Anticellular Effects of 9-(2-Hydroxyethoxymethyl) Guanine Against Herpes Simplex Virus-transformed Cells

(Accepted 30 May 1979)

SUMMARY

The guanine derivative 9-(2-hydroxyethoxymethyl) guanine (acycloguanosine), a potent inhibitor of herpes simplex virus (HSV) multiplication, was found to have marked anticellular activity against HSV-transformed thymidine kinase-positive cells. HSV type 1-transformed cells were more sensitive to the drug than HSV type 2-transformed cells.

Recent studies have indicated that 9-(2-hydroxyethoxymethyl) guanine (acycloguanosine) is a more effective antiviral drug against herpes simplex virus (HSV) than any previously known compound (Elion et al. 1977; Schaeffer et al. 1978). Acycloguanosine is selectively converted to a monophosphate by HSV-induced thymidine kinase (TK) and subsequently is converted to acycloguanosine di- and triphosphates. Acycloguanosine triphosphate inhibits HSV DNA polymerase and thereby inhibits virus replication. The same investigators also suggested that incorporation of acycloguanosine might result in chain termination of replicating virus DNA. A preliminary report has suggested that the compound inhibits replication of HSV-transformed cells (Furman et al. 1978). These observations have prompted us to examine whether acycloguanosine has specific anticellular activity against a variety of HSV-transformed cells produced in our laboratory.

A mouse cell line lacking TK activity (N clA cl10-TK-) obtained from Dr R. Goldberg of the National Institutes of Health was grown in Dulbecco's modified Eagle's medium containing 10% foetal calf serum and 20 µg/ml of 5-bromodeoxyuridine (BrdUrd). The BrdUrd was not added to medium in cell passages preceding an experiment. N clA cl10-TK-/HSV-1(NT) and N clA cl10-TK-/HSV-2(333) are cell lines converted to the TK-positive phenotype by infection of N clA cl10-TK- cells with ultraviolet (u.v.)-irradiated HSV-1 strain NT and HSV-2 strain 333, respectively. These cells were maintained in TK-selective MTAGG medium (Dulbecco's growth medium supplemented with 4 µg/ml of thymidine, 13 µg/ml of adenosine, 14 µg/ml of guanosine, 75 µg/ml of glycine and 0.28 µg/ml of methotrexate sodium) except for the passage immediately preceding an experiment. Revertant clones of N clA cl10-TK-/HSV-2(333) cells, referred to as H-2 BrdUrd, were selected for resistance to BrdUrd (20 µg/ml) and propagated in the same manner described for the parental N clA cl10-TK- cells. HSV-1 strain NT and HSV-2 strain 333 were primarily used throughout this study (Rapp & Turner, 1978).

To determine the dose response of acycloguanosine antiviral activity, plaque inhibition tests were carried out. Monolayers of rabbit kidney cells were infected with approx. 150 p.f.u. of HSV-1 or HSV-2 in 60-mm plastic dishes. After a 1 h adsorption period at 35 °C, the cultures were overlaid with 5 ml of 0.5% agarose in Dulbecco's growth medium containing appropriate concentrations of acycloguanosine and then incubated for 3 to 4 days at 37 °C. The plaques were counted after fixation with 5% formalin and stained with 0.7% crystal violet. As shown in Fig. 1(a), HSV-1 plaque formation was inhibited by 0.25 µM of acycloguanosine and was completely suppressed by 2 µM; in contrast, HSV-2 plaque formation was not inhibited completely by 8 µM.

In the following experiments, the anticellular effect of acycloguanosine was studied in
Fig. 1. The dose response of acycloguanosine antiviral and anticellular action. (a) Antiviral activity was measured by plaque reduction assays on rabbit kidney cells using HSV-1 (○--○) and HSV-2 (□--□), and the plaque counts were expressed as a percentage of the number obtained in control cultures. (b) Anticellular activity was measured in N clA clIo-TK-/HSV-1(NT) (○--●), N clA clIo-TK-/HSV-2(333) (□--□), N clA clIo-TK - cells (●--●) and N clA clIo-TK + cells (△--△). The number of cells was expressed as a percentage of the number obtained in control cultures.

HSV-transformed and non-transformed N clA clIo cells (Fig. 1b). Cells dispersed in growth medium containing appropriate concentrations of acycloguanosine were seeded in 60-mm plastic dishes at a concentration of 4 × 10⁴ cells/ml (2 × 10⁵ cells/dish) and incubated in a 5% CO₂ atmosphere. After 4 days of cultivation, the cells were washed, trypsinized and counted with a haemocytometer. The growth of N clA clIo-TK-/HSV-1(NT) and N clA clIo-TK-/HSV-2(333) cells was strongly inhibited by 10 μM of acycloguanosine. N clA clIo-TK-/HSV-1(NT) cells were more sensitive to the drug than N clA clIo-TK-/HSV-2(333). On the other hand, N clA clIo-TK - and N clA clIo-TK + cells showed far more resistance and grew well in the presence of 10 μmol of acycloguanosine. Fig. 2 shows the growth curves of the four cell lines described above and the revertant cell lines in the presence of 50 μM-acycloguanosine. Four revertant clones were selected from N clA clIo-TK-/HSV-2(333) cells and examined for acycloguanosine resistance. Fig. 2(c), however, shows the results of only two clones, since all clones produced almost the same results. In experiments, similar to those described above, 50 μmol of acycloguanosine caused extensive cell death in both HSV-transformed cell lines, whereas cell growth of N clA clIo-TK-, N clA clIo-TK + and revertant cells was minimally inhibited. We also tested the effect of acycloguanosine on the growth of 333-8-9 and 14-021-81 cells established from hamster embryo cell cultures treated with u.v.-irradiated HSV (Rapp & Li, 1975). However, these transformed cell lines did not demonstrate significant differences in sensitivity to acycloguanosine when compared to baby hamster kidney and hamster embryo cells (data not shown). This may reflect only small amounts of virus TK in the hamster cells.

The present study showed that acycloguanosine has specific anticellular effects on HSV-transformed TK + cells as well as antiviral effects on HSV. Initial studies on the metabolism of this drug have shown that acycloguanosine is phosphorylated far more effectively by HSV-induced TK than by cellular TK. The high specificity of virus-induced TK with this compound may be responsible for its anticellular effect against virus TK-positive cells, but a detailed mechanism by which acycloguanosine kills the cells is still unclear. One possible explanation is that cellular DNA synthesis may be blocked by incorporation of acycloguanosine into cellular DNA. The results described suggest that morphologically
transformed cells containing virus TK are specifically killed by acycloguanosine. It has been shown that HSV-2-specific TK activity is expressed not only in rat cells transformed by HSV-2 temperature-sensitive mutant 1, but also in about 50% of the tumour cells obtained a year later from rats injected as newborns with the transformed cells (J. M. Macnab, L. Visser, A. T. Jamieson and J. Hay, unpublished data). Although we could not show distinct effects of acycloguanosine on the growth of two HSV-transformed hamster embryo cell lines, it will be necessary to examine: (i) the frequency of incorporation of the HSV TK gene in morphologically transformed cells; (ii) the stability of maintenance of the HSV TK gene (Sugino et al. 1977); and (iii) the regulation of expression of the TK gene (Davidson et al. 1973; Kit & Dubbs, 1977).

We thank Koichi Yamanishi and Nancy Turner for their helpful discussions. This investigation was supported by contract NoI CP 53516 within the Virus Cancer Program of the National Cancer Institute and by grant CA 18450 awarded by the National Cancer Institute. The acycloguanosine was kindly supplied by Dr G. Elion, The Wellcome Research Laboratories, Burroughs-Wellcome Co., Research Triangle Park, NC, U.S.A.

Department of Microbiology
and Specialized Cancer Research Center
The Pennsylvania State University
College of Medicine
Hershey, Pennsylvania 17033, U.S.A.
REFERENCES


(Received 26 January 1979)