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Short Communication

In vitro inhibition of human cytomegalovirus replication by desferrioxamine

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Abstract

Desferrioxamine (DFO) is commonly used in therapy as a chelator of ferric ion in disorders of iron overload. We found that DFO inhibits human cytomegalovirus (HCMV) replication in infected cultures of human foreskin fibroblasts (HFF) at concentrations that have been achieved in humans with no significant adverse effects. The concentrations of DFO required for 50 and 90% reduction in the production of a HCMV-late antigen ranged for several HCMV strains from 3.1 to 4.9 μM and from 14.2 to 17.3 μM, respectively. DFO concentration of 60 μM had no significant effect on the viability of HFF cells. Inhibitory effects of DFO on HCMV replication were completely prevented by co-incubation with stoichiometric amounts of Fe^{3+}.

Key words: Human cytomegalovirus; Desferrioxamine; Ribonucleotide reductase; Iron

Several members of the herpes virus family including herpes simplex virus type 1 (HSV-1), HSV-2 (Averett et al., 1983, 1984; Dutia 1983), varicella zoster virus (VZV) (Spector et al., 1989) and Epstein-Barr virus (Goldschmidts et al., 1989) were found to encode their own ribonucleotide reductase enzymes which are biochemically distinct from the isofunctional cellular counterparts. Some iron-chelating substances including 2-acetylpyridine 4-(morpholinoethyl)thiosemicarbazone (A723U) (Spector et al., 1985), 2-acetylpyridine 5-[(dimethylamino)thiocarbonyl]thiocarbonohydrazone (1110U81 or

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A1110U) (Spector et al., 1989; Porter et al., 1990) and 2-acetylpyridine 5-[(2-chloroanalino)thiocarbonyl]thiocarbonohydrazone (348U87) (Spector et al., 1991) are potent inhibitors of herpes virus ribonucleotide reductases, while they have lesser effects on cellular counterparts. These agents inhibit replication of HSV-1 and potentiate the activity of acyclovir against HSV-1 (Spector et al., 1989; Porter et al., 1990; Spector et al., 1992). 1110U81 and A1110U also inhibit HCMV replication and potentiate anti-HCMV effect of ganciclovir (GCV) (Hamzeh et al., 1993). However, sequence homology studies or enzyme purification studies failed to show that HCMV encodes an active ribonucleotide reductase. Therefore, it is of interest to study effects of iron chelators other than 111081 and A1110U on HCMV replication.

Desferrioxamine (DFO) is a trihydroxamic acid which can complex with ferric ion to form ferrioxamine (Summers et al., 1979). This was the basis for use of DFO therapeutically as a chelator of ferric ion in disorders of iron overload (Pippard and Callender, 1983). In the present study, we observed in vitro effects of DFO, single or combined with Fe^{3+}, on HCMV replication.

GCV (Syntex, Palo Alto, CA) and DFO (Desferal; Ciba-Geigy, Basel, Switzerland) were prepared fresh (on the day of each experiment) in distilled water or dimethylsulfoxide, respectively. HCMV strain AD169 was purchased from American Type Culture Collection (ATCC; Rockville, MD). HCMV clinical isolates were obtained from urine specimens of patients with AIDS. HCMV strains were maintained and passaged in human foreskin fibroblasts (HFF) using Minimal Essential Medium (MEM) supplemented with 2% fetal bovine serum (FBS). HSV-1 (strain McIntyre), HSV-2 (strain MS), adenovirus 3 (strain GB) and poliovirus type 1 (strain Chat) were obtained from ATCC.

Antiviral activity of DFO and GCV against HCMV strains was determined in confluent HFF cultures using a rapid modification of a plaque reduction assay (Gerna et al., 1992). To measure antiviral activity of DFO against HSV-1, HSV-2, adenovirus 3 and poliovirus 1, virus yield reduction assay was performed as described previously (Cinatl et al., 1992) using Vero, HeLa and HFF cell lines.

Effects of DFO on cell proliferation and viability were tested in uninfected HFF cultures on the same day as the antiviral activity experiments. For this aim, HFF cells were seeded at a density 2×10^4 cells per cm^2 and DFO was added to non-confluent or confluent cultures 1 and 6 days after seeding, respectively. Viable cells were counted using a hemocytometer. The viability of the cells was determined by the dye exclusion method after staining with 0.5% trypan blue solution.

As shown in Table 1, the concentrations of DFO required for 50 and 90% reduction (EC_{50} and EC_{90}) of the formation of HCMV plaques in HFF cells ranged for several HCMV strains from 3.1 to 4.9 μM and from 14.2 to 17.3 μM, respectively. In contrast, a 60 μM DFO-concentration (maximum concentration tested) had no effect on replication of other viruses including HSV-1, HSV-2, poliovirus 1 and adenovirus 3 tested for all cell lines.

As shown in Table 2, the concentration of DFO required for 50% reduction (IC_{50}) of the cell proliferation in nonconfluent cultures was 9.8 μM, while in confluent cultures the IC_{50} was greater than 60 μM (maximum concentration tested). DFO had no significant effect on cell viability both in nonconfluent and confluent HFF cultures. The

Table 1
Effects of GCV and DFO on plaque formation by different HCMV strains

HCMV strain	GCV (μM)		DFO (μM)	
	EC_{50}^{a}	EC_{90}^{b}	EC_{50}	EC_{90}
AD 169	3.8 ± 0.72	58.1 ± 6.3	4.9 ± 0.51	17.3 ± 1.5
Patient 1	5.4 ± 0.68	55.4 ± 4.9	4.1 ± 0.42	14.2 ± 2.1
Patient 2	4.5 ± 0.49	61.2 ± 7.2	4.2 ± 0.38	15.8 ± 1.9
Patient 3	6.2 ± 0.81	54.3 ± 6.1	3.1 ± 0.37	14.9 ± 1.2
Patient 4	5.8 ± 0.48	58.5 ± 6.4	3.4 ± 0.41	16.3 ± 1.7

[a] The concentration of compound that reduced plaque formation by 50%.
[b] The concentration of compound that reduced plaque formation by 90%.
Results represent mean value (± S.D.) for two separate experiments.

selectivity index (ratio IC_{50}/EC_{50}) in confluent cultures (used in antiviral assays) was greater than 10.

We tested the role of iron in the anti-HCMV effect of DFO. AD169 infected HFF cells were co-incubated with DFO and $FeCl_3$. The results proved that the inhibitory effects of DFO were completely prevented by co-incubation with stoichiometric ammount of Fe^{3+} (Fig. 1).

We found that DFO is a nontoxic inhibitor of HCMV replication. The mechanism by which DFO exerts its antiviral activity is not clear. HCMV infection is characterized by several fold increases in the intracellular deoxynucleotides (Biron et al., 1985) which may be required for efficient viral replication. DFO is a potent inhibitor of the de novo synthesis of deoxyribonucleotides due to the inhibition of cellular ribonucleotide reductase (Hoffbrand et al., 1976; Hunting and Henderson, 1983). Thus, inhibition of the buildup of deoxynucleotide pool resulting from inhibition of cellular ribonucleotide reductase may explain inhibition of HCMV replication. On the other hand, mechanisms independent of ribonucleotide reductase could account for antiviral effects. In certain cellular models DFO and other iron chelators have been found to inhibit DNA synthesis by inhibition of ribonucleotide and deoxyribonucleotide incorporation into nucleic acids (Barankiewicz and Cohen, 1987). Such DFO effects may be deleterious only to the rapidly replicating viral DNA and not to the less replicating cellular DNA. The results dealing with a prevention of DFO antiviral activity by co-incubation with $FeCl_3$ suggest

Table 2
Effects of DFO on cell numbers in nonconfluent and confluent HFF cultures

DFO (μM)	Cell number per $cm^2 \times 10^{-4}$	
	nonconfluent	confluent
0	8.2 ± 1.1	9.5 ± 1.2
5	6.3 ± 0.3	9.1 ± 1.3
10	4.5 ± 0.7	8.8 ± 0.7
20	2.1 ± 0.4	8.2 ± 0.9
40	2.2 ± 0.3	7.9 ± 0.8
60	1.9 ± 0.4	8.1 ± 1.0

Results represent mean value (± S.D.) of triplicate culture from two independent two independent experiments. Cell viability was over 95% in all cases.

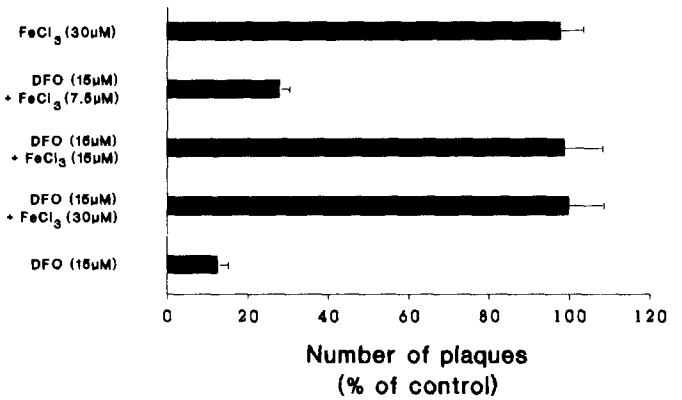

Fig. 1. Effect of $FeCl_3$ on antiviral activity of DFO against HCMV strain AD169. Each column represents mean value ($\pm$S.D.) from two separate experiments. Controls were infected with AD169 without any treatment.

that DFO exerts its primary effect by chelating ferric ion and subsequently inhibiting HCMV replication. This finding provides evidence for the role of iron in HCMV replication.

DFO failed to inhibit replication of several human pathogenic viruses including HSV-1 and HSV-2. The lack of inhibition of HSV is of interest since HSV mutants lacking ribonucleotide reductase grow inefficiently especially in nondividing cells (Goldstein and Weller, 1988) and DFO is a potent inhibitor of purified HSV ribonucleotide reductase (Porter et al., 1990). Therefore, the effects of DFO on viral ribonucleotide reductase should be examined in HSV infected cells. The present results demonstrate that antiviral effects of DFO and the regulation of virus replication by iron are highly selective for HCMV.

In conclusion, DFO is a promising substance for further testing to see whether it could be used in patients with HCMV infection. The concentrations which significantly inhibit HCMV replication in vitro can be easily achieved in vivo without recognisable adverse effects (Allain et al., 1987; Donfrancesco et al., 1990). Moreover, the observation of in vitro inhibition of HCMV by DFO suggests a new mechanism of HCMV inhibition.

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