Influence of cis-Dichlorodiammineplatinum (II) on Growth of SV 40 Virus in Green Monkey Kidney Cells

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With 1 Figure

Received April 28, 1972

Summary

Cis-dichlorodiammineplatinum (II) partially inhibits the replication of SV 40 virus in green monkey kidney cells. The results of both immunofluorescence and complement-fixation tests indicated that the production of the T antigen of SV 40 was unaltered, but V antigen production was suppressed. The partial block of V antigen synthesis was most marked in the early period after infection, subsequently it was less pronounced. Both infectious virus production and V antigen formation were most markedly inhibited in cells treated with DDP both before and after infection.

1. Introduction

The complex platinum compound, cis-dichlorodiammineplatinum (II) (referred to as DDP) has been reported to exhibit a variety of biologic effects. The presence of DDP in the nutrient medium can inhibit cell division in Escherichia coli and cause the development of long filamentous forms (8). DDP has also been shown to exhibit anti-tumour activity against Ehrlich ascites tumour in mice (3), Sarcoma 180 and Leukemia L 1200 in mice (9) as well as against Dunning ascitic leukemia and Walker 256 carcinosarcoma in rats (5). DDP has also been found to be a potent inducer of phage in some lysogenic bacteria (7). Further, it has been demonstrated that the percentage of Epstein-Barr virus positive cells increases after treatment of EB 3 Burkitt lymphoma cells with DDP (11).

In seeking the mechanism of the DDP action in Ehrlich ascites tumor cells, Howle and Gale (3) reported the preferential inhibition of the DNA synthesis, whereas the inhibition of RNA and protein syntheses were less marked. The results obtained by Harder and Rosenberg (2) in cultures of human amnion AV 3 cells were similar. Also Kára et al. (4) observed the inhibitory effect of DDP on the

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DNA synthesis and growth of rat sarcoma cells and Wistar rat embryo fibroblasts. This paper describes the inhibitory effect of DDP on the reproduction of SV 40 virus in green monkey kidney cells.

2. Materials and Methods

2.1. Virus

SV 40 virus was characterized in previous studies (10). The virus stock was stored at −30°C.

2.2. Cells

Secondary cultures from green monkey (Cercopithecus aethiops) kidney (GMK) cells were used. They were grown and maintained in a mixture of Melnick's medium B and Parker (199) medium, supplemented with 2% calf serum, 0.15% bicarbonate and antibiotics.

2.3. Inhibitors

Crystalline eis-dichlorodiammineplatinum (II) was obtained from Dr. B. Rosenberg. The substance was dissolved in saline at a concentration of 0.3 mg/ml (= 1 mMolar). Freshly prepared stock solutions were used in all experiments. The desired DDP concentration was achieved by diluting the stock solution in maintenance medium.

Commercially available 1-3-D Arabino-furanosyl-cytosine (ara-C) (The Upjohn Company, Kalamazoo, Michigan, U.S.A.) was dissolved in saline at a concentration of 0.2 mg/ml. The solution was stored in the dark at −30°C.

2.4. Virus Growth Experiments

A concentration of 3 μg of DDP/ml (= 10 μMolar) was used. The previous tests had revealed that at this concentration the substance when present in the medium employed was not toxic for the GMK cells and did not inactivate SV 40 over a period of 96 hours. The influence of DDP treatment on SV 40 growth was studied as follows. The medium was replaced in well grown tube cultures from GMK cells was replaced by fresh medium with or without DDP. After 20 hours incubation at 37°C, the medium was drained, the cells were washed and SV 40 virus was inoculated. The virus adsorption proceeded for 2 hours at 37°C; then the unadsorbed virus was rinsed out. To half of each of the DDP treated and untreated cultures 1.5 ml of medium with DDP was added; the other half of the cultures received medium without DDP. The cultures were placed in a roller drum (at 37°C). At intervals three tube cultures from each of the four groups were sampled and placed at −20°C. After all samples had been collected, the cultures were submitted to three cycles of freezing and thawing. Parallel cultures were pooled and cell debris was removed by low-speed centrifugation. For infectious virus titrations tube cultures from secondary GMK cells were used, five tubes per each ten-fold dilution. The infected cultures were incubated at 37°C in a roller drum. After 6 to 7 days the maintenance medium was replaced. The final reading was 19–21 days after inoculation.

2.5. Immunofluorescence (IF) Test

Approximately 2 × 10^6 GMK cells in 5 ml of medium were seeded into 60 mm Petri dishes containing cover slips. The cultures were incubated at 37°C in an atmosphere with 5% CO2. When confluent cell sheets were formed, the cultivation medium was replaced by fresh medium with or without 3 μg of DDP or 10 μg of ara-C/ml. After 20 hour incubation at 37°C, the cultures were washed with PBS and inoculated with SV 40. The virus adsorption proceeded at 37°C for 2 hours. Thereafter the inoculum was removed and infected cells were washed three times with PBS. Five ml of medium with or without inhibitor were then added to the cultures which were again placed at 37°C. At intervals, several cover slips were taken from each of the four culture groups, and examined in an indirect IF test for the presence of SV 40 tumor (T) and viral (V) antigens as described previously (10). A serum pool from hamsters bearing tumors.