

Inhibition of Herpes Simplex Virus Replication by Methyl Daunosamine

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Received 8 March 1982/Accepted 7 May 1982

Methyl daunosamine inhibited the replication of herpes simplex virus type 1 in a dose-dependent manner. The growth of the host Vero cells was not affected by daunosamine levels that had significant antiviral activity (2.5 mM) but was inhibited by concentrations of 5 mM or greater. Methyl daunosamine appears to be unique among the sugars with antiviral activity because at antiviral concentrations it did not inhibit the glycosylation of macromolecules.

Adriamycin is an anthracycline antibiotic commonly used in cancer chemotherapy. It also exerts antiviral activity against herpesvirus and vaccinia virus (2). However, at the drug concentration studied (1 μ M), adriamycin was cytotoxic and produced cell death. Because of our interests in anthracyclines and in the antiviral activity of 2-deoxy-D-glucose and other sugar analogs, we tested the ability of a methylated analog of the C₆ sugar portion of the adriamycin molecule, methyl 3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranoside (methyl daunosamine), to inhibit the replication of herpes simplex virus type 1 (HSV-1).

Effect of methyl daunosamine on HSV-1 replication. Monolayers of Vero cells in 16-mm culture wells (Costar, Cambridge, Mass.) grown in modified essential medium with Earle salts (Gibco Laboratories, Grand Island, N.Y.) and 10% newborn calf serum (Flow Laboratories, McLean, Va.) were infected at 2.5 PFU per cell with HSV-1 strain CL-101. At the end of a 1-h adsorption period at 37°C, the inocula were removed, the cells were washed with phosphate-buffered saline, and fresh medium with or without methyl daunosamine (Pfanstiehl Laboratories Inc., Waukegan, Ill.) was added. The cultures were frozen 24 h after infection at -70°C, and, after thawing, the total amount of virus present in the medium and associated with the cells was determined by a plaque assay. The virus was titrated in duplicate by infecting Vero cell monolayers with sequential 10-fold dilutions of the virus preparations. Medium containing immunoglobulin (Merck and Co., Rahway, N.J.) was added after a 1-h adsorption period, and the cells were incubated for 48 h. The medium was removed, the cells were stained with 1% methylene blue in water, and the plaques were scored.

A dose-response curve for the inhibition of HSV-1 replication by daunosamine is shown in Fig. 1. Significant inhibition of HSV-1 occurred at concentrations of 2.5 mM methyl daunosamine and above. This is similar to the dose-response curve for the inhibition by 2-deoxy-D-glucose of the replication of HSV-1 (3), fowl plague virus (7), Semiliki Forest virus (7), and respiratory syncytial virus (5). The amino sugar glucosamine exerts comparable activity against fowl plague virus, is slightly less active against Sindbis virus, and is a more potent inhibitor of Semiliki Forest virus replication (7). Several other sugars and sugar derivatives at concentrations of up to 10 mM or more were without effect on the replication of the viruses screened: mannose, galactose, fucose, 2-deoxy-D-galactose, ribose, arabinose, xylose, sorbose, fructose, 2-deoxyribose, rhamnose, glucuronic acid lactone, glucosaminic acid, galactosamine, α -methyl-D-glucopyranoside, and *N*-acetyl glucosamine (7). We also observed that 5 mM 3-*O*-methylglucose does not affect HSV-1 replication (unpublished data). Thus, the sugars and sugar derivatives capable of inhibiting virus replication (methyl daunosamine, glucosamine, 2-deoxy-D-glucose, and the 2-fluoro analogs of 2-deoxy-D-glucose, 2-deoxy-2-fluoro-D-glucose, and 2-deoxy-2-fluoro-D-mannose [10, 11] possess some unique structural features, since in general amino and deoxy sugars do not inhibit viral replication.

Effects of methyl daunosamine on macromolecule biosynthesis and accumulation. Monolayers of Vero cells in 16-mm culture wells grown in modified essential medium with Earle salts and 10% newborn calf serum were infected with HSV-1 strain CL-101 at a multiplicity of 2.5 PFU per cell. After 1 h of viral adsorption, the inocula were removed, the cells were washed with phosphate-buffered saline, and fresh medium containing 2% newborn calf serum with or without 2.5 mM methyl daunosamine was added.

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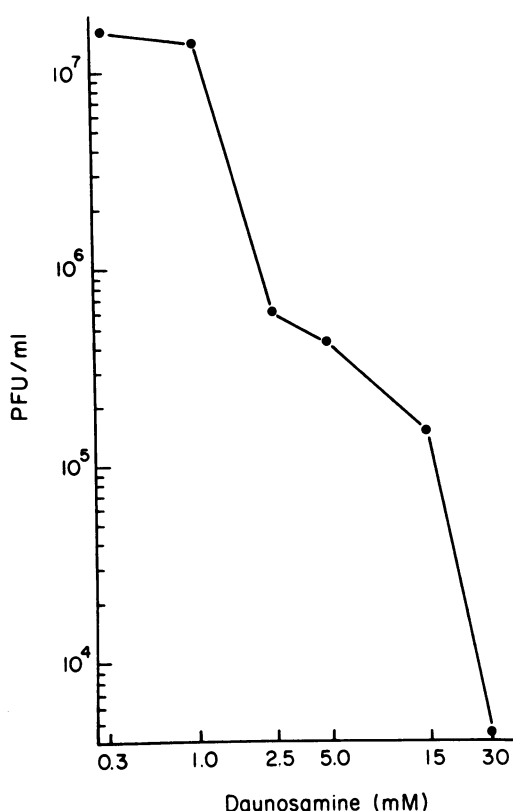


FIG. 1. Effect of methyl daunosamine on the yield of infectious HSV-1. Monolayers of Vero cells were infected at 2.5 PFU per cell. At the end of the adsorption period, fresh medium with or without methyl daunosamine was added. At 24 h after infection, the virus was harvested. The virus yield was determined by plaque assay in duplicate.

ed. At 3 h after the start of infection, 1 μ Ci of [14 C]thymidine (specific activity, 56 mCi/mM; Moravek Biochemicals, Inc., Brea, Calif.), 2 μ Ci of [3 H]uridine (specific activity, 20 Ci/mM;

Moravek), 0.5 μ Ci of [14 C]glucosamine (specific activity, 57.9 mCi/mM; Amersham Corp., Arlington Heights, Ill.), or 0.1 μ Ci of 14 C-protein hydrolysate (specific activity, 57 mCi/matom; Amersham) was added directly to the cultures in duplicate. The cells were harvested 20 h after infection by removing the medium and adding a lysis buffer of 1% Triton X-100 (Packard Instrument Co., La Grange, Ill.) in phosphate-buffered saline. The lysed cells were removed with a Pasteur pipette, and the culture wells were rinsed twice with lysis buffer. An equal volume of ice cold 10% trichloroacetic acid was added to each sample, and, after 10 min on ice, the precipitates were filtered through a 0.45- μ m Millipore filter type HA (Millipore Corp., Bedford, Mass.). The precipitates were washed three times with 5% trichloroacetic acid and once with 95% ethanol; after drying, they were counted in Econofluor (New England Nuclear Corp., Boston, Mass.) in a Beckman LS7500 scintillation counter (Beckman Instruments, Inc., Palo Alto, Calif.). Methyl daunosamine had only a modest effect on thymidine incorporation in both HSV-1-infected and uninfected Vero cells and, more significantly, did not inhibit amino acid incorporation or macromolecule glycosylation (Table 1). A 1-h pulse-labeling of macromolecule precursors at 5 to 6 h after infection provided results similar to those shown in Table 1 (data not shown), except that the inhibition of thymidine incorporation was 20% or less, so that the effects of methyl daunosamine on macromolecule synthesis and accumulation were similar. The inhibition of thymidine incorporation into macromolecules may be related to the antiviral action of methyl daunosamine, may be a low-level cytotoxic effect (see below), or may be the result of an effect on thymidine uptake or thymidine metabolite pool sizes. 2-Deoxy-D-glucose and D-glucosamine, at concentrations with antiviral activity comparable to that of 2.5 mM methyl daunosamine, both

TABLE 1. Effects of 2.5 mM methyl daunosamine on macromolecule synthesis in uninfected and HSV-1-infected Vero cells^a

Precursor	Uninfected cells		HSV-1-infected cells	
	cpm ^b	% of untreated control	cpm	% of untreated control
[14 C]deoxythymidine	47,200	65	39,500	75
[3 H]uridine	351,000	109	234,000	103
[14 C]glucosamine	24,600	91	45,200	98
14 C-amino acids	11,200	92	15,700	85

^a Vero cells were infected or mock infected with 2.5 PFU per cell in duplicate. Cells were labeled 3 to 20 h after infection as indicated and harvested at 20 h. Under identical conditions, 6 mM 2-deoxy-D-glucose reduced the incorporation of glucosamine in the macromolecules of infected and uninfected cells to 56 and 50%, respectively, of the untreated controls. HSV-1 grown in 6 mM 2-deoxy-D-glucose is 94 to 98% less infectious than control virus.

^b In the presence of methyl daunosamine.

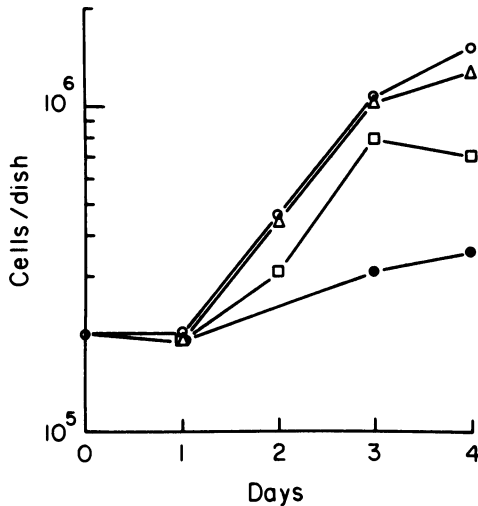


FIG. 2. Effect of methyl daunosamine on the growth of Vero cells. Cells, 2×10^5 per dish, were plated on day zero. On day 1, the medium was replaced with fresh medium with or without methyl daunosamine. Symbols: ○, control (no drug); △, 2.5 mM methyl daunosamine; □, 5 mM methyl daunosamine; ●, 15 mM methyl daunosamine. Cell numbers were determined in triplicate.

inhibit the incorporation of labeled sugars into the macromolecules of uninfected and virus-infected cells by 50 to 90% or more (4, 6, 9, 11, 12). The fluorosugars 2-deoxy-D-glucose and 2-deoxy-2-fluoro-D-mannose also affect protein glycosylation (10). Thus, methyl daunosamine appears to be unique as it is the only known sugar derivative with antiviral action that does not affect protein glycosylation.

Effect of daunosamine on Vero cell growth. One desirable characteristic for a useful antiviral agent is selective toxicity toward infected cells. Toxicity toward uninfected cells may present a severe limitation to the use of an antiviral agent. Thus, in the current work, it was important to determine the effects of daunosamine on the growth of uninfected host cells. Vero cells, 2×10^5 cells per dish, were plated in 35-mm culture dishes (Falcon Plastics, Oxnard, Calif.) on day zero. On day 1, fresh medium with or without daunosamine was added and remained present for the duration of the experiment. Cell numbers were determined with a hemacytometer in triplicate. Vero cell growth was virtually unaffected by 2.5 mM methyl daunosamine (Fig. 2), a concentration that inhibited HSV-1 replication by 1.3 log units (Fig. 1). The 50% effective dose was close to 5 mM since this concentration inhibited cell growth by 19% on day 3 and 53% on day 4. In contrast, glucosamine, the only other amino sugar with demonstrated antiviral

activity, is highly cytotoxic at antiviral concentrations (12). Even a 3-h exposure of glucosamine causes gross structural abnormalities in Ehrlich ascites and Sarcoma 180 cells (8).

Another sugar analog, 2-deoxy-D-glucose, has been reported to be promising for the topical treatment of venereal disease caused by HSV (1). It is relatively nontoxic to host cells as evidenced by the reversibility of the antiviral effects during the first 12 h of the virus replication cycle (5, 13). We have shown that a 24-h exposure of 2-deoxy-D-glucose does not inhibit Vero cell growth, but that a continuous exposure, as in the present experiments, inhibits Vero cell growth in a dose-dependent fashion (Spivack, Prusoff, and Tritton, submitted for publication). Since the antiviral and growth inhibitory activities of methyl daunosamine are very similar to those of 2-deoxy-D-glucose, this amino sugar deserves further study as a potentially useful agent for the treatment of HSV and perhaps other viral infections, particularly in combination with antiviral drugs that have different mechanisms of action.

This research was supported by U.S. Public Health Service grants CA 24955 and CA 28852 to T.R.T. and CA 05262 to W.H.P. from the National Cancer Institute, and grant CH 212 from the American Cancer Society and Research Career Development Award CA 00684 to T.R.T. J.G.S. was a predoctoral trainee under U.S. Public Health Service training grant CA 09085 from the National Cancer Institute.

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