Antiviral Activity of Papaverine and Nucleoside Analogs on the Human Cytomegalovirus Infection

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Human Cytomegalovirus 감염에 대한 파파베린과 뉴클레오사이드 유사체의 항바이러스 효과

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ABSTRACT: Antiviral activities of papaverine and nucleoside analogs, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG) and acyclovir, against human cytomegalovirus (HCMV) infection were compared *in vitro*. Papaverine and DHPG were effective in reducing infectious HCMV yields with ED_{50}s (effective dose 50: the concentraion at which 50% of virus yields was obtained) of approximately 1.02 and 0.45 μM, respectively; while acyclovir was less effective with an ED_{50} of about 10.4 μM. The relative cytotoxicity of these drugs was evaluated under the same conditions used to measure infectious HCMV yields. Papaverine and DHPG demonstrated little cellular toxicity as measured by their effect on the viability of confluent cells at concentrations in the range of those demonstrating potent inhibition of HCMV replication. Similarly, protein synthesis was largely unaffected by these drugs in stationary mock-infected cells as measured by the incorporation of isotopically labelled amino acids. In contrast, cellular DNA synthesis was invariably reduced in the presence of either drug. HCMV-specific DNA synthesis was also strongly inhibited by papaverine and DHPG.

KEY WORDS□HCMV, papaverine, DHPG, acyclovir

INTRODUCTION

Human cytomegalovirus (HCMV) infection is wide spread, and has been reported in all countries in which it has been investigated. Like other human herpesviruses, these infections are usually asymptomatic (Plotkin *et al.*, 1984) and result in the development of a life-long persistent infections which are usually latent. Life-threatening HCMV disease is observed particularly in the fetus and in immunocompromised patients. For example, severe neurologic diseases may develop following fetal or less frequently neonatal HCMV infection (Spector, 1983; Pass and Hutto, 1986). Reactivation of latent HCMV infection is particularly problematic in patients receiving organ transplants (Onorato *et al.*, 1985) or in those patients with AIDS (acquired immunodeficiency syndrome) (Rodgers *et al.*, 1983, Macher *et al.*, 1983).

Despite the clinical importance of HCMV infections, de velopment of effective preventive or therapeutic procedures has been elusive. Most attempts to develop therapeutic agents for HCMV infection have focused on the use of nucleoside analogs. These drugs have included adenine and cytosine arabinosides, acyclovir, and many fluoro-substituted nucleoside analogs. Unfortunately, none of these drugs has been entirely successful as an effective treatment for HCMV disease (Verheyden, 1988). Recently, a derivative of acyclovir, (9-[(1,3-dihydroxy-2-propoxy)methyl]

guanine: DHPG) has demonstrated some promising *in vitro* and *in vivo* findings. DHPG has been tested extensively *in vitro* (Cheng *et al.*, 1983; Tocci *et al.*, 1984) and *in vivo* (Shepp *et al.*, 1985; Collaborative DHPG Treatment Study Group, 1986) generally with good results.

Problems with the use of these nucleoside analog drugs persist, particularly toxicity and development of resistant mutants. Both *in vitro* (Schinipper and Crumpacker, 1980; Field *et al.*, 1981) and *in vivo* (Crumpacker *et al.*, 1982), resistant mutants to acyclovir have been observed. More recently human CMV mutants resistant to DHPG that is associated with a reduced level of DHPG-triphosphate has been identified (Biron *et al.*, 1986). Furthermore, toxic side effects associated with the clinical application of the nucleoside analogs have complicated their use.

Consistent with the importance of the role of cellular responses in the replication of HCMV (for a review see Albrecht *et al.*, 1989), alternative treatment modalities based on interfering with the cellular responses to HCMV infection have been explored recently. Papaverine, a compound known to have potent smooth muscle relaxing properties, has also demonstrated potent anti-HCMV activity *in vitro* (Albrecht *et al.*, 1987). Papaverine, which may represent a new class of anti-HCMV drugs (Albrecht *et al.*, 1987), is a natural product derived from opium poppy latex and is used currently to treat cardio-vascular disease. Since papaverine has been in clinical use for decades and its proposed mechanism of action in inhibiting HCMV replication appears to be quite different from that of nucleoside analogs, a careful evaluation of the relative effect of papaverine on HCMV replicaiton is warranted.

This study was undertaken to compare the antiviral activity of papaverine and well recognized nucleoside analogs (DHPG, acyclovir), especially with regard to their effect on HCMV DNA synthesis and relative cytotoxicity.

MATERIALS AND METHODS

Cell and virus

Human embryo fibroblast (LU) cells were used throughout these studies. LU cells were grown in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal calf serum (FCS) and 0.075% sodium bicarbonate. Confluent LU cells were maintained with EMEM supplemented with 2% FCS and 0.15% sodium bicarbonate. For cells incubated in a 5% CO_2 atmosphere the concentration of sodium bicarbonate was increased to 0.22%.

HCMV strain AD169 was used exclusively in this study. To produce HCMV stocks, confluent LU cells grown in 75 or 150 cm^2 flasks were infected with HCMV at a MOI (multiplicity of infection) of 0.01-0.03 PFU (plaque forming units) /cell. About 8-10 days after virus infection, the cells were frozen at $-80°C$. HCMV was harvested by rapidly thawing the cells at 37°C and sonicating the cells for 1 min. Virus stocks were maintained at $-80°C$.

Measurement of virus infectivity

To measure HCMV infectivity a modification of the procedure of Wentworth and French (1970) was used. Samples to be assayed for HCMV infectivity were serially diluted (10-fold) in maintenance medium and a small volume (0.2 m*l*) of appropriate dilutions was inoculated onto confluent LU cells in 35 mm dishes. Virus was allowed to adsorb at 37°C for 1 hr with occasional rocking of the dishes. Afterwards, the virus inoculum was aspirated and the cells were overlayed with a semisolid medium consisting of EMEM supplemented with 2% FCS, 0.225% sodium bicarbonate, 0.25% agarose (Type II, medium EEO, Sigma Chemical Co., St. Louis, MO, U.S.A.), 100 units/m*l* penicillin, 100 μg/m*l* streptomycin, and 1 μg/m*l* fungizone (Flow Lab., McLean, VA, U.S.A.). A second overlay was added after 7 days incubation and, after 14 days incubation, the cells were fixed with 10% formalin and stained with 0.03% methylene blue. Plaques were counted with the aid of a dissecting microscope.

Virus yield assay

The effect of the selected drugs on infectious yields of HCMV was determined in cultures of LU cells in 25 cm^2 plastic tissue culture flasks. Confluent LU cells were infected with HCMV at a multiplicity of at least 3 PFU/cell and the virus was permitted to adsorb for 1 hr at 37°C. Drugs were added immediately after virus adsorption (0 hr postinfection, p.i.). For experiments involving treatment for more than 48 hr, the media and drugs were replaced at 48 hr intervals. Virus replication was stopped by freezing the cells at $-80°C$ at times when infectious yields were near maximal (in the absence of drugs), usually 96 hr p.i. Virus was harvested by two cycles of freezing

and thawing followed by sonication for 15 seconds. Virus yields were determined by plaque assay as described previously. ED_{50} and ED_{90} (the concentrations at which the infectious yields were reduced by 50 or 90%, respectively) were calculated from plots of drug concentration ($\log_{10}$) relative to infectious virus yields ($\log_{10}$).

Drug toxicity tests

The cellular toxicity of drugs was tested by determining their concentration effect on cell viability as measured by the trypan blue exclusion method. Confluent LU cells in 25 cm^2 flasks were treated with selected increments of concentration of each drug and the medium and drugs were changed two days later. After a total of four days, the cells were dissociated with trypsin, collected by centrifugation, resuspended with a small volume of phosphate-buffered saline, and diluted 1:10 in trypan blue solution (Gibco, Grand Island Biological Company, Grand Island, NY, U.S.A.). Dye-excluding cells were enumerated with the aid of a heamocytometer.

The effect of drugs on macromolecular synthesis in LU cells was determined by measuring the relative rate of incorporation of radioactive precursors into trichloroacetic acid (TCA)-precipitable material. For evaluation of the effect of the drugs on protein synthesis, LU cells grown to a confluency in 35 mm dishes were treated with the drugs to be investigated. Seventy-two hr after the drug treatment, ^{3}H-leucine (specific activity=59.8 Ci/mmol) was added to culture fluids to a final concentration of 2 μCi/ml. Labelling was stopped 24 hr after the addition of ^{3}H-leucine by removing the culture medium, washing the cells, and adding 1 ml of cold lysis buffer (0.01 M Tris, 0.5% NP40, 0.5% sodium deoxycholate). The lysate was collected, clarified at 10,000 rpm for 10 minutes and a small volume (0.1 ml) of the supernatant fluids was spotted onto a Whatman No. 3 filter disk. The filter disk was washed three times in cold 5% TCA. After drying the radioactivity was measured by liquid scintillation spectrophotometry (Model LS9000, Beckman Instruments, Inc. Palo Alto, CA).

The effect of each drug on cellular DNA synthesis was evaluated by labelling LU cells with ^{3}H-thymidine (specific activity=20 Ci/mmol, final concentration=10 μCi/ml) from 72 to 96 hr after drug treatment. Cells were treated with drug in the same manner as described above for evaluating their effect on protein synthesis. Incorporation

of label was stopped by freezing cells at $-20°C$. Later the cells were thawed and digested overnight by the addition of 0.06 ml protease [type XIV, Sigma Chemical Co.; 1% in 1X SSC (150 mM NaCl, 15 mM sodium acetate, pH 7.0)], 0.03 ml ethylenediamine tetraacetic acid (EDTA, 0.02 M) and 0.03 ml sodium lauroyl sarcosinate-30 (Chemical Additive Co., Farmingdale, NY; 2% in 0.1XSSC) per ml of medium. A small volume (0.1 ml) of the digest was spotted onto Whatman No. 3 disks. The filter disks were washed three times with 5% TCA, dried, and the radioactivity was measured as before.

Measurement of HCMV DNA synthesis

To determine the effect of the antiviral drugs on synthesis of DNA in cells infected with HCMV, LU cells grown in 35 mm dishes were infected (MOI=approximately 3 to 5 PFU/cell) or mock-infected and treated with selected concentrations of each drug. ^{3}H-thymidine (specific activity=6.7 Ci/mmol) was added to a final concentration of 10 μCi/ml at 72 hr p.i. Labelling was stopped at 96 hr p.i. by freezing cells at $-20°C$. The cells were lysed and the DNA released as described for cellular DNA.

The specific effect on HCMV DNA synthesis was determined by separating cellular and HCMV DNA using isopycnic ultracentrifugation in CsCl as described previously in detail (Albrecht *et al.*, 1980).

Drugs

Papaverine was purchased from Eli Lilly Co., Indianapolis, IN, U.S.A. as a solution (30 mg/ml) prepared for intravenous injection. DHPG and acyclovir were kindly provided by Syntex (Mountain View, CA, U.S.A) and Burroughs Wellcome (Research Triangle Park, NC, U.S.A.), respectively. Stocks of the latter two drugs were made immediately prior to use by dissolving with 1N NaOH (for DHPG) or distilled water (for acyclovir).

RESULTS

Effect of papaverine, DHPG, and acyclovir on infectious HCMV yields

The antiviral activity of papaverine and the nucleoside analogs DHPG and acyclovir was evaluated by yield reduction assays. The results are shown in Fig. 1. There was a nearly linear relationship between infectious HCMV yields and the concentration for each of the three drugs
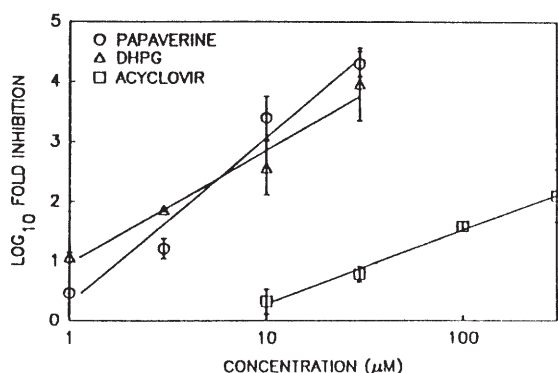
Fig. 1. *Inhibition of HCMV multiplication by papaverine, DHPG, and acyclovir. Confluent LU cells grown in 25 cm² flasks were infected with HCMV (strain AD169) at MOI of 3-5 PFU/cell. Maintenance medium containing the selected concentrations of drug was added at 0 hr p.i. Medium and drugs were replaced at 48 hr p.i. and the virus yields were determined by plaque assay at 96 hr p.i*
(○), papaverine; (△), DHPG; (□), acyclovir.

tested (98.4% fitness for papaverine, 98.5% fitness for DHPG, 99.6% fitness for acyclovir). DHPG was the most effective in reducing infectious HCMV yields with an ED_{50} of approximately 0.45 μM. Papaverine was similarly effective with a calculated ED_{50} of about 1.02 μM. Acyclovir was the least effective in reducing HCMV yields with an ED_{50} of approximately 10.4 μM. The calculcated ED_{90}'s for papaverine, DHPG, and acyclovir were approximately 1.8, 1.05, and 38 μM, respectively. The slopes for papaverine and DHPG indicate that DHPG was slightly more effective than papaverine at concentrations lower than 5.73 μM while reverse observation was obtained at concentrations higher that 5.73 μM in inhibiting HCMV multiplication.

Effect of papaverine, DHPG, and acyclovir on the viability of stationary LU cells

To determine if the inhibitory effect of the drugs on virus yields resulted from cellular toxicity, cells were treated with each of the drugs for the same interval of time required for the infectious yields assay and over the same range of concentrations. Cell viability was measured by the trypan blue-exclusion assay as described in Materials and Methods. The data summarized in

Table 1. *Effect of papaverine, DHPG, and acyclovir on the viability of stationary human embryo lung cells[a]*

Drug	Concentration (μM)	Number of viable cells ($\pm SD^b \times 10^6$)	Percent of control[c]
None		1.87±0.21	100
Papaverine	1	1.90±0.31	102
	3	1.92±0.11	103
	10	1.85±0.25	99
	30	1.88±0.09	101
	100	1.62±0.13	87
DHPG	1	2.03±0.41	109
	3	1.86±0.12	99
	10	1.95±0.21	104
	30	2.18±0.35	117
	100	1.80±0.33	96
Acyclovir	10	1.79±0.11	96
	30	1.83±0.29	98
	100	1.68±0.30	90
	300	1.52±0.18	81
	1000	1.40±0.21	75

[a] LU cells were grown to a confluency in 25 cm² flasks. Selected concentrations of drug in maintenance medium were added to LU cell cultures two days after cells reached confluency. Media and drugs were replaced 48 hr after drug treatment and the number of viable cells was determined 48 hr later by trypan blue exclusion.
[b] Standard deviation
[c] Percent of control = (the number of viable cells in the presence of drug) ÷ (the number of viable cells in the absence of drug) × 100

Table 1 suggest that at the highest concentrations tested papaverine was marginally toxic for stationary LU cells, while DHPG exerted no toxic effect at the highest concentration evaluated. Neither drug affected the viability of LU cells at concentrations (3-30 μM) which demonstrated substantial reductions of infectious HCMV yields while acyclovir diminished the viability of LU cells at concentrations (100 μM or greater) required for even lower level of reduction in infectious HCMV yields. Thus, acyclovir was relatively more toxic to LU cells than either papaverine or DHPG.

Effect of papaverine, DHPG, and acyclovir on macromolecular synthesis in LU cells

To determine the effect of the selected antiviral

Table 2. Effect of papaverine, DHPG, and acyclovir on the synthesis of proteins and DNA in mock-infected LU cells

Drug[b]	Concentra-tion (μM)	Radioactivity[a]			
		^{3}H-leucine		^{3}H-thymidine	
		CPM±S.D.	Percent of control[c]	CPM±S.D.	Percent of control[c]
None		5388±337	100	18320±1149	100
Papaverine	3	4341±143	81	4904±486	27
	10	4540±208	84	2600±404	14
	30	4662±223	87	1196±59	7
DHPG	3	5390±425	100	11340±1033	62
	10	5994±331	111	9263±693	51
	30	5608±172	104	9050±2445	49
Acyclovir	30	5329±222	99	12816±3166	70
	100	5594±77	104	10372±644	57
	300	5594±424	104	9381±2871	51

[a] Cells were labelled from 72 to 96 hr after treatment with drug.
[b] Fresh maintenance medium and drugs were added at 48 hr intervals.
[c] Precent of control=(the number of viable cells in the presence of drug)÷(the number of viable cells in the absence of drug)×100.
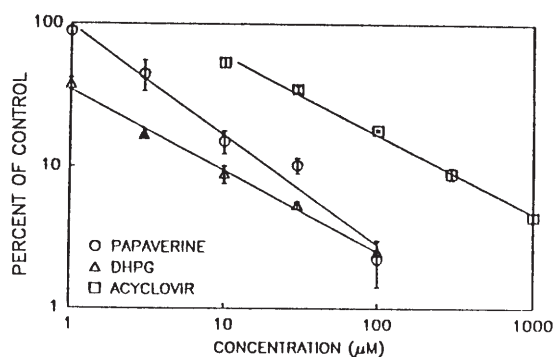

Fig. 2. Effect of papaverine, DHPG, and acyclovir on the synthesis of DNA in HCMV-infected cells. LU cells in 35 mm petri dishes were infected with HCMV (strain AD169) at a MOI of 3.7 PFU/cell Media containing the selected concentrations of the drugs were added to the dishes at 0 hr p.i. and replaced at 48 hr p.i. At 72 hr, p.i. a small volume (20 μl) of concentrated ^{3}H-thymidine (specific activity=6.7 Ci/mmol, 1 mCi/ml) was added to the medium supporting the infected cells to make a final concentration of 10 μCi/ml. Radioactivity was determined at 96 hr p.i. as described in Materials and Methods.
($\bigcirc$), papaverine; ($\triangle$), DHPG; ($\square$), acyclovir.

compounds on cellular metabolism, the rate of synthesis of proteins and DNA was measured in mock-infected cells. Table 2 summarizes the effect of the three drugs on the incorporation of ^{3}H-leucine or ^{3}H-thymidine into TCA-precipitable material from 72 to 96 hr p.i. at the highest three concentrations tested for antiviral activity (Fig. 1). The rate of protein synthesis in LU cells was not adversely affected by either DHPG or acyclovir. Papaverine treatment resulted in a sligh inhibition of cellular protein synthesis from 13 to 19 percent, however, the inhibitory effect did not increase with increasing concentrations of papaverine.

Cellular DNA synthesis, on the other hand, was affected by the three drugs. DHPG and acyclovir inhibited cellular DNA synthesis from 30% to 51 %. Papaverine inhibited cellular DNA synthesis in a concentration-dependent manner. As concentrations increased from 3 to 30 μM, papaverine inhibited cellualr DNA synthesis from 73 to 93%.

Inhibition of HCMV DNA synthesis

In parallel with the studies of cellular DNA synthesis, the effect of the three drugs on HCMV DNA synthesis was investigated by determining the radioactivity incorporated into TCA-precipitable meterial following labelling of HCMV-infected cells with ^{3}H-thymidine trom 72 to 96 hr p.i. Since it has been previously shown that nearly all of the DNA synthesized during this time interval is HCMV DNA, acid-insoluble radioactivity measured at this interval after infection should provide a good indication of the rate of HCMV DNA synthesis (Albreacht et al., 1987). As shown in Fig. 2, DHPG and papaverine
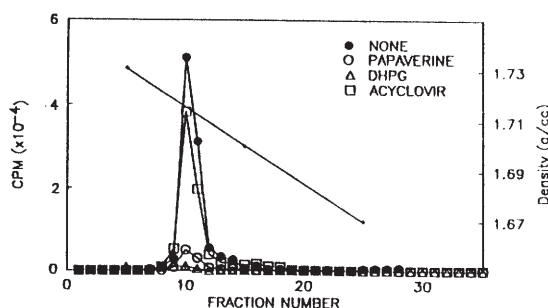
Fig. 3. *Isopycnic ultracentrifugation of DNA isolated from HCMV-infected cells. LU cells were infected with HCMV (strain AD169, MOI = 3.7 PFU/cell) and treated with 10 µM of papaverine, DHPG, or acyclovir. Cells were labelled with ^{3}H-thymidine (final concentration = 10 µCi/ml) from 72 to 96 hr p.i.*
(●), No drug; (○), papaverine; (△),DHPG; (□), acyclovir.

Table 3. *ED$_{50}$s and ED$_{90}$s for papaverine, DHPG and acyclovir against HCMV multiplication and DNA synthesis*

Drugs	HCMV multi-plication[a]		HCMV DNA synthesis[b]	
	ED$_{50}$ (µM)	ED$_{90}$ (µM)	ED$_{50}$ (µM)	ED$_{90}$ (µM)
Papaverine	1.02	1.8	2.4	19.3
DHPG	0.45	1.05	0.54	8.9
Acyclovir	10.4	38	14.2	264

[a] Determined by the effect of the drugs on the production of HCMV infectious yields. Summarized from the data presented in Fig. 1.
[b] Determined by the effect of the drugs on the incorporation of ^{3}H-thymidine to DNA of HCMV-infected cells. Summarized from the data presented in Fig. 2.

were similarly effective in inhibiting the incorporation of ^{3}H-thymidine into DNA in HCMV-infected cells, although papaverine was somewhat less effective than DHPG. Acyclovir was at least 10 times less effective than DHPG or papaverine. There was a good correlation between the concentration of and the level of inhibtion of DNA synthesis by the three drugs (98.7% fitness for papaverine, 99.7% fitness for DHPG, 99.7% fitness for acyclovir). The concentration required for 50% inhibition of DNA synthesis in HCMV-infected cells was calculated from the regression lines to be about 2.4, 0.54, and 14.2 µM for papaverine, DHPG, and acyclovir, respectively. Similarly, 90% inhibition of DNA synthesis in HCMV-infected cells was observed at 19.3, 8.9 and 264 µM of papaverine, DHPG and acyclovir, respectively.

To confirm that the radioactivity incorporated into DNA at 72-96 hr p.i. was nearly entirely HCMV DNA as suggedted by an earlier studies and that the concentration effect of these three drugs for HCMV DNA synthesis was as suggested by the data presented above, HCMV and cellular DNA from the infected cells treated or not treated with each of the three drugs at a concentration of 10 µM were separated by isopynic centrifugation (Fig. 3). At 10 µM, acyclovir reduced HCMV DNA synthesis by approximately 27%. At same concentration, papaverine inhibited HCMV DNA synthesis by about 90%,

and DHPG by about 98%. Table 3 summarizes the data presented in Figs. 1 and 2.

DISCUSSION

The findings from this study suggest that like DHPG, papaverine is a potent inhibitor of HCMV replication and of HCMV DNA synthesis *in vitro*. The ED$_{50}$ for DHPG was found to be about 0.45 µM in this study, the ED$_{50}$s from other studies range from 0.1 to 7.0 µM(summarized by Verheyden, 1988). The ED$_{50}$ for papaverine determined in this study was about 1 µM, and for acyclovir was about 10 µM. These data suggest that papaverine and DHPG are similarly effective in reducing HCMV yields *in vitro*.

The antiviral activity and mechanism of action of DHPG and acyclovir have been reviewed by Matthews and Boehme (1988). Although DHPG is much more effectively phosphorylated than acyclovir (appoximately 10-fold) by cellular kinases (Biron *et al.*, 1985; Smee *et al.*, 1985), herpes simplex virus (HSV)-coded thymidine kinase (TK) may accerlerate the phosphorylation of the both drugs. Since HCMV is not known to encode a virus-specific TK, the anti-HCMV activity of DHPG may be greater than acyclovir because of differing efficiences of phosphorylation by cellular enzymes. Similar reasoning might also explain why DHPG was substantially more effective than acyclovir in inhibiting cellular and viral DNA synthesis.

The mechanism of papaverine inhibition of HCMV replication is currently unclear. Although previous studies suggested that papaverine inhibits HCMV replication through modulation of calcium (Nokta *et al.,* 1987) and/or cyclic nucleotide metabolism (Lee and Albrecht, 1987), virtually nothing is known of the virus-directed molecular events susceptible to papaverine inhibition that result in reduced HCMV yields. The data for inhibition of HCMV-specific DNA synthesis are in close correlation with those for the reduction of infectious HCMV yields (Table 3). The data also suggest that infectious yield assay is more sensitive method than DNA synthesis assay in evaluating the effect of the drugs on HCMV replication. The potent inhibition of cellular and HCMV DNA synthesis by papaverine is somewhat surprising. Papaverine has been used clinically as an effective smooth muscle relaxant on the basis of its effect on cyclic nucleotide levels (Triner *et al.,* 1970; Wells *et al.,* 1975). Based on the recognized mechanism of action of papaverine our findings could suggest that DNA synthesis is more sensitive to papaverine and to papaverine-sensitive changes in cellular cyclic nucleotide levels than is cellular protein synthesis.

Otherwise, the differential effectiveness on HCMV yields and HCMV DNA synthesis of papaveirne and DHPG could be explained by their different mechamisms of action. Since pa-paverine is not a direct inhibitor of DNA synthesis, the apparent higher level of inhibition of HCMV yields than HCMV DNA synthesis compared to DHPG at higher concentration may be explained by papaverine's effect on cellular responses to HCMV infection (Albrecht *et al.,* 1989). Papaverine may affect HCMV replication by altering the intracellular environment required for efficient virus replication including DNA synthesis. This alteration may include the changes in the levels in secondary messengers as discussed above, inositol lipid hydrolysis (Valyi-Nagy *et al.,* 1988), or Na^+ entry (Nokta *et al.,* 1988). Apparantly these effects accumulate as the concentration of papaverine increases. In other words, the inhibition of HCMV multiplicaiton by papaverine may be due to its effect on several cellular events with differential sensitivitiy to papaverine ultimately affecting HCMV DNA synthesis and perhaps other events in HCMV replication.

The therapeutic application of drugs, however, cannot be based solely on measurements of antiviral activities *in vitro*. Cytotoxicity must be considered as well. Papaverine was aparently somewhat more cytotoxic than DHPG *in vitro*. At concentrations where substantial reduction of the infectious HCMV yields of about 10^4-fold were observed, however, both papaverine or DHPG exerted no significant cytotoxicity as determined by the viability of stationary LU cells.

적   요

파파베린과 두가지의 뉴클레오사이드 유사체인 DHPG와 acyclovir의 HCMV 증식에 대한 억제효과를 비교해 보았다. 파파베린과 DHPG는 ED$_{50}$ (바이러스 증식을 50% 억제하는 농도)가 각각 1.02 μM과 0.45 μM로 HCMV 증식을 효과적으로 억제한 반면 acyclovir는 비교적 그 효과가 떨어져 ED$_{50}$가 10.4 μM인 것으로 나타났다. 이들 약제의 세포독성을 알아본 결과 HCMV 증식을 효과적으로 억제하는 농도내에서는 세포의 생존에는 큰 영향을 미치지 않는 것으로 나타났다. 단백질합성 역시 크게 영향을 받지는 않았으나 DNA합성은 각 약제의 농도에 따른 억제현상을 보였다. 이러한 세포성 DNA합성의 억제는 이들 약제에 의한 바이러스 DNA합성 억제와 밀접한 관계가 있는 것으로 생각된다.

ACKNOWLEDGEMENT

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