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Antiviral and immunomodulatory activity of the metal chelator ethylenediaminedisuccinic acid against cytomegalovirus in vitro and in vivo

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Abstract

Antiviral activity of the metal chelator ethylenediaminedisuccinic acid (EDDS) was examined in vitro against human cytomegalovirus (HCMV) wild type strains and strains that are resistant against ganciclovir (GCV) and cidofovir (HPMPC). EDDS inhibited the replication of wild-type as well as GCV- and HPMPC-resistant strains with a 50% effective concentration of 7.4–12 μg/ml. At concentrations of 100 μg/ml EDDS, unlike GCV or HPMPC, suppressed HCMV-induced up-regulation of intercellular adhesion molecule-1 (ICAM-1) and reduced T-cell adhesion to HCMV-infected cells in a monolayer adhesion model. In vitro EDDS inhibited murine cytomegalovirus (MCMV) replication (EC_{50} 8.6 μg/ml) and caused in mice some protection against MCMV induced mortality at a non-toxic dose. Since immunopathological factors may play a significant role in HCMV disease it will be of interest to further study whether EDDS is effective in terms of modulation of inflammatory responses to HCMV infections.

Keywords: Ethylenediaminedisuccinic acid; Cytomegalovirus; Antiviral activity; Immunomodulatory activity

1. Introduction

Human cytomegalovirus (HCMV) is recognized as a major pathogen in immunocompromised patients responsible for high morbidity and mortality (Vogel et al., 1997; Emery, 2001). Despite the use of strong inhibitors of HCMV DNA synthesis

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such as ganciclovir (GCV), foscarnet (PFA) and cidofovir (HPMPC), relapse of disease may occur after cessation of therapy and the virus may become resistant following long-term treatment (Grossi and Baldanti, 1997; Jabs et al., 1998). On the other hand, long-term use of GCV is limited by the appearance of neutropenia and thrombocytopenia whereas PFA and HPMPC may cause nephrotoxicity (Vogel et al., 1997; Field, 1999). Numerous pathogenic effects of HCMV may stem from virus replication in permissive cells resulting in their lysis. In addition, immunopathological factors may play a role in CMV disease, such as HCMV retinitis or HCMV pneumonia characterized by leukocyte infiltration of infected tissues. In vitro experiments demonstrated that HCMV induces up-regulation of several adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) (Grundy and Downes, 1993; Craigen and Grundy, 1996; Cinatl et al., 1999). ICAM-1, an important inducible cell adhesion glycoprotein of the immunoglobulin supergene family, is responsible for enhanced functional binding of leukocytes to infected cells. This is a major step in leukocyte transendothelial migration to sites of inflammation induced by stimuli including HCMV (Span et al., 1991; Tuder et al., 1991; Grundy et al., 1993; Blaheta et al., 1994). Further investigations revealed that HCMV also induces enhanced production of both C-X-C (i.e. IL-8 and GRO-α) and C-C (i.e. RANTES, MCP-1 and MIP-1α) chemokines in different cell types independently of virus replication (Grundy, 1998; Michelson et al., 1997; Murayama et al., 1997). Importantly, secretion of C–X–C chemokines by HCMV-infected endothelial cells resulted in increased transendothelial neutrophil migration (Craigen et al., 1997). Therefore, novel antiviral treatments including those preventing the inflammatory potential of HCMV are highly desirable.

Previously it was demonstrated that metal chelators such as desferrioxamine (DFO) or diethylenetriaminepentaacetic acid (DTPA) posses anti-HCMV activity (Cinatl et al., 1994, 1996a). Moreover, these agents proved to be effective in down-modulation of chemokines or virally induced proinflammatory molecules or adhesion molecules, both in vitro and in vivo (Cinatl et al., 1995; Kloover et al., 1999; Martelius et al., 1999). We have therefore studied the effect of the metal chelator ethylenediaminedisuccinic acid (EDDS) on the replication of HCMV as well as the impact of EDDS on HCMV induced up-regulation of cell surface molecule ICAM-1. Functional studies to prove the biological relevance were performed using a monolayer adhesion assay. To investigate in vivo antiviral activity, effects of EDDS on murine cytomegalovirus (MCMV) induced mortality in SCID mice were evaluated.

2. Materials and methods

2.1. Cells and viruses

Human embryonic lung fibroblasts (HEL) obtained from American type culture collection (ATCC, Manassas, VA) were used at low passage. They were grown in 96-well microtiter plates and used for HCMV evaluation. The human T-cell leukemia line Jurkat (ATCC) was used as a model for activated human T cells in functional studies on EDDS effects. C127I cells were obtained from ATCC (ATCC: CRL-1616) and were used for the anti-MCMV assay.

The following strains of HCMV were used: AD169 (ATCC: VR-538) and Davis (ATCC: VR-807), wild-type clinical isolates (CMV5usa), GCV resistant isolates (CMV6usa, Ly9990, U9070) HPMPC/GCV resistant isolate (Der530) (Snoeck et al., 1996). Stocks of murine cytomegalovirus (ATCC: VR-194) were prepared from homogenates of salivary glands (10% w/v) of NMRI mice infected with a sublethal dose of MCMV as described previously (Neyts et al., 1993).

2.2. Compounds

Ganciclovir (GCV, Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany), desferal (DFO, Ciba-Geigy, Basel, Switzerland), ethylenediaminedisuccinic acid (EDDS, AnalytiCon, Potsdam, Germany) and complex of calcium trinatrium DTPA (CaDTPA, Astrapin, Pfaffen-Schwabenheim, Germany) were prepared freshly (on the day of experiment) in distilled water.

Cidofovir (HPMPC, Pharmacia & Upjohn, Erlangen, Germany) was dissolved in distilled water and stored at aliquots at −20 °C. 2,2′-Bipyridine (BPD) obtained from Sigma (Deisenhofen, Germany) was prepared fresh in dimethylsulfoxide.

2.3. Cytotoxicity assay

In vitro toxicity of EDDS was determined for HEL cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction in active mitochondria (Mosmann, 1983). The method was performed as described previously (Cinatl et al., 1996b). In brief, HEL cells were seeded at 4000 cells/100 μl in 96-well microtiter plates and cultured for 12 h at 37 °C to attach to the plates. Different dilutions of the test compounds in minimal essential medium (MEM) or control medium without drug were added. After cells were allowed to proliferate for 4 days the MTT solution was added and absorbance at 570 nm was determined using a multiwell ELISA reader. After subtracting the background absorbance results were expressed as percentage of control cultures receiving no drug. The in vitro toxicity was expressed as CC_{50}, i.e. the cytotoxic concentration required to inhibit cell growth of exponentially growing cells by 50%.

2.4. Plaque reduction assay

HEL cells were grown in 96-well microtiter plates and were inoculated with 100 PFU virus per well. Following a 2-h adsorption period, the virus inoculum was removed and serial dilutions of the test compounds were added. To investigate the inhibitory effects of EDDS on HCMV replication when the compound was added at different points in the replication cycle (of strain AD169), compounds were added either together with virus or 2, 24, 48, 72, 96, 120 h after virus adsorption, respectively. HCMV infected cultures were incubated at 37 °C for 7–8 days. Cells were fixed with 70% ethanol and stained with 2.5% Giemsa solution. The cytopathic effect (CPE) was evaluated microscopically using a scale from 0 to 5, with 0 indicating an absence of CPE and 5 being 100% CPE (comparable to the untreated controls).

The minimal antiviral inhibitory concentration was expressed as EC_{50} i.e. the concentration required to inhibit virus induced CPE or plaque formation by 50%. EC_{50}s are estimated from (semilogarithmic) graphic plots of the percentage of CPE compared to control as a function of the concentration of the test compound.

Inhibitory effects of EDDS on MCMV replication were investigated using confluent cultures of C127 I cells grown in 96-well plates. The cells were infected with 50 PFU/well of MCMV. Following a 2-h adsorption period the virus was removed and serial dilutions of the test compounds were added. Virus induced cytopathic effects were recorded at day 5 or 6 post infection.

2.5. HCMV ELISA

HCMV Elisa was performed as described previously (Cinatl et al., 1996b). Briefly, HEL cells in 96-well plates were infected at MOI 0.01, 0.1 and 1 and selected drug concentrations of EDDS were added after a 1-h adsorption period. Following a 6–7-day incubation, the cells were fixed with methanol-acetone and stained with mouse monoclonal antibodies directed against 67-kDa HCMV late nuclear protein. Horseradish–peroxidase-conjugated goat anti-mouse IgG was used as a secondary antibody. The substrate used was POD blue (Roche, Mannheim, Germany). The absorbance was measured at 450 nm. Drug effects were calculated as a percentage of the reduction in absorbance in the presence of each drug concentration compared to absorbance obtained with virus in the absence of EDDS.

2.6. Effects of EDDS on expression of cell surface molecules

To determine the effects of EDDS on HCMV up-regulated cell surface molecule ICAM-1 expression in HEL cell cultures, numbers of cells expressing ICAM-1 were visualized by immunoperoxidase staining and quantified by flow cytometry. The cells were pre-treated with EDDS concentrations of 10–1000 μg/ml before infection. Then HEL cells were infected or mock-infected with HCMV strain AD169 at MOI of 1. After a

90-min incubation period cells were washed three times with phosphate-buffered saline (PBS) and medium with or without different concentrations of EDDS was added, respectively. To perform immunoperoxidase staining HEL cells were fixed three days after virus inoculation using a 1:1 mixture of acetone and methanol. ICAM-1 positive cells were determined using mouse monoclonal antibody (mAb) (Roche, Mannheim, Germany) directed against ICAM-1. Immunoperoxidase staining was performed according to methods described previously. Moreover, quantitative analysis of the ICAM-1 expression was performed by fluorometric measurements (Cinatl et al., 2000a). In brief, 5×10^5 mock, or HCMV-infected HEL cells were fixed for 10 min in 4% buffered formaldehyde. After washing twice with PBS containing 0.5% Tween-20, cells were incubated for 30 min with mouse monoclonal antibody (mAb) against ICAM-1 (R&D Systems, Wiesbaden-Nordenstadt, Germany). After washing cell pellets twice in PBS the FITC-conjugated goat-anti-mouse IgG (Becton Dickinson, Heidelberg, Germany) was added for 30 min. Fluorescence intensities were measured by flow cytometry (FACScan, Becton Dickinson). Data collected from 1×10^4 cells were analyzed using 'CellQuest' software (Becton Dickinson). All experiments were repeated at least three times.

2.7. Monolayer adhesion assay

Functional studies using a monolayer adhesion assay were performed according to a protocol described previously (Cinatl et al., 2000b). In brief, fibroblast monolayers were pre-treated with EDDS concentrations of 100 μg/ml and infected or mock-infected with HCMV strain AD169 at MOI of 0.25. Mock-infected/treated monolayers served as controls. After a 90-min incubation period cells were washed three times with phosphate-buffered saline (PBS) and medium with or without EDDS (100 μg/ml) was added, respectively. About 5×10^5 Jurkat cells, as a model for activated human T cells, were added to each monolayer for 60 min. Non-adherent Jurkat cells were washed off using warm (37 °C) MEM. Remaining cells were fixed with 1% glutaraldehyde (Merck, Darmstadt, Germany). Adherent Jurkat cells were counted in five different fields (5×0.25 mm^2) using a phase contrast microscope ($20 \times$ objective). The mean cellular adhesion rate was obtained by calculating the mean of five counts.

2.8. Mice

Animal experiments were carried out according to the guidelines for use of vertebrate animals and were approved by the 'Ethical Committee: Animal Studies' of the University of Leuven. Five to six-week-old SCID mice (C.B-17 scid/scid inbred strain) of about 20 g were bred under specific pathogen-free conditions and were housed under germ-free conditions during the experiments. Infection experiments were performed as described previously (Neyts et al., 1992). SCID mice were inoculated intraperitoneally (i.p.) with 10^5 plaque forming units (PFU) of MCMV per 0.2 ml MEM. Test compounds were administered subcutaneously (s.c.) in 0.5 ml volumes twice a day for 10 consecutive days. Treatment was started 2 h after infection.

2.9. Statistics

Statistics were performed using Jandel SigmaStat™ (Version 2.0, Jandel GmbH, Erkrath, Germany). Data groups were considered significantly different when $P < 0.05$. For comparison of two groups we used the Student's t-test in case of equal variances and normality of data. Otherwise, the Rank sum test was used. If more groups were compared a one-way analysis of variance was used, when data passed equal variance test and test of normality. Otherwise, the Kruskal–Wallis analysis of variance on ranks was used. For individual comparison of the different groups all pairwise multiple comparison procedure (Student–Newman–Keuls method) was used.

3. Results

3.1. Antiviral effects of EDDS in vitro

As shown in Table 1 EDDS was in vitro

equally effective (EC_{50} between 7.4 and 12 μg/ml) against wild-type HCMV strains and strains resistant to GCV or to a strain resistant to both GCV and HPMPC. Growth of HEL cells was only slightly affected ($<10\%$) by EDDS at concentrations up to 1250 μg/ml, the CC_{50} values for inhibition of HEL cell growth were 3615 ± 401, 242 ± 39.5 and >10 μg/ml for EDDS, GCV and HPMPC, respectively. In C127I cells EDDS and HPMPC proved active against MCMV (EC_{50}: EDDS: 8.6 ± 1.0 μg/ml; EC_{50} HPMPC: 0.01 ± 0.007 μg/ml).

In order to evaluate the therapeutic index (TI) of EDDS against HCMV we compared its effects with previously investigated metal chelators such as DTPA, DFO and BPD (Cinatl et al., 1996a). Our experiments proved EDDS to have the highest therapeutic index which is similar to that of GCV (Table 2). To obtain further evidence as to when EDDS elicits its antiviral activity in the replication cycle of HCMV the drug was added at different times post infection (Table 3). When added only during the virus adsorption period, EDDS proved, unlike dextran sulfate, inactive. This indicates that EDDS does not act on virus binding. Statistical comparison of the EC_{50} values of EDDS given at different time points after virus inoculation, using one way analysis of variance, showed statistically significant differences ($P = 0.001$) between the application times. Inhibitory effects of the different treatment times were compared by all pairwise multiple comparison procedure (Student–Newman–Keuls method). No statistical significant differences were detected between treatment groups at 2, 24 or 48 h post infection, indicating no significant loss of activity of EDDS after delayed addition up to 48 h. In contrast to this, addition of EDDS 72 h after virus infection proved a significant less potent antiviral effect compared to EDDS administration after 2, 24 or 48 h. Moreover, addition of EDDS 96 h and also 120 h post infection resulted in a further significant decrease of antiviral activity compared to the four earlier time points of addition.

We next investigated whether the anti-HCMV activity depends on the multiplicity of infection and monitored this by assessing HCMV late antigen expression. As shown in Fig. 1, EDDS (50 μg/ml) completely inhibited expression of HCMV-LA in cells infected at MOI 0.01 and 0.1 whereas in cultures infected at MOI 1 virus replication was not completely suppressed even when EDDS was used at concentrations up to 100 μg/ml (data not shown).

Table 1
Inhibitory effect of EDDS on different clinical isolates of HCMV cultured in HEL cells

Virus strain	EC_{50} (μg/ml)		
	EDDS	GCV	HPMPC
($GCV^R + HPMPC^R$)	$11.7 \pm NT$[a]	$11.7 \pm NT$[a]	$0.1 \pm NT$
CMV5usa (WT)	$11.7 \pm NT$[a]	$0.7 \pm NT$	$0.1 \pm NT$
Ly9990 (GCV^R)	7.6 ± 2.5	≥ 26.2	0.4 ± 0.04
U9070 (GCV^R)	7.9 ± 0.9	5.6 ± 0.6	0.3 ± 0.04
Der530 ($GCV^R + HPMPC^R$)	7.4 ± 2.8	9.6 ± 1.4	2.4 ± 1.1
Davis	11.8 ± 1.3	0.7 ± 0.1	0.1 ± 0.02
AD169	9.1 ± 1.7	1.1 ± 0.1	0.2 ± 0.01

[a] Mean ± S.D., $n = 3$; NT = not tested.

Table 2
Effects of different chelators on HCMV replication (strain AD169) in HEL cells

Drug	EC_{50} (μg/ml)	CC_{50} (μg/ml)	TI[b]
DFO	4.4 ± 0.55[a]	6.37 ± 0.99	1.5
BPD	10.31 ± 1.13	10.47 ± 1.19	1
CaDTPA	4.53 ± 0.51	83.56 ± 13.93	18
EDDS	9.1 ± 1.7	3615 ± 401	397
GCV	1.1 ± 0.1	242 ± 39.5	220

[a] Values are mean ± S.D. from three independent experiments.
[b] Therapeutic index.

3.2. In vitro effects of EDDS on adhesion molecule expression

To study the ability of the metal chelator EDDS to modulate HCMV stimulated adhesion

Table 3
Inhibitory effects of EDDS, dextran sulfate and HPMPC on HCMV (strain Davis, MOI = 1) replication when added at different time points post infection

Time of addition	EC_{50} (μg/ml)		
	EDDS	Dextran sulfate (M_W 5000)	HPMPC
Compound present			
Only at the time of viral adsorption	>100	0.1	NT
2 h after virus adsorption	9.97 ± 1.89[a]	>20[b]	0.16 ± 0.05
24 h after virus adsorption	11.8 ± 1.31	>20	0.12 ± 0.02
48 h after virus adsorption	12.4 ± 1.83	>20	0.53 ± 0.08
72 h after virus adsorption	15.6 ± 1.24	>20	0.99 ± 0.29
96 h after virus adsorption	17.3 ± 1.36	>20	1.39 ± 0.16
120 h after virus adsorption	27.4 ± 2.36	>20	1.73 ± 0.32

[a] Mean ± S.D., $n = 4$.
[b] Maximum concentration tested;
NT = not tested.

molecules expression (which is relevant for leukocytes transendothelial migration to sites of inflammation), the effect of EDDS on HCMV up-regulation of ICAM-1 expression in HEL cells was evaluated by immunoperoxidase staining (data not shown) and quantified by flow cytometry. HCMV infected HEL cell cultures exhibited an increased ICAM-1 expression relative to the mock-infected cells at 72 h post infection. In contrast to mock infected cell cultures, EDDS (at 100 μg/ml) reduced the up-regulation of ICAM-1 expression by 65% in HCMV infected cells as determined by flow cytometry (Fig. 2). Neither GCV nor HPMPC, at concentrations that completely inhibited viral replication, markedly reduced HCMV up-regulated ICAM-1 expression. Functional studies using a monolayer adhesion assay proved the biological relevance of the modulating effect of EDDS on HCMV up-regulated adhesion molecule expression. In accordance to ICAM-1 down-regulation the adhesion of Jurkat cells to EDDS treated CMV-infected cells was reduced significantly as compared to untreated controls (Fig. 3) ($P < 0.05$).

3.3. Effects of EDDS in vivo on MCMV-induced mortality in SCID mice

The effect of EDDS on MCMV-induced mortality was studied in SCID mice, an animal model described previously (Neyts et al., 1992). As shown in Table 4 EDDS, at a dose of 1000 mg/kg/day, delayed MCMV-induced mortality in SCID mice by 5 days (MDD: 17.3 ± 4.4; 1000 mg/kg/day) as compared to untreated mice (MDD: 12.6 ± 2.7) when given for 10 consecutive days ($P < 0.05$). Toxicity determinations in mice showed that EDDS doses up to 6000 mg/kg/day (highest dose tested) had only minor toxic side effects on treated mice, whereas anti-MCMV effect was not enhanced. HPMPC at a concentration of 25 mg/kg/day resulted in survival of all animals at day 25 post infection.

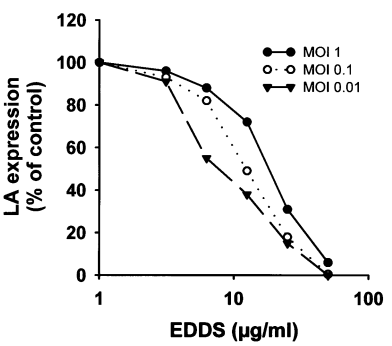

Fig. 1. Effect of different EDDS concentrations on HCMV late antigen expression in HEL (strain AD169) when different MOI are used. HCMV late antigen expression was measured by HCMV ELISA 6–7 days after virus inoculation.

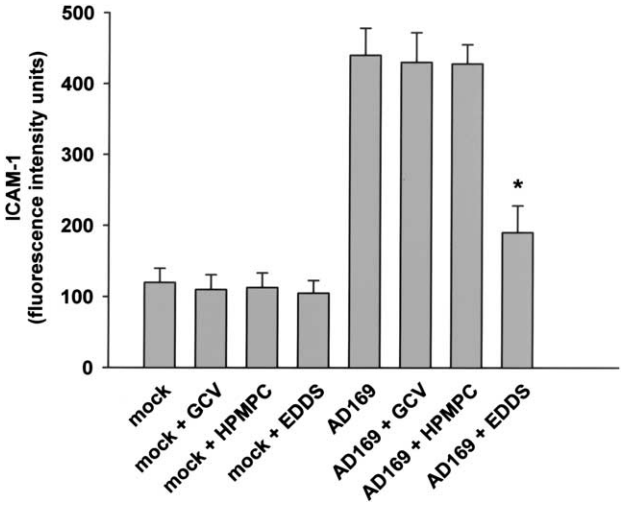

Fig. 2. Effects of EDDS (100 μg/ml), GCV (20 μg/ml) or HPMPC (0.5 μg/ml) on HCMV (strain AD169, MOI = 1) induced ICAM-1 expression as determined by flow cytometry at 72 h after infection. Data are means ± S.D. from a representative experiment performed in triplicates. $*P < 0.05$.

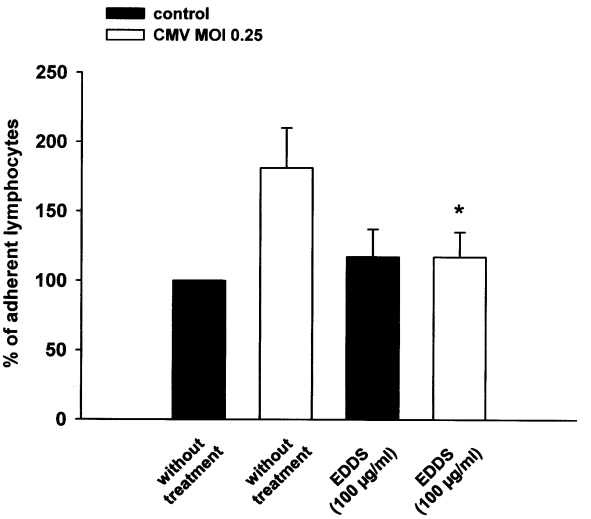

Fig. 3. Number of Jurkat cells binding to mock- or HCMV (strain AD169, MOI = 0.25) infected fibroblasts after incubation with EDDS (100 μg/ml). Measurement was performed at 72 h post infection. Effect is given as percentage of the total number of cocultured Jurkat cells. Data are mean ± S.D. from a representative experiment performed in triplicate. $*P < 0.05$.

4. Discussion

EDDS inhibited at non toxic concentrations the growth of several HCMV strains including those resistant to GCV and HPMPC. Time of addition experiments indicated that EDDS, unlike dextran sulfate (inhibitor of viral entry process), does not act on virus adsorption but inhibits later phases of the virus replication cycle.

In vitro EDDS showed at least a 20-fold higher therapeutic index against HCMV compared to the related chelator DTPA. Moreover, EDDS exhibited significant effects in SCID mice against infections with the murine cytomegalovirus whereas the related compound DTPA was not protective against a generalized infection with rat CMV (Kloover et al., 1999). EDDS proved thus to be superior to other chelators in term of its anti-cytomegalovirus activity.

An infection model in SCID mice was chosen, because this model reflects more the clinical situation where mostly severely immunosuppressed patients (transplant recipients and AIDS-patients), but not immunocompetent persons, may develop severe generalized CMV infections (Vogel et al., 1997; Emery, 2001). In MCMV-infection models in immunocompetent mice, animals may survive a lethal infection following a relatively limited reduction in viral titers as a result of treatment with an antiviral drug. Indeed, because such reduction may allow the immune system sufficient time to overcome the infection. In the MCMV/SCID model, any protective effect observed is the result of a direct antiviral effect (Neyts et al., 1992). A

Table 4
Effect of EDDS on MCMV-induced mortality in SCID mice

Condition	Mean day of death	Survivors[a]
Virus control	12.6 ± 2.7	0/13
EDDS[b]		
1000 mg/kg/day	17.3 ± 4.4*	0/8
500 mg/kg/day	10.8 ± 0.7	0/8
250 mg/kg/day	10.5 ± 1.1	0/8
HPMPC		
25 mg/kg/day	>25	8/8[c]

[a] Number of animals alive at 25 days post infection.
[b] The compound was given subcutaneously for 10 consecutive days. Treatment was initiated 2 h after virus inoculation.
[c] Animals were terminated at 40 days post infection.
* $P < 0.05$.

delay of 5 days in the mean day of death of MCMV-infected SCID mice, as observed here following treatment with 1000 mg/kg/day of EDDS may thus entirely be ascribed to an antiviral effect.

Although the mechanism of antiviral activity was not studied, several scenarios may be proposed for the antiviral action of EDDS. The antiviral activity may stem from affecting viral but also cellular enzymes which require metal ions. It was shown that iron chelators with antiviral activity inhibit cellular and viral ribonucleotide reductases (Spector et al., 1985, 1989, 1991; Cooper et al., 1996). However, this mechanism is not very probable. The structure of EDDS is related to that of the more intensively investigated DTPA. Both are highly hydrophilic substances that are under physiological conditions negatively charged. Therefore, as already demonstrated for DTPA (Aisen and Listowsky, 1980), cellular penetration of EDDS resulting in the inhibition of cellular ribonucleotide reductase is rather unlikely. Another effect of metal chelators relevant for the anti-HCMV activity might be the modulation of signal transduction from the cell membrane, which plays a role in HCMV infection (Fortunato et al., 2000). The influence on the signal transduction pathways by sequestering metal ions could contribute to the modulation of cellular transcription factors important for the control of viral genes. In fact, DTPA was shown to inhibit nuclear factor (NF)-κB dependent up-regulation of HCMV major immediate early promoter (Prösch et al., 1998; Kanekiyo et al., 2000). However, this mechanism is unlikely since EDDS can be added to infected cultures without losing anti-HCMV activity at time points many hours after the expression of immediate early antigens in HCMV-infected cells.

In addition to their direct effects on viral replication, metal chelators like DFO and DTPA were shown to have immunomodulatory/anti-inflammatory properties. Both DFO and DTPA prevented HCMV induced up-regulation of ICAM-1 and C–X–C chemokines in different cell types (Cinatl et al., 1995; Scholz et al., 1996). DFO also suppressed inflammatory responses in a rat liver allograft-model infected with rat cytomegalovirus (Martelius et al., 1999). Importantly, DFO treatment of a HIV-infected patient with cytomegalovirus retinitis refractory to treatment with GCV and PFA resulted in improvement of visual activity without toxic side-effects (Gümbel et al., 1998).

In vitro and in vivo experiments demonstrated that HCMV-infection is paralleled by enhanced ICAM-1 expression on different cell types including endothelial cells, fibroblasts and epithelial cells (Grundy 1998, Cinatl et al., 2000a,b). Moreover, HCMV-induced ICAM-1 was shown to contribute to leukocyte adhesion and transendothelial penetration which may be important for the recruitment of leukocytes in sites of inflammation (Grundy et al., 1998, Sedmak et al., 1994). Indeed, these mechanisms may explain the frequently observed occurrence of HCMV infection in conjunction with solid organ transplant rejection. In the present study we observed effects of EDDS on HCMV-induced ICAM-1 expression and adhesion of Jurkat cells to infected fibroblasts. The human T-cell leukemia line Jurkat was frequently used as a model for studying adhesion properties of activated human T-cells (Farrokh-Siar et al., 1999, van Seventer et al., 1998). Suppression of HCMV-induced up-regulation of ICAM-1 and the ability to inhibit functional binding of lymphocytes to HCMV-infected cells demonstrated that EDDS, similar to other metal chelators, possess anti-inflammatory activity (Cinatl et al., 1995; Kloover et al., 1999).

In conclusion, in vitro EDDS elicited significant anti-HCMV and anti-MCMV activity at non-toxic concentrations. Some protective effect was also observed against a lethal MCMV infection in SCID mice. Moreover, suppression of HCMV induced ICAM-1 expression, but also inhibitory effects on T-cell adhesion to EDDS treated HCMV-infected fibroblasts, underlines that EDDS has the potential to suppress the harmful inflammatory responses to HCMV infection.

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