrow and spleen cells was weaker than that of DAG (Fig. 1).

Unlike DAG, however, Diac-DAG had virtually no effect on DNA synthesis in liver cells, which could indicate that this compound does not exhibit hepatotoxicity (Fig. 1).

The higher therapeutic index of Diac-DAG (3.4) compared with DAG (1.7) [12] is probably due not only to the direct cytotoxic action on the tumor, but also to the lower toxicity of Diac-DAG to the vitally important systems of the body.

During the planning of optimal schemes of treatment of malignant neoplasms with Diac-DAG, there would seem to be a good case for giving a second injection of the compound after an interval of not less than 60 h, i.e., at a time of complete restoration of DNA synthesis in normal actively dividing cells, which would reduce its toxic action on bone marrow, the epithelium of the small intestine, and the spleen.

The authors are grateful to Dr. S. Somfai-Relle for taking part in the discussion of the results.

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