Vol. 86, No. 2, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1979 Pages 312-318

POLYAMINE METABOLISM IN MRC5 CELLS INFECTED WITH DIFFERENT HERPESVIRUSES

A.S. Tyms, Elizabeth Scamans and J.D. Williamson

Department of Virology, St. Mary's Hospital Medical School,
Paddington, London W2 1PG

Received December 6,1978

SUMMARY: Both methyglyoxal bis(guanylhydrazone), an inhibitor of S-adenosyl-L-methionine decarboxylase (EC.4.1.1.50) and DL-α-methylornithine, an inhibitor of ornithine decarboxylase (EC.4.1.1.17), are shown to be potent inhibitors of the replication of human cytomegalovirus (HCMV) in MRC-5 cells. These compounds, both inhibitors of polyamine biosynthesis, do not affect the replication of either herpes simplex virus type 1 (HSV-1) or herpes simplex virus type 2 (HSV-2). This difference in antiviral effect is shown to be related to the stimulation of spermidine and spermine synthesis in host cells following HCMV infection and the inhibition of polyamine metabolism in HSV-1 or HSV-2-infected cells. Inhibition of HCMV replication by the inhibitors of polyamine biosynthesis is accompanied by a marked decrease in the formation of intranuclear, DNA-containing inclusions characteristic of HCMV infection. These results suggest significant differences in the mechanisms of replication of different herpesviruses.

The aliphatic polyamines spermidine and spermine together with their diamine precursor putrescine have properties which suggest a regulatory role in macromolecular biosynthesis (1). Many studies have shown elevated ornithine decarboxylase activity accompanied by increases in the formation of putrescine with concomitant accumulation of spermidine and spermine in model systems of stimulated growth and the presence of polyamines has been shown to affect DNA, RNA and protein synthesis in cell-free systems. Virus replication is associated with virus-induced modification of host-cell macromolecular synthesis: the detection of polyamines in some animal viruses (2,3,4) suggests that synthesis of polyamines is required for the replication of these viruses. Some support for this hypothesis is provided by the effect of specific inhibitors of polyamine metabolism on virus replication. Methylglyoxal bis (guanylhydrazone) (MGBG) has been shown to inhibit spermidine and spermine

Abbreviations used are: α-MeOrn, DL-α-methyl ornithine; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; HCMV, human cytomegalovirus; MGBG, methylglyoxal bis(guanylhydrazone).

0006-291X/79/020312-07$01.00/0

biosynthesis in mammalian cells (5). This inhibitor has been shown to prevent the replication of foot-and-mouth disease virus, poliovirus, influenza virus and vaccinia virus in cell cultures (6,7). The replication of herpes simplex virus type 1 (HSV-1), however, is not affected by MGBG (8).

To investigate further this apparent inconsistency, the effect of inhibitors of polyamine metabolism on the replication of other viruses belonging to the Herpesvirus group, herpes simplex virus type 2 (HSV-2) and human cytomegalovirus (HCMV), was studied. The inhibitors used were MGBG and α-methylornithine (α-MeOrn), the latter a potent inhibitor of putrescine and spermidine biosynthesis (9). The results obtained show that the replication of HSV-1 and HSV-2 is unaffected by either compound whereas the replication of HCMV is inhibited by MGBG or α-MeOrn. This difference is related to changes in polyamine metabolism in cell cultures following virus infection.

MATERIALS AND METHODS:

MRC-5 human diploid fibroblasts were grown in Eagle's minimum essential medium containing 10% or 2% foetal calf serum for growth and maintenance, respectively. HSV-1 and HSV-2 were isolated in this laboratory and the 'Rawles' strain of HCMV was used. Infectivity titrations were made by plaque formation in Vero cells with HSV-1 and HSV-2 or in MRC-5 cells with HCMV.

Polyamine metabolism in uninfected and virus-infected cells was monitored using appropriate radioactively-labelled precursors: both DL-[5-^{3}H] ornithine dihydrochloride and [1,4-^{14}C] putrescine dihydrochloride were obtained from the Radiochemical Centre, Amersham, England. Polyamines were extracted from cells and the dansyl derivatives prepared essentially as described by Seiler (10). Thin-layer chromatography was carried out on 0.25 mm Silica gel G plates (Anachem, Luton, England) and the plates developed with ethyl acetate: cyclohexane (2:3, v/v). Appropriate areas were recovered from the plates after chromatography by reference to fluorescent standards and radiolabelled material recovered by elution into a liquid scintillation system (3.5 g PPO, 50 mg POPOP/ litre in Metapol: toluene, 1:2, v/v). Protein estimations were made with the Folin phenol reagent (11).

The appearance of intranuclear, DNA-containing inclusions in HCMV-infected cells was visualized by an acridine orange staining method (12). Unlabelled polyamines, used for preparing dansyl standards, were supplied by Sigma Chemical Co., MGBG was obtained from Aldrich Chemical Co. Inc. Milwaukee and α-MeOrn was a generous gift from Centre de Recherche Merrell International, Strasbourg, France.

RESULTS:

Preliminary experiments to determine any antiviral effect were made using various concentrations of MGBG or α-MeOrn added to the maintenance medium supplied to MRC-5 cell cultures immediately after infection. In all experiments cell cultures were infected using a multiplicity of infection between 1 and 5 p.f.u./cell. The production of infectious, progeny virus is complete within 24 hours post-infection (p.i.) in human fibroblasts infected with HSV-1 or HSV-2 but progeny virus does not begin to appear in HCMV-infected cells until 2 days p.i. and continues for several days subsequently (13). Consequently, infectivity titrations were made at 24 hours p.i. with HSV-1 or HSV-2-infected cultures and at 5 days p.i. with HCMV-infected cultures. In the presence of medium containing either 0.1 mM-MGBG or 10 mM-α-MeOrn the production of infectious, progeny virus from HCMV-infected cultures was completely inhibited but the infectivity titres of HSV-1 and HSV-2 under these conditions were relatively unaffected. The results from a typical experiment are shown in Table 1. Similar results were obtained with HSV-1 or HSV-2-infected cultures maintained from 24 hours in the presence of the compounds prior to infection or by using washed virus preparations to infect the cell cultures. Examination at 7 days p.i. of HCMV-infected cultures treated with the inhibitors failed to detect infectious virus. These results demonstrate that the replication of HCMV is inhibited by MGBG or α-MeOrn whereas the replication of HSV-1 and HSV-2 is unaffected.

This difference in antiviral effect was found in further studies to be related to changes in polyamine metabolism in MRC-5 cells following virus infection. In these experiments radiolabelled putrescine (0.1 μCi/ml) or ornithine (0.5 μCi/ml) was added to maintenance medium for a 24 hour period immediately after infection with HSV-1 or HSV-2 and from 2 days p.i. with HCMV-infected cultures. Control cultures were "sham" infected and exposed to radiolabel for identical periods. At the end of the labelling period the

Table 1. The effect of 0.1 mM-MGBG or 10 mM-α-MeOrn on HSV-1, HSV-2 and HCMV replication in MRC-5 cells.

	Infectivity titres (p.f.u./ml)			
	MGBG		α-MeOrn	
Virus	Control	Test	Control	Test
HSV-1	4.5×10^6	1.9×10^6	7.8×10^6	2.4×10^6
HSV-2	2.8×10^6	1.8×10^6	4.7×10^7	4.9×10^7
HCMV	2.2×10^5	$<10^1$	1.1×10^5	$<10^1$

Table 2. The effect of virus infection and 0.1 mM-MGBG on polyamine metabolism in MRC-5 cells.

	Radioactivity (dpm/mg cell protein)	
Experiment	Spermidine	Spermine
1. Sham infected (0-24 h)	23,119	3,707
2. HSV-1-infected (0-24 h p.i.)	2,835	1,090
3. HSV-2-infected (0-24 h p.i.)	3,490	763
4. Sham infected (48-72 h)	5,246	595
5. HCMV-infected (48-72 h p.i.)	34,288	48,300
6. HCMV-infected + 0.1 mM-MGBG (48-72 h p.i.)	843	138

radioactivity associated with spermidine and spermine extracted from uninfected and virus-infected cells was determined as described.

Conversion of [^{14}C]-putrescine to labelled spermidine and spermine in MRC-5 cells infected with HSV-1 or HSV-2 was reduced compared with uninfected controls. In HCMV-infected cultures, however, the amount of radioactivity associated with these polyamines increased markedly (Table 2). These results show that spermidine and spermine synthesis is inhibited in HSV-1 or HSV-2-infected MRC-5 cells but stimulated at least six-fold following HCMV infection. Other results show that in HCMV-infected cultures the conversion of [^{14}C]-putrescine or [^{3}H]-ornithine to spermidine and spermine was inhibited by 0.1 mM-MGBG or 10 mM-α-MeOrn, respectively (Table 2 and Table 3). Thus, the concentrations of these compounds which have an effect on HCMV replication are effective in inhibition of polyamine metabolism.

Table 3. The effect of virus infection and 10 mM-α-MeOrn on polyamine metabolism in MRC-5 cells.

Experiment	Radioactivity (dpm/mg cell protein) Spermidine	Spermine
1. Sham infected (48-72 h)	122	92
2. HCMV-infected (48-72 h p.i.)	1,392	1,020
3. HCMV-infected + 10 mM-α-MeOrn (48-72 h p.i.)	122	88

Table 4. The effect of 10 mM-α-MeOrn on the formation of intranuclear, DNA-containing inclusions and production of infectious, progeny virus in HCMV-infected MRC-5 cells.

α-MeOrn (mM)	Infected cells with inclusions (%)*	Infectivity titre (p.f.u./ml)
0	84	1.1×10^5
1.0	79	2.5×10^4
3.2	13	1.2×10^3
10.0	7	$<10^1$

* A minimum of 200 infected cells were examined at each concentration of the inhibitor.

The replication of HCMV occurs in the nucleus of the infected cell and synthesis of virus DNA is accompanied normally by the formation of intranuclear, DNA-containing inclusions. Such inclusions can be visualized by acridine orange staining of HCMV-infected cells (14). Examination of MRC-5 cells at 3 days after infection with HCMV revealed the presence of intranuclear inclusions in a large proportion of infected cells. In the presence of increasing concentrations of α-MeOrn the frequency of such inclusions declined progressively and this decrease was paralleled by a reduction in infectivity titres. At 10 mM-α-MeOrn less than 10% of infected cells possessed DNA-containing inclusions and the yield of infectious, progeny virus was completely inhibited (Table 4). Similar results were obtained with HCMV-infected cultures treated with 0.1 mM-MGBG. These results show that the formation of intranuclear, DNA-containing inclusions in HCMV-infected cells is reduced markedly in the presence of concentrations of the inhibitors which affect the yield of infectious, progeny virus.

DISCUSSION:

A previous investigation has shown that spermidine and spermine synthesis from putrescine is inhibited in LS cells infected with HSV-1 and that virus replication was not affected by MGBG (8). The present study has confirmed these observations and shown also that infection with HSV-2 has similar effects. Conversely, the replication of HCMV, another member of the Herpesvirus group, has been found to be inhibited by MGBG or α-MeOrn. Since this second compound is effective against ornithine decarboxylase (EC 4.1.1.17), the effect of both inhibitors indicates that putrescine, spermidine and spermine synthesis is required for HCMV replication. This conclusion is consistent with the marked stimulation of polyamine synthesis found to occur in HCMV-infected cells. Thus, the different antiviral effects of MGBG and α-MeOrn against the herpesviruses studied are related to the effect of virus infection on polyamine metabolism in the host cell.

Since the replication of HSV-1 and HSV-2 is not affected by MGBG or α-MeOrn this suggests that the synthesis of spermidine and spermine is not essential for virus replication. Indeed, inhibition of polyamine metabolism in HSV-1-infected cells has been ascribed to the inhibition of host-cell protein synthesis resulting from virus infection (8). Cellular macromolecular synthesis is stimulated in HCMV-infected cells but virus DNA synthesis is not dependent on host-cell DNA synthesis (15). In the present study the formation of intranuclear, DNA-containing inclusions was inhibited by MGBG or α-MeOrn at concentrations shown to inhibit virus replication. Both compounds have been shown to inhibit DNA replication in mammalian cells (16,17). These observations suggest that virus DNA synthesis requires concomitant polyamine synthesis in HCMV-infected cells. This hypothesis is supported by the requirements for virus DNA synthesis in vaccinia virus replication. Infection with vaccinia virus inhibits host-cell macromolecular synthesis but polyamine metabolism continues after infection and virus DNA synthesis is affected by MGBG (4,12,18). Thus, the apparent lack of a

requirement for de novo synthesis of spermidine and spermine in the replication of HSV-1 and HSV-2 is anomalous. Further studies will investigate the nature of continued polyamine biosynthesis in HCMV-infected cells and polyamine metabolism in cells infected with other herpesviruses.

This work was supported by grants from the Medical Research Council.

1. Bachrach, U. (1973) Function of Naturally Occurring Polyamines, pp.82-95, Academic Press, New York.
2. Gibson, W. & Roizman, B. (1971) Proc. Natl. Acad. Sci. U.S.A. 68, 2818-2821.
3. Shortridge, K.F. & Stevens, L. (1973) Microbios 7, 61-68.
4. Lanzer, W. & Holowczak, J.A. (1975) J. Virol. 16, 1254-1264.
5. Williams-Ashman, H.G. & Schenone, A. (1972) Biochem. Biophys. Res. Commun. 46, 288-295.
6. Ferrari, W., Loddo, B. & Gessa, G.L. (1965) Ann. N.Y. Acad. Sci. 130, 404-411.
7. Küchler, C. Küchler, W. & Schulze, W. (1968) Acta Virol. 12, 441-445.
8. McCormick, F.P. & Newton, A.A. (1975) J. Gen. Virol. 27, 25-33.
9. Abdel-Monem, M.M., Newton, N.E. & Weeks, C.E. (1974) J. Med. Chem. 17, 447-450.
10. Seiler, N. (1970) Methods Biochem. Anal. 18, 327-330.
11. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
12. Williamson, J.D. (1976) Biochem. Biophys. Res. Commun. 73, 120-126.
13. Fenner, F. & White, D.O. (1976) Medical Virology, pp.297-319, Academic Press, New York.
14. McAllister, R.M., Straw, R.M., Filbert, J.E. & Goodhert, C.R. (1963) Virology 19, 521-523.
15. DeMarchi, J.M. & Kaplan, A.S. (1976) J. Virol. 18, 1063-1070.
16. Fillingame, R.H., Jorstad, C.M. & Morris, D.R. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 4042-4045.
17. Mamont, P.S., Bohlen, P., McCann, P.P., Bey, P., Schuber, F. and Tardif, C. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 1626-1630.
18. Hodgson, J. & Williamson, J.D. (1975) Biochem. Biophys. Res. Commun. 63, 308-312.