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

Lee, Chan Hee¹ and T. Albrecht²

¹Department of Microbiology, Chungbuk National University, Cheongju, Korea
²Department of Microbiology, University of Texas Medical Branch, Galveston, Texas, U.S.A

Human Cytomegalovirus 감염에 대한 파파베린과 뉴클레오사이드 유사체의 항바이러스 효과

이찬희¹・T. Albrecht²

¹충북대학교 자연과학대학 미생물학과
²Department of Microbiology, University of Texas Medical Branch, Galveston, Texas, U.S.A

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 ED₅₀ (effective dose 50; the concentration at which 50% of virus yields was obtained) of approximately 1.02 and 0.45 μM, respectively; while acyclovir was less effective with an ED₅₀ 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 widespread, 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, development 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 fluorosubstituted 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 (Schnipper 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 replication 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% CO₂ 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 cm² 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 ml) 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/ml penicillin, 100 μg/ml streptomycin, and 1 μg/ml 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 cm² 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. ED₅₀ and ED₉₀ (the concentrations at which the infectious yields were reduced by 50 or 90%, respectively) were calculated from plots of drug concentration (log₁₀) relative to infectious virus yields (log₁₀).

**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 cm² flasks were treated with selected increments of concentrations 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 hemocytometer.

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 confluence in 35 mm dishes were treated with the drugs to be investigated. Seventy-two hr after the drug treatment, ³H-leucine (specific activity = 59.8 Ci/mmole) was added to culture fluids to a final concentration of 2 μCi/ml. Labelling was stopped 24 hr after the addition of ³H-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 ³H-thymidine (specific activity = 20 Ci/mmole, 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 lauryl sarcosinate-30 (Chemical Additive Co., Farmingdale, NY; 2% in 0.1X SSC) 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. ³H-thymidine (specific activity = 6.7 Ci/mmole) 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.
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 ED₉₀ of approximately 0.45 μM. Papaverine was similarly effective with a calculated ED₉₀ of about 1.02 μM. Acyclovir was the least effective in reducing HCMV yields with an ED₉₀ of approximately 10.4 μM. The calculated ED₉₀’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 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.

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μM)</th>
<th>Number of viable cells (±SD×10⁶)</th>
<th>Percent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>1.87±0.21</td>
<td>100</td>
</tr>
<tr>
<td>Papaverine</td>
<td>1</td>
<td>1.90±0.31</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.92±0.11</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.85±0.25</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.88±0.09</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.62±0.13</td>
<td>87</td>
</tr>
<tr>
<td>DHPG</td>
<td>1</td>
<td>2.03±0.41</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.86±0.12</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1.95±0.21</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.18±0.35</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.80±0.33</td>
<td>96</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>10</td>
<td>1.79±0.11</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.83±0.29</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.68±0.30</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.52±0.18</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1.40±0.21</td>
<td>75</td>
</tr>
</tbody>
</table>

*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.

Standard deviation

Percent of control=(the number of viable cells in the presence of drug)÷(the number of viable cells in the absence of drug)×100

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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μM)</th>
<th>3H-leucine CPM±S.D.</th>
<th>Percent of control</th>
<th>3H-thymidine CPM±S.D.</th>
<th>Percent of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>5388±337</td>
<td>100</td>
<td>18320±1149</td>
<td>100</td>
</tr>
<tr>
<td>Papaverine</td>
<td>3</td>
<td>4341±143</td>
<td>81</td>
<td>4904±486</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4540±208</td>
<td>84</td>
<td>2600±404</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>4662±223</td>
<td>87</td>
<td>1196±59</td>
<td>7</td>
</tr>
<tr>
<td>DHPG</td>
<td>3</td>
<td>5390±425</td>
<td>100</td>
<td>11340±1033</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5994±331</td>
<td>111</td>
<td>9263±693</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>5608±172</td>
<td>104</td>
<td>9050±2445</td>
<td>49</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>30</td>
<td>5329±222</td>
<td>99</td>
<td>12816±3166</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5594±77</td>
<td>104</td>
<td>10372±644</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>5594±424</td>
<td>104</td>
<td>9381±2871</td>
<td>51</td>
</tr>
</tbody>
</table>

*Cells were labelled from 72 to 96 hr after treatment with drug.

*Fresh maintenance medium and drugs were added at 48 hr intervals.

*Percent of control = (the number of viable cells in the presence of drug) ÷ (the number of viable cells in the absence of drug) x 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 3H-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.

○ papaverine; (△) DHPG; (□) 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 3H-leucine or 3H-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 slight 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 cellular 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 material following labelling of HCMV-infected cells with 3H-thymidine from 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 (Albrecht et al., 1987). As shown in Fig. 2, DHPG and papaverine
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 inhibition 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 $\mu$M 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 $\mu$M 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 suggested 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 $\mu$M were separated by isopycnic centrifugation (Fig. 3). At 10 $\mu$M, 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 $\mu$M in this study, the $ED_{50}$ from other studies range from 0.1 to 7.0 $\mu$M (summarized by Verheyden, 1988). The $ED_{50}$ for papaverine determined in this study was about 1 $\mu$M, and for acyclovir was about 10 $\mu$M. 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 (approximately 10-fold) by cellular kinases (Biron et al., 1985; Smeck et al., 1985), herpes simplex virus (HSV)-coded thymidine kinase (TK) may accelerate 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 efficiencies 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.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>HCMV multiplication* ($\mu$M)</th>
<th>HCMV DNA synthesis* ($\mu$M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$ED_{50}$</td>
<td>$ED_{50}$</td>
</tr>
<tr>
<td>Papaverine</td>
<td>1.02</td>
<td>2.4</td>
</tr>
<tr>
<td>DHPG</td>
<td>0.45</td>
<td>5.45</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>10.4</td>
<td>264</td>
</tr>
</tbody>
</table>

* Determined by the effect of the drugs on the production of HCMV infectious yields. Summarized from the data presented in Fig. 1.

* 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.
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 papaverine and DHPG could be explained by their different mechanisms of action. Since papaverine 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). Apparently these effects accumulate as the concentration of papaverine increases. In other words, the inhibition of HCMV multiplication by papaverine may be due to its effect on several cellular events with differential sensitivity 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 apparently somewhat more cytotoxic than DHPG in vitro. At concentrations where substantial reduction of the infectious HCMV yields of about 104-fold were observed, however, both papaverine or DHPG exerted no significant cytotoxicity as determined by the viability of stationary LU cells.

**ACKNOWLEDGEMENT**

This study was partially supported by a grant from the National Science Foundation. (Grant No. 893-0407-106-2).

**REFERENCES**


(Received February 25, 1991)
(Submitted September 13, 1991)