Volume 231, number 1, 207-211 FEB 05774 April 1988

Megalomycin C, a macrolide antibiotic that blocks protein glycosylation and shows antiviral activity

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Received 24 December 1987; revised version received 14 February 1988

Megalomycin C, a natural macrolide antibiotic showed a potent antiherpetic activity. At concentrations that efficiently prevented HSV-1 multiplication, the compound had no cytotoxic or antiproliferative effects. Viral DNA and protein synthesis took place at normal levels in the presence of the antibiotic, suggesting that neither the translation of viral mRNA, nor the synthesis of viral nucleic acids was affected. The incorporation of mannose and galactosamine into viral proteins was blocked and precursor, but not mature, HSV-1 glycoproteins were detected in the presence of megalomycin C. Non-infectious HSV-1 viral particles were formed when the compound was present, but their glycoproteins were not properly glycosylated.

Megalomycin C; Protein glycosylation; Antiviral activity

1. INTRODUCTION

Glycosylation inhibitors have been devised as potential antiviral agents [1,2]. Although all such compounds interfere with the correct glycosylation of proteins, the effect on different viruses varies [3–11]. In fowl plague virus the glycoproteins synthesized in the presence of tunicamycin are degraded by proteases [5]. Underglycosylated G protein of vesicular stomatitis virus is not transported to the membrane and the egress of new virions is diminished [6]. Similarly, virions of Semliki Forest and Sindbis virus do not incorporate immature proteins [5,7]. With other viruses, such as influenza, some virion particles are formed in the presence of glycosylation inhibitors, but their glycoproteins are underglycosylated [8]. The absence of mature glycoproteins does not block the assembly of new herpes simplex virions [4,9–11]. Moreover, the viral particles thus formed are able to bind to and enter cells. However, these virions are non-infectious since they do not decapsidate [1]. In a search for new antiviral agents we found that megalomycin C, a macrolide antibiotic produced by *Micromonospora megalomicea*, showed a good antiviral activity against herpes simplex virus type 1 (HSV-1) and swine fever virus [3,12]. In this report we describe the mode of action of megalomycin C against HSV-1.

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2. MATERIALS AND METHODS

2.1. *Cells and viruses*

The source and propagation of HeLa cells and HSV-1 virus have been described [3,13]. To measure cell growth 10^5 HeLa cells per well were seeded in 24-well plates. Where appropriate cells were infected with HSV-1 at a multiplicity of 10 PFU per cell. Then, megalomycin C was immediately added and cells were incubated at 37°C as described in section 3. Cells were trypsinized and viable cells were counted by the trypan blue exclusion test in a Neubauer hemocytometer.

2.2. *Analysis of proteins by polyacrylamide gel electrophoresis*

After incubation of cells in methionine-free medium in the presence of 100 μCi of [^{35}S]methionine per ml for the period of time indicated in each experiment the proteins were analyzed as described [13]. To analyze the glycoproteins cells were incubated for the period of time indicated in each experiment in medium without glucose in the presence of 100 μCi/ml of D-[6-^{3}H]glucosamine (35 Ci/mmol), or D-[2,6-^{3}H]mannose

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00145793/88/$3.50

(45 Ci/mmol), or D-[6-^{3}H]galactose (30 Ci/mmol) per ml (all purchased from the Radiochemical Centre, Amersham). Labeling and sample preparation for electrophoresis were carried out as described [13].

2.3. *Purification of radiolabeled HSV-1 particles*

HeLa cells were infected with HSV-1 at 10 PFU/ml. At 10 h post infection megalomycin C was added and the cells labeled with [^{35}S]methionine (5 μCi/ml), or [^{3}H]glucosamine (20 μCi/ml). At 20 h post infection, the cells were harvested, disrupted as described above, and then centrifuged at 15000 × *g* for 10 min. Supernatants were layered onto a cushion containing 3 ml of 30% sucrose in 50 mM Tris-HCl, pH 7.4, 0.85% NaCl and centrifuged at 40000 rpm in a Sorvall Ti65 rotor. Pellets were dissolved in 50 μl of sample buffer, and electrophoresis was done as described above.

2.4. *Dot-blot analysis of HSV-1 DNA*

HeLa cells were grown in 60 mm dishes and infected with HSV-1 at 30 PFU/ml. After 16 h of infection, infected cells were collected and the DNA isolated as described [14]. Twofold dilutions of total DNA were spotted on a nitrocellulose filter and hybridized to a nick-translated ^{32}P-labeled pBR332 fragment, containing an *Eco*RI fragment which includes the TK gene of HSV-1. This plasmid was generously provided by Dr E. Tabarés (Madrid, Spain). Conditions for hybridization were described [15].

3. RESULTS

The presence of 10 μM megalomycin C in the culture medium inhibited the replication of HSV-1 in HeLa cells as observed by the prevention of the disruption of the cell monolayer (fig.1A). 50 μM megalomycin C fully prevented the cell monolayer from destruction by HSV-1 when the multiplicity of infection was lower than 0.5 PFU/cell, suggesting that this compound blocks the production of a new infectious virus. At these concentrations it had no cytotoxic effects as determined by phase-contrast microscopy and produced very little inhibition of protein synthesis in control cells even after 2 days of incubation (fig.1A). The reduction of infectious HSV-1 was about 2 logs in the presence of 50 μM megalomycin C (fig.1B). At these concentrations cell proliferation was only slightly reduced over a 3-day period (fig.1C).

Addition of megalomycin C from the beginning of HSV-1 infection had no inhibitory effect on the expression of viral proteins, since they appeared at the same time as in the absence of this antibiotic (fig.2A). This result suggested that neither viral protein synthesis nor viral mRNA synthesis was affected by megalomycin C. The synthesis of DNA was also analyzed, even 100 μM megalomycin C did not prevent HSV-1 DNA replication (fig.2B).

The glycosylation of proteins in the presence of 50 μM of megalomycin C was analyzed by the incorporation of several radioactive sugars. This compound had no effect on translation when added at the same time as the radioactive methionine

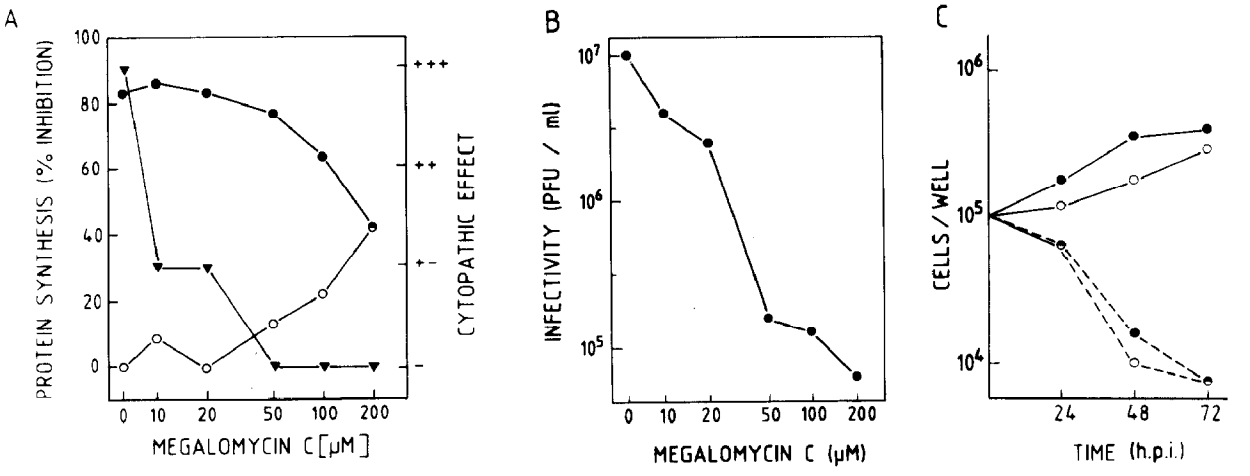

Fig.1. Antiherpetic and anticellular effects of megalomycin C. (A) Effect of different concentrations of megalomycin C on the cytopathic effect (CPE) induced by HSV-1 infection of HeLa cells (10.5 PFU/cell) at 48 h post infection (▲). −, + −, + +, + + + represent CPE on a progressive scale (+ + + is the maximum). Protein synthesis was measured as indicated in section 2 in uninfected HeLa cells (○) and in HSV-1 infected cells (●). (B) Production of infectious HSV-1 in a multistep growth cycle in the presence of several concentrations of megalomycin C. (C) Proliferation of infected and noninfected cells in the presence of megalomycin C. (−) Uninfected cells; (− − −) cells infected with HSV-1 at high multiplicity (10 PFU/cell); (●) untreated cells; (○) cells treated with 50 μM megalomycin C from the beginning of infection. h.p.i., hours post infection.

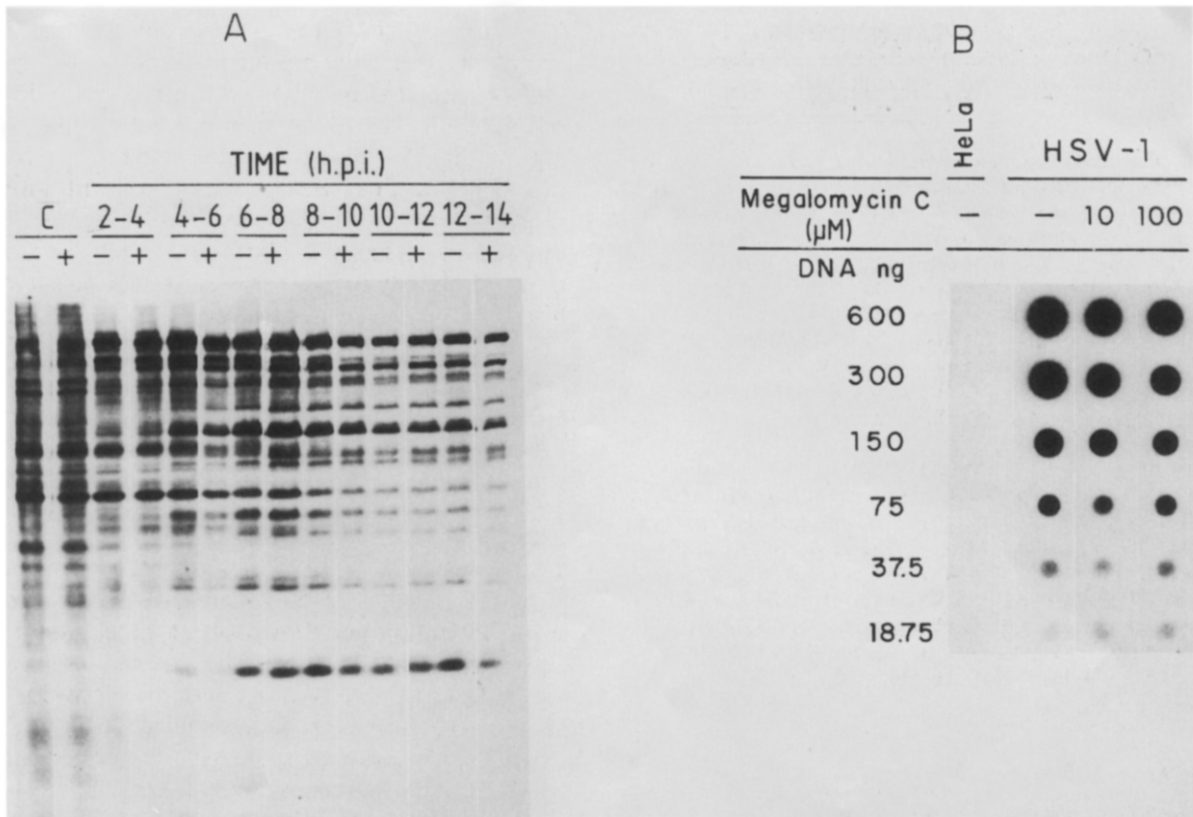

Fig.2. Effect of megalomycin C on the viral protein synthesis and viral DNA synthesis. (A) Time course of protein synthesis in HeLa cells infected with 10 PFU/ml HSV-1 in the presence (+) or absence (−) of 100 μM megalomycin C from the beginning of infection. Proteins were labeled with [^{35}S]methionine at the indicated hours post infection (h.p.i.). (B) Effect of megalomycin C on HSV-1 DNA. Total cellular DNA from control cells (−) or cells incubated with 10 and 100 μM megalomycin C was hybridized to the TK radioactive probe as described in section 2.

(fig.3). Moreover, no inhibition was observed on either protein synthesis or protein glycosylation in uninfected HeLa cells (fig.3). However, it completely blocked the incorporation of mannose and galactose to viral proteins. Furthermore, the incorporation of [^{3}H]glucosamine into the mature glycoproteins gC, gB and gD was also inhibited, although less effect was found on the incorporation into the precursor forms pC, pB and pD. As expected, the precursor forms were glycoproteins rich in mannose, whereas the mature forms contained galactose. Finally, viral particles were labeled with [^{35}S]methionine, [^{3}H]glucosamine or [^{3}H]galactose and their proteins analyzed by polyacrylamide gel electrophoresis. Fig.4 shows that the [^{35}S]methionine label in HSV-1 viral proteins was similar in the presence or absence of megalomycin C, but the sugar radioactivity was lower in non-infectious particles obtained from megalomycin-treated cells. Some galactose labeling appeared in this preparation in the presence of megalomycin C, perhaps because it was concentrated by centrifugation. These results agree with previous observations obtained with other inhibitors [4,9–11] and suggest that HSV-1 non-infectious particles could be formed in the presence of the antibiotic, and their glycoproteins are underglycosylated.

4. DISCUSSION

The data presented in this contribution suggest that the mature forms of viral glycoproteins were decreased in the presence of megalomycin C,

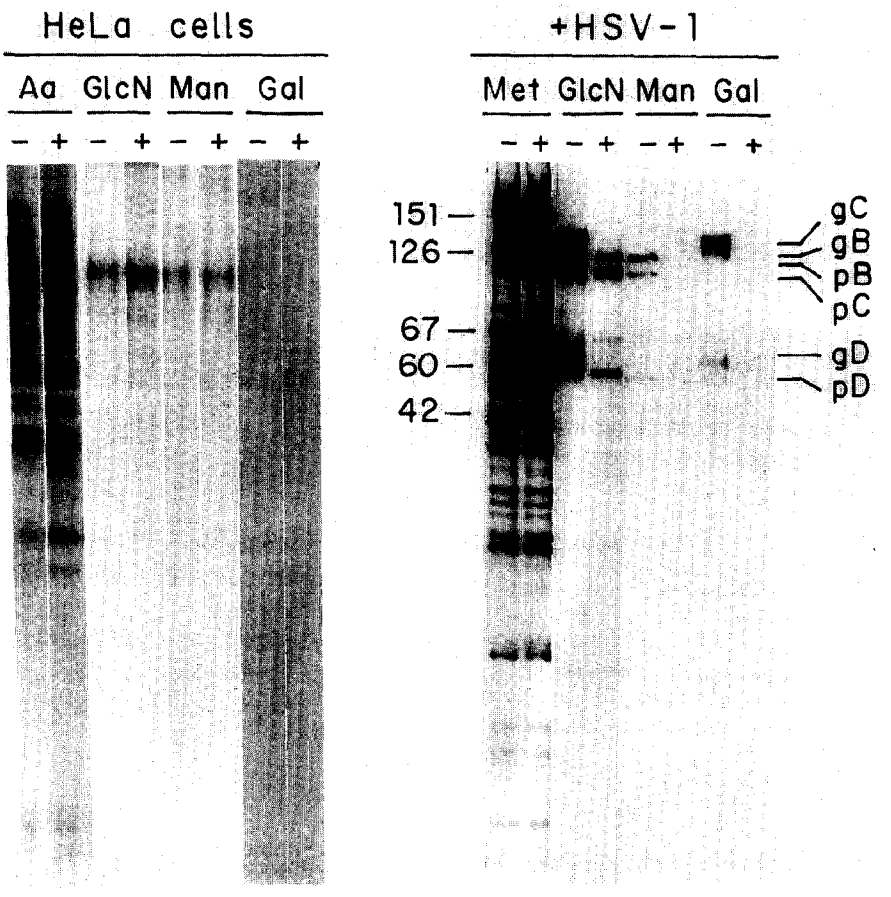

Fig.3. Protein synthesis and protein glycosylation in HSV-1 infected HeLa cells in the presence (+) or absence (−) of 50 μM megalomycin C analyzed by SDS-PAGE. HSV-1 infected cells were labeled between 15 to 17 h post infection with [^{35}S]methionine (Met), [^{3}H]glucosamine (GlcN), [^{3}H]mannose (Man) or [^{3}H]galactose (Gal). Megalomycin C was added 1 h before and maintained during the labeling period. HeLa control cells were incubated in the presence of 50 μM megalomycin C (+) and in absence of the compound (−). Host proteins were labeled with [^{3}H]amino acid mixture (Aa), [^{3}H]glucosamine (GlcN), [^{3}H]mannose (Man) and [^{3}H]galactose (Gal).

whereas the glycoprotein precursors were present. These results could indicate that the trimming of glycoproteins is the step blocked by megalomycin C. However, this is not so clear because mannose-rich glycoproteins are synthesized in the presence of trimming inhibitors, whereas megalomycin C blocked the addition of mannose residues to the proteins. More probably, this compound may inhibit the formation, or the transfer of oligosaccharides from the lipid donor (dolichol-phosphate) to the nascent peptide chain. Studies with cell-free systems will be necessary to decide between these possibilities.

Glycosylation inhibitors have been devised as potential antiviral agents [1]. However, the most active members of this group of inhibitors, as tunicamycin and streptovirudines, are very toxic to cells [16,17]. Other glycosylation inhibitors such as 2-deoxyglucose or glucosamine show little selectivity and decrease virus production in culture cells only at millimolar concentrations [3,4]. In this respect, our finding that megalomycin C acts on glycosylation and shows a potent antiviral activity is of particular interest. The antiherpetic activity of megalomycin C is most probably based on the formation of underglycosylated viral proteins.

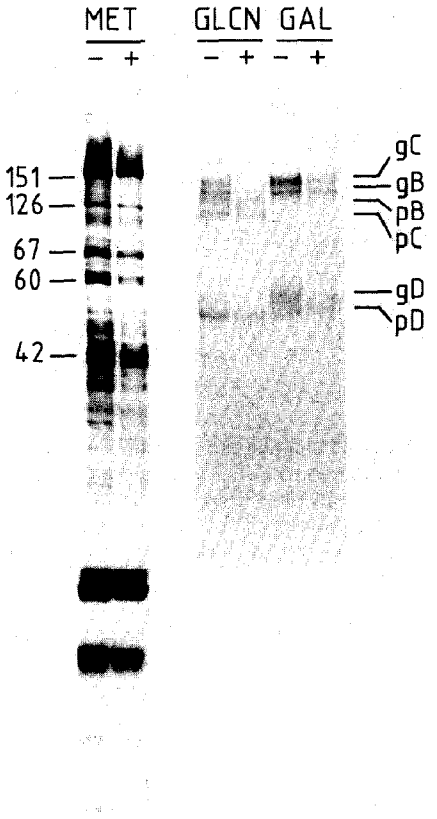

Fig.4. Analysis of proteins and glycoproteins of HSV-1 purified virions. MET, virions obtained from [^{35}S]methionine labeled infected cells; −, from untreated cells; +, from cells treated with 50 μM megalomycin C. The GLCN panels show [^{3}H]glucosamine incorporation. The GAL panels show [^{3}H]galactose incorporation.

Acknowledgements: B.A. is a fellow of the Ministerio de Educación y Ciencia. FISS and CAICYT (Plan Concertado 41/82, Antibióticos, SA) are acknowledged for financial support. Megalomycin C complex was a generous gift from Schering Corporation (Bloomfield, USA). We thank Dr M.A. Alonso for his help in the dot-blot analyses.

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