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Antiviral effects of 6-diazo-5-oxo-L-norleucin on replication of herpes simplex virus type 1

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Abstract

An L-glutamine antagonist, 6-diazo-5-oxo-L-norleucin (L-DON), inhibits replication of vesicular stomatitis virus, poliovirus and paramyxoviruses in cultured cells. We tested the antiviral activity of L-DON against different strains of herpes simplex virus type 1 (HSV-1) in Vero cells. In the presence of a physiological plasma concentration of L-glutamine (0.5 mM) L-Don inhibited 50% production of virus plaques at concentrations ranging from 7.9 to 16 μM. At concentrations of 40 μM L-Don inhibited infectious virus yield by 99%. The antiviral activity of L-DON decreased with increasing L-glutamine concentrations. A concentration of 5000 μM of L-Don had no significant effects on the viability of Vero cells. Transmission electron microscopical investigations showed that L-DON prevented mainly envelopment of viral nucleocapsids in the cytoplasm. The immunoprecipitation experiments demonstrated selective inhibition of synthesis of HSV-1 glycoproteins in L-DON treated cells. The results showed that L-DON inhibits HSV-1 replication at a late stage in the virus replication cycle, probably the cytoplasmic maturation of virions and subsequent virion egress from the cells.

Keywords: HSV; Acyclovir; 6-diazo-5-oxo-L-norleucin; Virus-resistance

1. Introduction

Specific amino acids have been shown to be essential for replication of some viruses, for example (i) L-glutamine for Sendai virus (Ito et al., 1974), Maloney leukemia virus (MLV) (Gloger et

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al., 1985), human parainfluenza virus type 2 (HPIV-2), mumps and vesicular stomatitis virus (Nishio et al., 1990), murine cytomegalovirus and reovirus type 3 (Keast and Vasquez, 1992); (ii) L-arginine for herpesvirus (Tankersley, 1964; Inglis, 1968; Spring et al., 1969; Courtney et al., 1970), adenovirus (Rouse and Schlesinger, 1967), respiratory syncytial virus (RSV) (Levine et al., 1971) and Newcastle disease virus (NDV) (Inuma et al., 1973); (iii) L-lysine for reovirus (Loh and Oie, 1969); (iv) phenylalanine for RSV (Levine et al., 1971); (v) L-serine for NDV (Ito et al., 1969); (vi) methionine for measles virus (Hirayama et al., 1985); and (vii) isoleucine for MLV (Gloger et al., 1985). These findings suggest that either specific antagonists against some amino acids, or specific inhibitors of enzymes responsible for the metabolism of some amino acids might be candidates as anti-virus substances.

A glutamine analogue, 6-Diazo-5-oxo-L-norleucin (L-DON), was isolated from culture broths of an unidentified *Streptomyces* (Ehrlich et al., 1956). L-DON specifically inhibits γ-glutamyl transpeptidase (Hartman and McGrath, 1973; Novogrodsky et al., 1977) and also inhibits nucleic acid production by interfering with the amino acid transfer reaction during purine and pyrimidine synthesis (King et al., 1978; Weber, 1983). L-DON showed growth-inhibitory activity towards bacteria, yeasts and some tumors (Ehrlich et al., 1956; Earhart et al., 1990) and inhibited replication of different viruses including poliovirus, vesicular stomatitis virus (Goldstein and Guskey, 1984), HPIV-2, mumps (Nishio et al., 1990), RSV (Huang et al., 1994) and human cytomegalovirus (HCMV) (Wachsman et al., 1996). In these studies virus replication was inhibited by L-DON at concentrations which were not toxic to host cells and the inhibitory effects were reversed by the addition of L-glutamine to the culture medium (Goldstein and Guskey, 1984; Nishio et al., 1990). In cell cultures infected with HPIV-2, L-DON mainly decreased the amounts of viral glycoproteins (Nishio et al., 1990). In contrast, in the cells infected with RSV, L-DON inhibited synthesis of all viral proteins without significant selective effects on viral glycoproteins (Huang et al., 1994). These results suggest that the mechanism of action may be different even though both viruses are in the same family.

In the present study we observed effects of L-DON on herpes simplex type 1 (HSV-1) replication in Vero cells. Immunoprecipitation of viral proteins from L-DON treated or untreated cultures was performed to show effects of the drug on biosynthesis of HSV-1 specific glycoproteins. For further elucidation of the mechanism of action, we determined the effect of the drug on viral morphogenesis using transmission electron microscopy.

2. Materials and methods

2.1. Cell culture

Monkey kidney cell line Vero was obtained from the American Type Culture Collection (Rockville, MD) and the cells were cultured in Eagle's minimum essential medium (MEM), supplemented with 10% fetal bovine serum (FBS) containing 2 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml streptomycin.

2.2. Compound

L-DON was purchased from Sigma-Aldrich (Deisenhofen, Germany). A 100 mM stock solution was prepared in MEM and stored at -70°C. Appropriate dilutions were prepared immediately prior to use.

2.3. Viruses

HSV-1 laboratory strain MacIntyre was obtained from ATCC while the laboratory strain KOS was provided by Professor H. Eggers (Institut für Virologie, Universität zu Köln, Germany). HSV-1 clinical isolates were obtained from samples of patients with AIDS. The identity of viruses was confirmed using indirect immunofluorescence staining with monoclonal antibodies (Syva, Palo Alto, CA). The viruses were propagated in Vero or HeLa cells as described previously (Cinatl et al., 1992a) and virus stocks were quantified in terms of the 50% tissue culture infective dose

($TCID_{50}$) by endpoint dilution, with the infectious titre determined by the method of Reed and Muench (1938).

2.4. Antiviral assay

Antiviral activity of L-DON was assessed using the plaque reduction assay and virus yield assay. The plaque reduction assay was performed on Vero cells monolayers as described previously (Cinatl et al., 1992b). Briefly, cells in 35 mm dishes were incubated for 1 h with 100 plaque forming units in MEM supplemented with 2% FBS. After virus adsorption, overlay medium containing 1% carboxymethylcellulose with or without different drug concentrations was added. Numbers of plaques were determined 3–4 days after infection.

For the virus yield assay, Vero cells were infected at multiplicity of infection (MOI) of 2 $TCID_{50}$/cell. After virus adsorption (1 h), cells were incubated with MEM with 2% FBS containing different drug concentrations. At 24 h post-infection infected cultures were frozen and thawed twice to liberate cell-associated virus. Virus titre was determined by the endpoint dilution method and expressed as $TCID_{50}$/ml (see above).

2.5. Measurement of cell growth and viability

Effects of L-DON on cell proliferation and viability were tested in uninfected Vero cultures on the same day as the antiviral activity experiments. For this aim, Vero cells were seeded in 35 mm culture dishes at a density of 4×10^4 cells/cm^2 and incubated in MEM supplemented with 2% FBS and 0.5 mM L-glutamine. To measure effects on non-confluent and confluent cultures L-DON was added immediately or 5 days after seeding, respectively. On day 4 of treatment, viable cells were counted using an hemocytometer. The viability of the cells was determined by the dye exclusion method after staining with 0.5% trypan blue solution.

2.6. Electron microscopy

For ultrastructural investigations of virus morphogenesis, Vero cells were infected with HSV-1 strain MacIntyre at an MOI of 2 $TCID_{50}$/cell and incubated with or without 1000 μM L-DON. At 18 h post infection cells were processed for ultrastructural analysis as described previously (Cinatl et al., 1994). Briefly, cells were pelleted and fixed with 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in Durupan-Epon. Thin sections were contrasted with uranyl acetate and lead citrate and viewed with a Joel, JEM, 2000 CX microscope. For quantitative examination of numbers of virus-infected cells, 100 cells in electron micrographs obtained from cultures without or with drug treatment were assessed.

2.7. Immunoprecipitation of HSV-1 specific proteins

Effects of L-DON on the biosynthesis of HSV-1 specific proteins were studied in Vero cells infected with the MacIntyre strain at an MOI of 2 $TCID_{50}$/cell. After virus adsorption, the cells were incubated with or without L-DON at a concentration of 200 μM. At 18 h post infection, the cells were depleted of methionine and then pulse labeled with ^{35}S-methionine (at a final concentration of 100 μCi/ml) for 1 h. To immunoprecipitate virus-specific proteins, cellular extracts were prepared and incubated with polyclonal anti HSV-1 rabbit antibody (Dako) or mouse monoclonal antibodies directed against HSV-1 glycoprotein D (gD) and glycoprotein E (gE) (all obtained from Advanced Biotechnologies, Columbia, MD) as described previously (Cinatl et al., 1994). The precipitates were subjected to SDS-PAGE, under reducing conditions and fluorography.

3. Results

3.1. Antiviral effects of L-DON

In the previous studies L-DON inhibited replication of different viruses when added to the cell culture medium simultaneously with or after virus adsorption (Nishio et al., 1990; Huang et al., 1994). Accordingly, we observed antiviral effects

Table 1
Antiviral effects of ACV and L-DON on the growth of different HSV-1 strains in the plaque reduction assay

HSV-1	EC_{50} (μM)	
	ACV	L-DON
MacIntyre	0.61 ± 0.058[a]	8.0 ± 0.95
KOS	0.29 ± 0.042	16 ± 2.1
Patient 1	0.42 ± 0.066	7.9 ± 0.83
Patient 2	0.57 ± 0.051	12 ± 1.5

[a] Values are the mean ($\pm$S.D.) of a quadruplicate culture from two independent experiments.

of L-DON on HSV-1 replication when the drug was added to the culture medium after virus adsorption. As shown in Table 1, L-DON in the presence of a physiological plasma concentration of L-glutamine (0.5 mM) inhibited efficiently plaque formation of different HSV-1 strains. The results demonstrated that both laboratory strains and clinical isolates of HSV-1 were similarly sensitive to inhibition by L-DON. L-DON inhibited by 50% production of plaques at 50% effective concentrations (EC_{50}) ranging from 7.9 to 16 μM. These concentrations were 10–50-fold higher than EC_{50} of acyclovir (ACV).

The effects of L-DON on virus yield in Vero cells infected with MacIntyre or KOS strain (incubated in medium containing 0.5 mM L-glutamine) are shown in Table 2. L-DON inhibited virus production in a dose-dependent manner; L-DON at a concentration as low as 8 μM inhibited virus yield by more than 50%. At a concentration of 40 μM of L-DON, 99% of viral replication was inhibited. However, L-DON was not able to completely inhibit the infectious viral titre. At a concentration of 1000 μM of L-DON, the virus titre was reduced by more than 10^4 fold; in this case, titres as high as 1.5×10^1 and 2.15×10^2 were found in Vero cells infected with the MacIntyre or KOS strain, respectively.

Table 2
Effects of L-DON on virus yields of MacIntyre and KOS

L-DON concentration (μM)	Virus yield (log $TCID_{50}$ ml)	
	MacIntyre	KOS
0	7.79 ± 0.04[a]	7.95 ± 0.02
8	7.32 ± 0.03	7.43 ± 0.04
40	5.72 ± 0.02	5.92 ± 0.03
200	4.76 ± 0.03	4.95 ± 0.04
1000	1.17 ± 0.03	2.15 ± 0.02

[a] Values are the mean ($\pm$S.D.) of a triplicate culture from two independent experiments.

L-DON also inhibited cell fusion in Vero cells infected with the MacIntyre strain (Fig. 1A–D). The cells infected at MOI 2 and treated with 1000 μM showed no evidence of cell fusion 18 h post infection and cultures treated with L-DON at a concentration of 200 μM showed only occasional formation of multinucleated syncytia.

3.2. Effects of L-glutamine on antiviral activity of L-DON

In Vero cells infected with HPIV-2, L-glutamine partly reversed the inhibitory effect of L-DON on virus replication (Nishio et al., 1990). We studied whether L-glutamine may also influence the antiviral effects of L-DON in Vero cells infected with the MacIntyre strain. Similar numbers of HSV-1 plaques developed both in medium with and without L-glutamine (data not shown). However, as shown in Table 3, the EC_{50} of L-DON in plaque reduction assay increased with increasing L-glutamine concentrations. EC_{50} of L-DON was 3.1 μM in medium without L-glutamine, i.e. three-fold and ten-fold lower than in medium with 0.5 and 2 mM L-glutamine, respectively; L-glutamine had no effect on antiviral activity of ACV (Table 3).

3.3. Effects of L-DON on cell viability and growth

As shown in Table 4, the concentration of L-DON required for 50% reduction (IC_{50}) of the cell proliferation in non-confluent cultures was 10 μM, while in confluent cultures the IC_{50} was greater than 5000 μM (maximum concentration tested). L-DON had no significant effect on cell viability both in non-confluent and confluent Vero cultures. The selectivity index (ratio IC_{50}/EC_{50}) in confluent cultures (used in antiviral assays) was

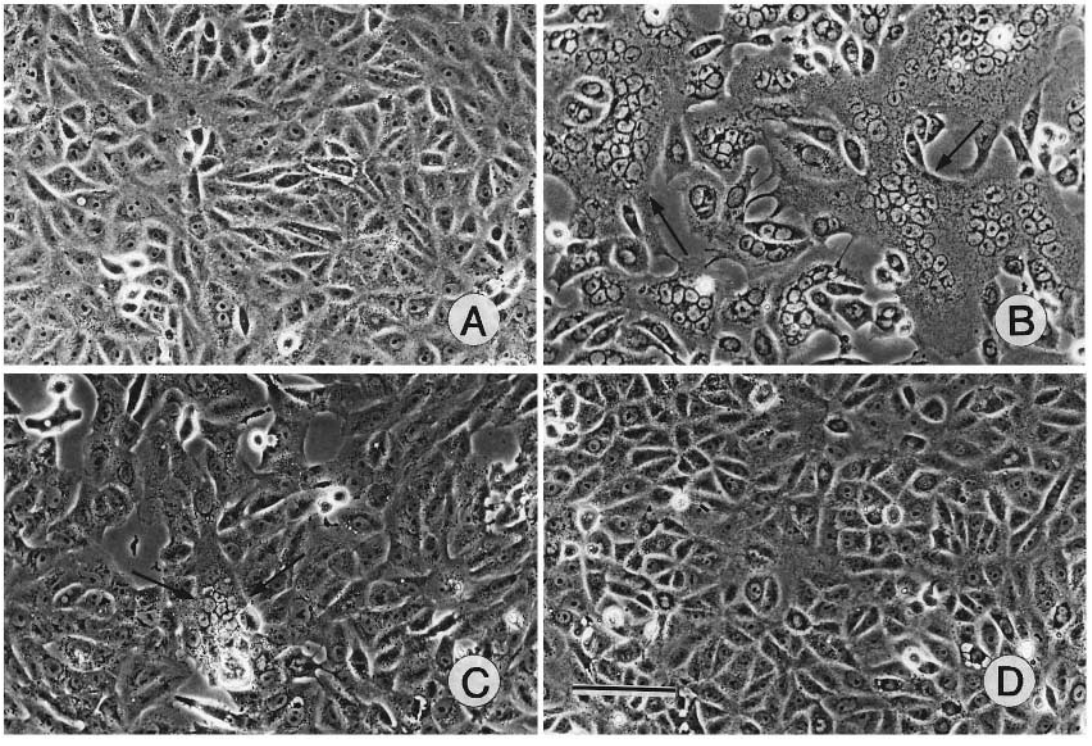

Fig. 1. Effects of L-DON on the cell fusion by HSV-1 in Vero cells. (A) Uninfected cells without any L-DON treatment. (B) HSV-1-infected cells without L-DON treatment showing numerous multinucleated cells (arrows). (C) HSV-1-infected, L-DON (200 μM) treated cells showing only occasional multinucleated cells (arrow). (D) HSV-1-infected, L-DON (1000 μM)-treated cells, without any cytopathic effect. Bar represents 50 μM.

greater than 300 for different HSV-1 strains. Moreover, in concert with the previous studies (Nishio et al., 1990; Huang et al., 1994), we found that cellular protein synthesis was not influenced by L-DON treatment as measured by the incorporation of ^{35}S-methionine and cysteine (data not shown).

Table 3
Effects of L-glutamine on antiviral activity of ACV and L-DON in the plaque reduction assay

L-glutamine (μM)	EC_{50} (μM)	
	ACV	L-DON
0	0.75 ± 0.11[a]	3.1 ± 0.23
0.125	0.62 ± 0.058	4.3 ± 0.56
0.25	0.53 ± 0.078	5.6 ± 0.48
0.5	0.81 ± 0.074	8.9 ± 1.1
1	0.66 ± 0.12	19 ± 2.5
2	0.54 ± 0.081	36 ± 3.1

[a] Values are the mean ($\pm$S.D.) of a quadruplicate culture from two independent experiments.

Table 4
Effects of L-DON on cell number in non-confluent and confluent Vero cultures

L-DON (μM)	Cell no./cm$^2 \times 10^{-4}$	
	Non-confluent	Confluent
0	21.5 ± 1.9	22.3 ± 2.8
8	11.5 ± 1.4	21.2 ± 3.1
40	4.9 ± 0.62	23.1 ± 1.8
200	4.3 ± 0.38	22.3 ± 2.4
1000	3.9 ± 0.41	20.4 ± 1.6
5000	4.1 ± 0.56	21.8 ± 2.9

Results represent the mean value ($\pm$S.D.) of a triplicate culture from two independent experiments. Cell viability was over 96% in all cases.

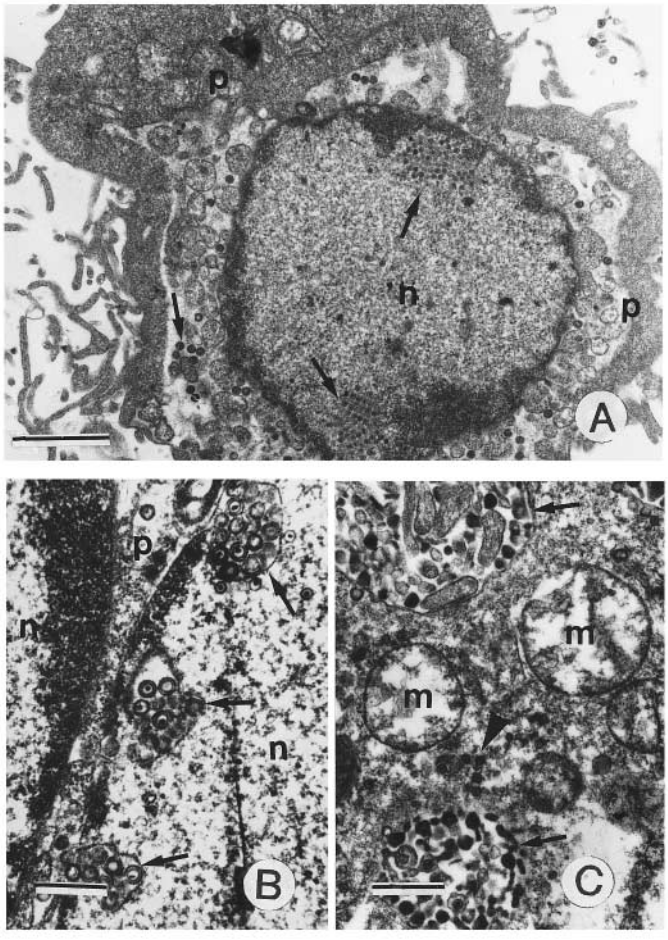

Fig. 2. Electron microscopic observations of a HSV-1-infected, untreated Vero cell. (A) Overview of a Vero cell with numerous nucleocapsids in the nucleus (n) (arrows), enveloped virions in the cytoplasm (arrow) (p) and in the extracellular space. (B) Accumulation of enveloped virus particles in the perinuclear space (arrows). (C) The cytoplasma with a few naked nucleocapsids (arrowhead) and enveloped particles enclosed in vacuoles (arrows) and mitochondria (m). Bars represent 3 μM (A), 500 nm (B) and 600 nm (C).

3.4. Effects of L-DON on virus morphogenesis

Ultrastructural observations of Vero cells at late HSV infectious cycle, i.e. 18 h post infection showed significant differences in the number of infected cells in untreated cultures and cultures treated with 1000 μM L-DON. In control cultures 98% of cells were infected with HSV-1 (containing nucleocapsids in the nucleus and/or enveloped virus particles in the cytoplasma), while only 36% of infected cells were found in treated cultures. In untreated cultures all of infected cells contained naked nucleocapsids in the nucleus (Fig. 2A and 2B). Nucleocapsids and enveloped virus particles in perinuclear space were observed in 48% of cells in untreated cultures (Fig. 2B). Cytoplasmic enveloped particles frequently enclosed in vacuoles as well as few naked nucleocapsids were observed in 56% of cells in untreated cultures (Fig. 2C); enveloped virions were observed also in extracellular spaces (Fig. 2A). In the case of infected cells treated with L-DON only 36% of the cells con-

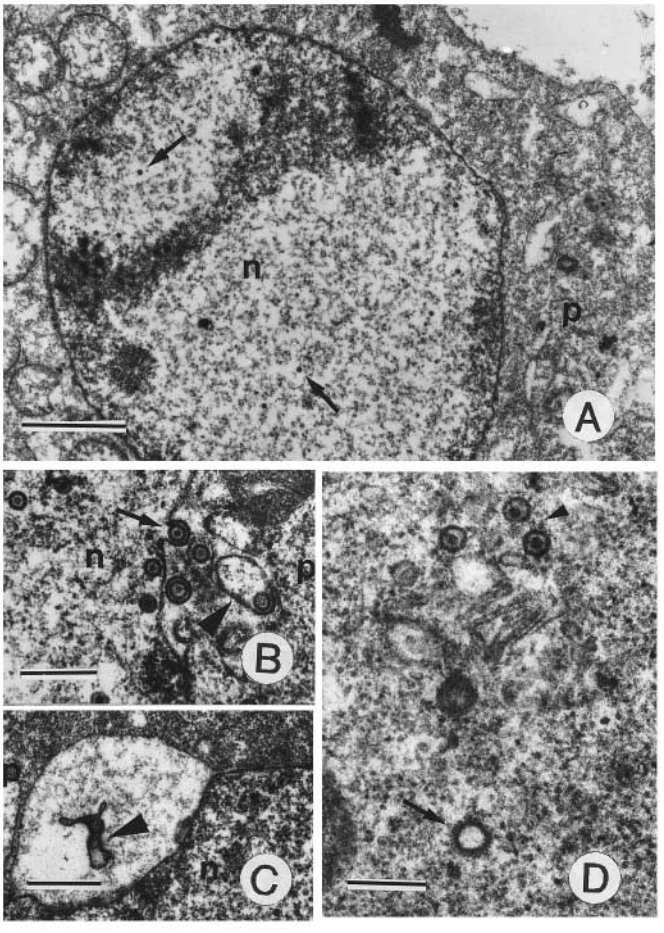

Fig. 3. Electron microscopic observations of HSV-1-infected, L-DON (1000 μM)-treated Vero cell. (A) Overview of Vero cell with nucleocapsids in the nucleus (n) (arrows) and lacking enveloped particles in the cytoplasm (p) or extracellular space. (B) Nucleocapsid budding through the inner nuclear membrane (arrow) and enveloped virions in the perinuclear space. Virions with swollen envelopes were observed (arrowhead). (C) Perinuclear space with an aberrantly enveloped virion (arrowhead). (D) The cytoplasma with few naked nucleocapsids with dense cores (arrowhead) and a non-enveloped, empty capsid (arrow). Bars represent 4 μm (A), 300 nm (B), 1 μm (C) and 300 nm (D).

tained naked nucleocapsids in the nucleus; moreover, significantly lower numbers of nucleocapsids per cell nucleus were found than in untreated cultures (Fig. 3A and 3B). Nucleocapsids and enveloped virions in the perinuclear space were found in 8% of cells in treated cultures (Fig. 3B); however, in the perinuclear space also aberrantly enveloped particles characterized by swollen envelope frequently occurred which were not observed in untreated cells (Fig. 3B and 3C). In the cytoplasm of L-DON treated cultures 6% of cells contained few naked nucleocapsids, while enveloped virus particles were not found (Fig. 3D). No extracellular virus particles were observed in Vero cells treated with L-DON.

3.5. Effects of L-DON on biosynthesis of virus specific proteins

L-DON has previously been shown to decrease the amounts of viral glycoproteins in cell cultures infected with HPIV-2 (Nishio et al., 1990). How-

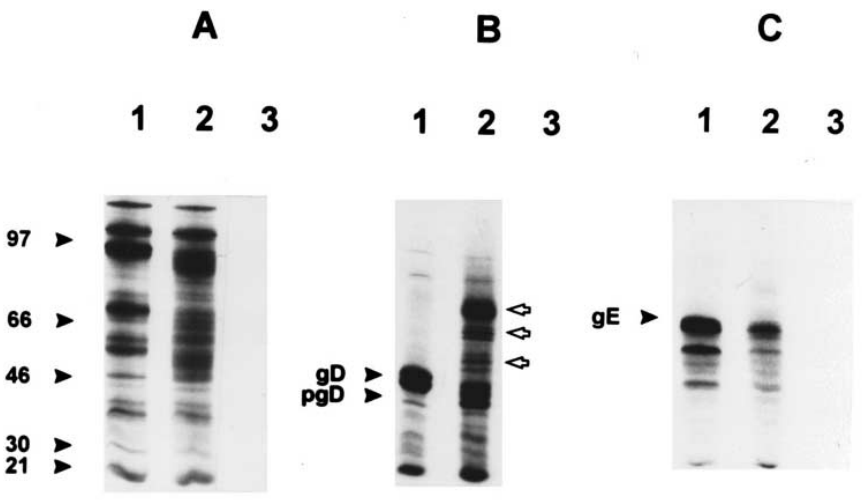

Fig. 4. Effects of L-DON on the biosynthesis of HSV-1-specific proteins in treated and untreated cells. (A) HSV-1 proteins were immunoprecipitated using rabbit antiserum to HSV-1. (B) HSV-1 proteins were immunoprecipitated using monoclonal antibody to gD. Arrowheads show the position of gD and pgD and arrows show position of additional bands. (C) HSV-1 proteins were immunoprecipitated using monoclonal antibody directed to gE. Lane 1, HSV-1-infected cells without L-DON treatment; Lane 2, L-DON (200 μM)-treated cells; Lane 3, mock-infected cell.

ever, in the cells infected with RSV L-DON had no significant effect on the synthesis of viral glycoproteins and inhibited synthesis of all viral proteins to a similar extent (Huang et al., 1994). Therefore, it was of interest to study the effects of L-DON on viral protein synthesis in HSV-1 infected Vero cells. L-DON at a concentration of 200 μM had no significant effects on synthesis of most viral proteins as demonstrated in pulse-labeled cells using anti-HSV-1 polyclonal serum (Fig. 4A). However, significant changes in the expression of bands with relative mobilities corresponding to those of viral glycoproteins were observed. Therefore, we tested whether biosynthesis of HSV-1 specific glycoproteins is influenced in infected cells treated with 200 μM L-DON using monoclonal antibodies directed against gD and gE. The synthesis of gD was significantly impaired. In this case a band with a higher relative mobility than that of mature gD (59 kDa) was found (Fig. 4B), probably representing the immature gD precursor (Cheung et al., 1991). However, in L-DON-treated cells several bands with a significantly lower relative mobility than that of mature gD were found, suggesting profound effects of the drug on gD processing (Fig. 4B). L-DON also influenced synthesis of gE (80 kDa); however, in this case only a low amount of gE was synthesized with a relative mobility similar to that of gE synthesized in untreated infected cultures (Fig. 4C).

4. Discussion

L-DON is a potent inhibitor of HSV-1 growth in cell culture. At a concentration of 40 and 200 μM L-DON inhibited infectious virus yields in Vero cells infected with HSV-1 or HSV-2 strains by 90 and 99%, respectively. These results suggest that HSV-1 virus is more sensitive than HPIV-2, mumps and VSV as reported by Nishio et al. (1990) and less sensitive than RSV as demonstrated by Huang et al. (1994). Antiviral effects of L-DON were not cell specific since it had similar effects in HSV infected HeLa and human dermal fibroblast cells (data not shown). Wachsman et al. (1996) tested antiviral activity of different inhibitors of pyrimidine de-novo biosynthesis including L-DON on replication of HSV-1 and human cytomegalovirus. They found that L-DON had no significant activity at a 200 μM concentra-

tion on the replication of HSV-1 (strain F) in human embryonal lung fibroblasts, while it had significant antiviral effects against human cytomegalovirus. In our hands not only virus production but also the cytopathic effect, virus morphogenesis and synthesis of viral glycoproteins were significantly influenced by 200 μM L-DON. It would be of interest to show whether strain F differs from the HSV-1 strains used in the present study in their sensitivity to L-DON and/or whether the use of human embryonal fibroblasts may affect L-DON antiviral activity.

To elucidate the mechanism of L-DON antiviral activity in HSV infected cells we investigated its effects on virus morphogenesis and synthesis of viral glycoproteins in Vero cells infected with the MacIntyre strain. There is a consensus that viral nucleocapsids, formed in the nucleus, acquire a lipid envelope containing immature forms of the HSV glycoproteins at the inner nuclear envelope and that these particles transiently accumulate in the perinuclear space, i.e. the region between the inner and outer nuclear membranes (Compton and Courtney, 1984; Whealy et al., 1991; Torrisi et al., 1992). However, there is controversy over the process by which these particles reach the cell surface. One possibility is that enveloped virus particles are transported to the cell surface in the interior of a series of membranous vesicles or tubules derived from the ER and Golgi apparatus (Johnson and Spear, 1982; Campadelli-Fiume et al., 1991; Torrisi et al., 1992). Other investigators suggested that enveloped particles present in the perinuclear space can fuse with cellular membranes (e.g. the outer nuclear envelope) producing unenveloped cytoplasmic capsids. These unenveloped capsids are derived membranes modified with viral glycoproteins (Whealy et al., 1991; Gershon et al., 1994). The present results seem to fit with the latter model of HSV envelopment. We observed enveloped nucleocapsids in perinuclear space of L-DON treated cells; however, in the cytoplasma mostly naked nucleocapsids were found. This suggests that L-DON did not block movement of particles to the cytoplasm but may interfere with the acquisition of an envelope derived from the Golgi-apparatus. These effects of L-DON on HSV-1 morphogenesis are similar to those observed in HSV infected cultures treated with brefeldin A, which causes a functional disintegration of the Golgi complex (Cheung et al., 1991; Whealy et al., 1991). However, in the perinuclear space of L-DON-treated cells abberrantly enveloped particles frequently accumulated which were not observed in untreated control cultures. It is possible that L-DON antiviral activity also results at least in part from a disturbance of viral envelopment at the inner nuclear membrane.

L-DON is known to interfere with glycoprotein synthesis by inhibiting the production of glucosamine (King et al., 1978). It has been suggested that inhibition of glycoprotein synthesis may contribute to antiviral activity of L-DON against HPIV-2 (Nishio et al., 1990). HSV-1 encodes at least 11 glycoproteins and five of these glycoproteins gB, gD, gH, gK and gL, are essential for productive infections in cultured cells and entry of extracellular virus particles into cells (Ligas and Johnson, 1988; Forrester et al., 1992; Hutchinson and Johnson, 1955). The remaining glycoproteins are not essential for virus infection and replication in cultured cells. However, some of these nonessential glycoproteins such as gI or gE may be important for cell-to-cell spread (Balan et al., 1994; Dingwell et al., 1995). It is well known that changes in the glycosylation, processing and/or transport of HSV-1 glycoproteins may have a profound effect on HSV growth. Immunoprecipitation experiments using polyclonal and monoclonal antibodies directed against gD and gE demonstrated that L-DON impaired the biosynthesis of both essential and nonessential glycoproteins. Thus, L-DON might contribute to the inhibition of HSV proliferation due to effects on the metabolism of virus glycoproteins. Expression of viral glycoproteins and their transport to the cell surface may also be important for induction of cell fusion (Knipe, 1996). It is possible that inhibition of virus-induced cell fusion by L-DON results at least in part from its effects on viral glycoproteins.

L-DON has recently been used in clinical trials as an antisarcoma agent. Patients treated with L-DON did not experience any severe side effects even at high doses (Earhart et al., 1990). This indicates that L-DON has potential use as an

antiviral agent in humans especially against those that are highly sensitive to the drug such as RSV. However, it is not clear whether L-DON could be used as an antiviral agent with a significant therapeutic ratio in patients infected with HSV. The clinically tolerated levels of L-DON are about 2 mg/l (Ahluwalia et al., 1990), i.e. similar to the EC_{50} observed in cell culture. On the other hand, L-DON may be a useful drug for topical treatment of HSV disease especially since its mechanism of action differs from that of currently used antiviral agents.

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