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Effect of desferrioxamine (DFO) and calcium trinatrium diethylenetriaminepentaacetic acid (DTPA) on rat cytomegalovirus replication in vitro and in vivo

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Abstract

Cytomegalovirus (CMV) infection is a major problem in the immunosuppressed patient. It is thought that besides direct CMV induced cell lysis, immunological damage is part of CMV pathogenesis. New antiviral drugs, which combine immunomodulating and antiviral qualities, could be beneficial. Recently, it has been described that desferrioxamine (DFO) and calcium trinatrium diethylenetriaminepentaacetic acid (DTPA) exhibit both properties. In this report the antiviral effects of both compounds against rat CMV (RCMV) are described in vitro and in vivo using a generalised and local infection model. In vitro, both compounds exhibited a significant antiviral effect, DTPA being more potent than DFO. However, in the generalised infection model no effect was seen on mortality, morbidity or presence of virus in internal organs. In rats infected subcutaneously in the hind paw, no effect was seen locally on paw thickness, presence of viral antigens and inflammatory response. In addition, these rats suffered from a generalised infection of low magnitude at 15 days post infection, although both DFO and DTPA were able to lower the level of viral replication. In conclusion, our data indicate that despite in vitro activity, in vivo usage of DFO or DTPA for acute CMV infection is not warranted.

Keywords: Rat cytomegalovirus; DFO; DTPA; In vitro; In vivo

1. Introduction

Cytomegalovirus (CMV) infection is common in the general population. Clinical symptoms are observed in immunosuppressed patients. Since the onset of the human immunodeficiency virus (HIV)

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epidemic, the number of patients suffering from a disseminated cytomegalovirus (CMV) disease has increased markedly. Currently, this progression has been deminished with the use of highly active antiretroviral therapy (HAART) but nevertheless, it is still a major problem in medical health care. The brain, retina and the gastro-intestinal tract are frequently infected in these patients (Dieterich and Rahmin, 1991; Peters et al., 1991; Arribas et al., 1996). Infection may impair the patient's homeostasis drastically and could eventually evolve into a life-threatening event. Also, in transplant (TX) patients and especially in heart TX recipients CMV infection is a major cause of morbidity and mortality (Grattan et al., 1989). Similar observations were made in liver and kidney TX recipients (O'Grady et al., 1988; Pouteil Noble et al., 1993; Reinke et al., 1994; Lautenschlager et al., 1997) and bone marrow TX (BMTX) patients (Emanuel, 1990).

Attempting to control the CMV induced pathology several treatment regimens have been developed. Nowadays, mostly 9-(1,3-dihydroxy-2-propoxymethyl)guanine (ganciclovir) trisodium phosphonoformatic acid (foscarnet) and (*S*)-1-(3-hydroxy-2-phosphorylmethoxypropyl)cytosine (cidoforin) are the treatment modalities available for combating CMV disease. In BMTX patients, ganciclovir is able to abolish a disseminated CMV disease when the patient is treated prophylactically (Goodrich et al., 1991). All three compounds have been shown to drastically improve CMV retinitis in immunocompromised patients, although relapses are common when treatment with ganciclovir or foscarnet is ceased (Bach et al., 1985; Felsenstein et al., 1985; Singer et al., 1985; Walmsley et al., 1988). Already in 1986 De Clercq et al. (1986) and De Clercq et al. (1987) described the nucleotide analogue (*S*)-1-(3-hydroxy-2-phosphononylmethoxypropyl)-cytosine (HPMPC, cidofovir) which is active against various DNA viruses, including CMV. Cidofovir has been extensively tested in animal models (Stals et al., 1991; Neyts et al., 1992; Stals et al., 1993 and is now available for treating patients (Lea and Bryson, 1996).

Despite the strong antiviral capacities of these compounds, usage is limited due to unwanted side effects. One of the major side effects of ganciclovir is neutropenia especially when ganciclovir is given to patients receiving other drugs such as trimethoprim-sulfamethoxazole (Gordin, 1984) or azidothymidine (AZT) (Richman et al., 1987). Proteinuria and an elevated serum creatinine level indicating nephrotoxity are adverse effects noted following foscarnet and cidofovir treatment. Besides, foscarnet should be administered intravenously route hereby limiting its clinical use (Walmsley et al., 1988).

Another complicating factor is that some strains of CMV are less sensitive or even resistant to ganciclovir (Erice et al., 1989; Drew et al., 1991; Gerna et al., 1992) and to a lesser extent foscarnet (Jacobson et al., 1991; Leport et al., 1993). Sarasini et al. (1995) and Baldanti et al. (1996) described multiple CMV strains resistant to both ganciclovir and foscarnet. In addition, cidofovir-resistant strains have been described in vitro (Lea and Bryson, 1996) and in vivo (Harada et al., 1997).

Recently, progress has been made in the search for new antiviral drugs. Cinatl et al. described two compounds desferrioxamine (DFO) and calcium trinatrium diethylenetriaminepentaacetic acid (DTPA), which were able to inhibit human CMV replication in vitro (Cinatl et al., 1994, 1995b). Both compounds are metal chelators, and DFO is already used clinically in case of an iron overload (Pippard and Callender, 1983). An AIDS patient suffering from a progressive CMV retinitis, did not respond to combination therapy consisting of ganciclovir and foscarnet, but when DFO was added to the treatment regimen, progression was inhibited (Gumbel et al., 1995).

Although antiviral effects of DFO and DTPA on human CMV replication were noted in vitro, very little data are available concerning the antiviral properties of both compounds on in vivo replicating virus. For this purpose we used a rat model and the appropriate virus (rat CMV, RCMV) to study the effects of DFO and DTPA on viral dissemination after systemic or local inoculation of virus.

2. Materials and methods

2.1. Animals and immunosuppression

Specific pathogen-free (SPF) Lewis rats were used in the in vivo experiments. The animals were bred at the Department of Experimental Animal service at the University of Maastricht, the Netherlands. Rats were kept under normal housing conditions and were fed normal rat chow ad libitum. Depending on the experiment, animals were used at an age of 4 (60–90 g), 6 (120–140) or 8 (180–200 g) weeks. In order to enhance the infection, rats received a total body irradiation (TBI) of 5 Gy 8 h before infection as discussed by Stals et al., 1990.

2.2. Virus, infection and treatment

Rats infected intraperitoneally (i.p.) were given 3×10^5 plaque forming units (PFU) of RCMV. The RCMV inoculum consisted of a pool of salivary glands from acutely infected rats as described previously (Bruggeman et al. 1983). Local infection was established by injecting rats subcutaneously (s.c.) in the right hind paw with 1×10^5 PFU (dissolved in 200 μl) of RCMV, which underwent several in vitro passages. The left hind paw was injected with supernatant of uninfected fibroblasts (200 μl) and served as an internal control.

Calcium trinatrium diethylenetriaminepentaacetic acid (DTPA; purity > 98%) purchased from Heyl, Berlin, Germany and desferrioxamine (DFO; Desferrioxamine mesilate; purity approx. 95%) purchased from Ciba-Geigy, Wehr, Germany were tested for their antiviral effect. For in vivo experiments both compounds were dissolved in distilled water and were given i.p. at a dose of 100 mg/kg dissolved in 0.2 ml twice a day. When the dose of the compounds exceeded 100 mg/kg twice a day, rats died due to cytotoxity of the compounds (personal communication from T. Martelius, Fourth Department of Surgery, Helsinki University Central Hospital, Helsinki, Finland). Control (non-infected) rats received sterile posphate-buffered saline (PBS) twice a day.

2.3. Experimental design

For the in vivo experiments two models depending on the mode of inoculation were used.

Intraperitoneal inoculation will lead to a fast dissemination of virus in the body, as described earlier (Stals et al., 1990). A subcutaneous infection in the hind paw, however, concentrates the viral load to the hind paw with slow release of virus to the systemic circulation (Bruggeman et al., 1985; Persoons et al., 1998). In the i.p. infected rats a lethal and a sublethal infection was established by infecting 4 and 8-week-old rats with 3×10^5 PFU of RCMV, respectively.

All rats were weighed and checked for CMV-induced illness daily and survival was recorded. The sublethally infected rats were sacrificed at 4 and 21 days post infection (p.i.). Effect of treatment was determined in the sublethal experiment by measuring viral load by plaque assay and viral DNA by polymerase chain reaction (PCR) in important target organs such as the salivary gland, spleen and liver. In the second model a local infection was established in the rat hind paw using rats of 6-weeks-old (Persoons et al., 1998). The paw thickness of the right and left hind paw was measured at a fixed point on both hind paws on a daily basis. Rats were sacrificed at 4, 8 and 15 days p.i. Effect of DFO or DTPA on the presence of viral antigens in the rat hind paw and on the amount of infectious virus in internal organs was measured. In addition, the effect of treatment on local inflammatory response (paw thickness and presence of inflammatory cells) was determined.

2.4. Plaque assay and PCR

The amount of infectious virus was determined by plaque assay using rat embryonic fibroblasts (REFs), as described before (Bruggeman et al., 1982) and is expressed as the number of PFU/g tissue homogenate.

Presence of viral DNA in the extracted organs was determined by a semiquantitative nested PCR, as described previously (van Dam et al. 1997).

2.5. Immunohistochemistry

Harvested tissues were fixed in a 3.7% formaldehyde solution in PBS for 24 h and embedded in paraffin. RCMV antigens were detected on 4 μm tissue sections with a mixture of two mouse monoclonal antibodies (MoAb) 8 and 35 (Bruning et al., 1987). MoAb W3-13 (Sera-lab, Crowley Down, UK) reacting with rat T-lymphocytes and MoAb ED-1 (gift from Dr C. Dijkstra, Amsterdam, The Netherlands) specific for rat monocytes and macrophages were used to characterize infiltrated cells. Expression of interleukin-2 receptor (IL-2R) reflecting T-cell mediated immune activation was determined using the appropriate antibody (OX39, Sera-lab, Crowley Down, UK). Positive cells were detected by using the immunoperoxidase method, as described earlier (Stals et al., 1990). Slides were evaluated semiquantitatively (− = no positive cells, − / + = sporadic cells, + = few cells, + + = average number of cells, + + + = many cells, + + + + = very many cells) by two independent observers.

2.6. Antiviral properties of DTPA and DFO

For detection of antiviral activity an immunofluoresence assay was used. For this purpose REFs were cultured in 96 well culture dishes and were used at confluency. RCMV was added at a multiplicity of infection (MOI) of 0.01 or 0.1. For optimal infection the cells were centrifuged at 700 × g for 45 min at 20°C. Directly after infection, cells were treated with DTPA or DFO at a concentration of 0, 4, 8, 16, 32, 64, 128 or 256 μM. Cells were fixed at 3, 5 or 7 days post infection, and the presence of RCMV antigens was detected by indirect immunofluorescence using RCMV specific monoclonal antibodies as described above. Cellular DNA was counterstained with 4′-6-diamino-2-phenyl indole (DAPI) (0.5 μg/ml). The number of RCMV positive cells per well was scored by counting five visual fields (200 × magnified), and the ratio of the number of positive cells over the total number of cells (infection index) was determined. Each experiment was performed in quintuplicate.

2.7. Statistical analysis

In all experiments the two sided non parametrical Mann–Whitney U-test was used and $P < 0.05$ was regarded as significantly different.

3. Results

3.1. In vitro

3.1.1. Viral inhibition and cytotoxity

Confluent REFs were infected at a MOI of 0.01 and were fixed at day 5 p.i., respectively. Addition of DTPA and DFO at a concentration of 4 μM to cells infected at a MOI of 0.01 resulted in a significant reduction of the infection index when compared to control (i.e. non-treated) cells (Fig. 1A). When DTPA was used at a concentration of 8 μM a complete reduction in the number of RCMV-infected cells was found. In contrast, there was no reduction in infection index seen DFO at this concentration. Even at high concentrations (16 and 32 μM) of DFO, no effect was seen.

The antiviral effect of DFO and DTPA was dependent on the concentration of input virus, as shown in Fig. 1B. REFs were infected at a MOI of 0.1 and fixed at day 3 p.i. At this MOI, DFO was not able to decrease infection index significantly at a concentration of 4 μM. However, a significant reduction in infection index was observed in DPTA-treated cells from 0.7 in control cells to 0.09 in cells treated with 8 μM DTPA. In this case no complete reduction was seen when DTPA concentrations were elevated.

Cellular cytotoxity was determined by counting the number of (non-infected) cells treated with DFO or DTPA at various concentrations. The concentration at which the compounds gave a 50% reduction in cell number (CC_{50}) was calculated. For DTPA the CC_{50} was 150, 52 and 20 μM after 3, 5 or 7 days of incubation, respectively, while for DFO the CC_{50} was 288, 100 and 84 μM.

As shown in Fig. 1C, the number of cells did not change when concentrations of DFO up to 16 μM were used. For DTPA no effect on the num-

ber of cells was observed when concentrations up to 8 μM were used. At these concentrations DTPA exhibited an antiviral effect, as described above.

3.2. In vivo

3.2.1. Lethal intraperitoneal infection

3.2.1.1. Effect on mortality. To investigate whether DTPA and DFO could avert a lethal CMV challenge, immunosuppressed rats of 4-weeks-old were inoculated i.p. with a lethal dose of 3×10^5 PFU of RCMV, as described before (Stals et al., 1990). At 6 days p.i., rats started to show symptoms of CMV disease, such as subcutaneous and mucosal haemorrhages, development of ascites, hypokinesia and a relative weight loss.

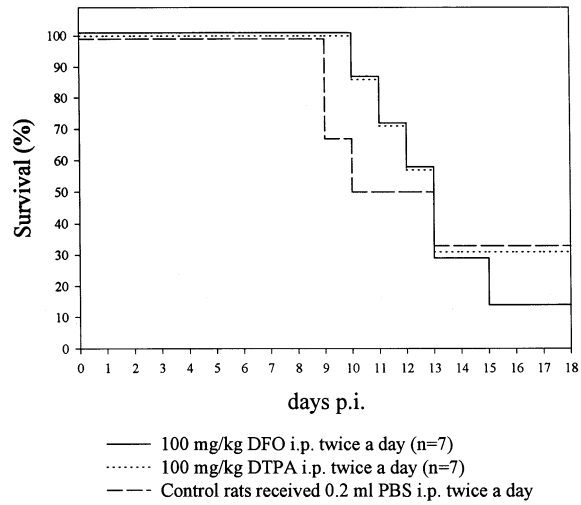

Fig. 2. Survival of 4-week-old immunosuppressed rats infected intraperitoneally with 3×10^5 PFU RCMV.

The increase in body weight per day during the experiment was 1.6, 2.0 and 2.6 g for DFO-treated, DTPA-treated and control rats, respectively. There was no significant difference between the DFO- and DTPA- treated and the control rats in severity of clinical symptoms and in time of onset of these symptoms. Infection led to death in four out of six control rats, in six out of seven DFO-treated and in five out of seven DTPA-treated rats (Fig. 2). Rats surviving the CMV inoculation were followed for a period of 2 weeks and all these animals recovered completely.

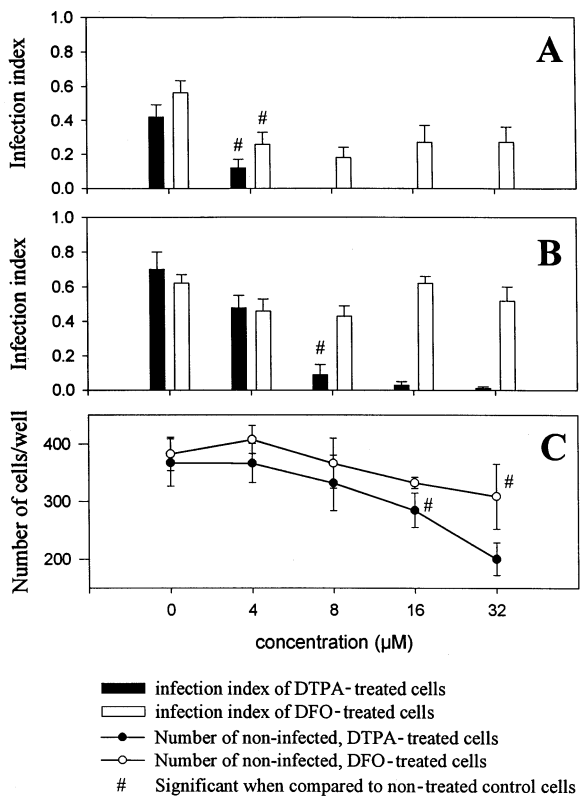

Fig. 1. Antiviral properties of DTPA and DFO in vitro. REFs were infected with RCMV at a MOI of 0.01 and fixed at 5 days p.i. (A) or infected with a MOI of 0.1 and fixed at 3 days p.i. (B) Viral nuclear antigens were stained with MoAb8. The bar chart shows the ratio between infected versus total number of counted cells (infection index). The line chart (C) shows the number of uninfected cells treated with DFO or DTPA.

3.3. Sublethal intraperitoneal infection

3.3.1. Presence of virus in liver, spleen and salivary gland

In order to investigate the antiviral properties of DTPA and DFO in rats suffering from a generalized infection, rats of 8-weeks-old were i.p. infected and the effect of DFO or DTPA treatment on viral replication in internal organs was investigated. As shown in Table 1, salivary gland

Table 1
Presence of infectious virus[a] in organs of intraperitoneally by infected rats[b] with and without drug treatment

Treatment	Salivary gland[c]		Spleen[d]		Liver[d]	
DFO[e]	7.63 ± 1.6[g]	5/5[h]	1.04 ± 1.43	7/7	0.00 ± 0.00	0/7
DTPA[e]	7.98 ± 1.5	5/5	2.50 ± 1.42	7/7	0.00 ± 0.00	0/7
None[f]	8.65 ± 0.6	5/5	0.00 ± 0.00	0/7	0.00 ± 0.00	0/7

[a] Measured by plaque assay.
[b] Rats received 3×10^5 PFU RCMV by intraperitoneal injection.
[c] Harvested at day 21 post infection ($n = 5$/group).
[d] Harvested at day 4 post infection ($n = 7$/group).
[e] Compound given intraperitoneally at a dose of 100 mg/kg twice a day.
[f] Control rats received 0.2 ml PBS intraperitoneally twice a day.
[g] Expressed as log PFU per gram tissue.
[h] Number of rats harbouring infectious virus per total number of rats.

homogenates yielded high amounts of infectious virus in non-treated animals (8.65 ± 0.6 log PFU/ml) at 21 days p.i.. Although treatment with DFO or DTPA showed a slight decrease in viral load (7.63 ± 1.6 and 7.98 ± 1.5, respectively) in this organ, this decrease was not significant. In spleens harvested at day 4 p.i. a low amount of infectious virus could be detected in DFO- or DTPA-treated rats while in the non-treated rats no infectious virus was detectable in this organ. No infectious virus could be detected in spleen samples harvested at day 21 for all groups.

Presence of viral DNA in organ samples was determined semiquantitatively. Spleen and liver samples yielded viral DNA in 10^{-5} and 10^{-1} dilutions at day 4 p.i., respectively. In salivary gland samples harvested at day 21 p.i. viral DNA was found at a 10^{-8} dilution. No differences were seen in amount of viral DNA between non treated, DFO-treated or DTPA-treated rats (data not shown).

Detection of viral antigens by immunohistochemistry revealed RCMV-positive cells in the salivary glands in all experimental groups at 21 days p.i. Treatment with DFO or DTPA did not result in a reduction of the number of RCMV-positive cells.

In conclusion, using the intraperitoneal infection route, no antiviral effect was observed in DFO- and DTPA treated animals when compared to controls.

3.4. Subcutaneous infection in the hind paw

3.4.1. Macroscopy

As is depicted in Fig. 3, in this infection model the course of infection could be divided in four phases. Phase 1 (day 1–5) is the incubation stage with no increase in paw thickness. Phase 2 (day 6–9) is characterized by a drastic increase in thickness and massive inflammation. Although the infected paw was painful, functionality was preserved until 12 days p.i.. The next phase (day

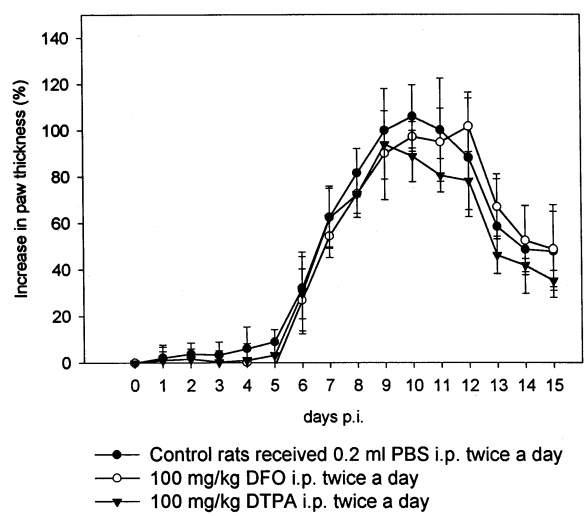

Fig. 3. Paw thickness of rats after subcutaneous injection of 1×10^5 PFU RCMV in the dorsum of the right hind paw. (day 0–4: $n = 15$/group, day 5–8: $n = 10$/group, day 9–15: $n = 5$/group).

Table 2
Presence of infectious virus[a] in organs of locally RCMV infected rats[b] with and without drug treatment

Treatment	Liver			Spleen			Salivary gland		
Days p.i.	4	8	15	4	8	15	4	8	15
DFO[c]	0/5[e]	0/5	1/5[f]	0/5	0/5	0/5[f]	0/5	0/5	5/5
DTPA[c]	0/5	0/5	0/3[f]	0/5	0/5	0/3[f]	0/5	0/5	3/3
Control[d]	0/5	0/5	5/5	0/5	1/5	5/5	0/5	0/5	5/5

[a] Measured by plaque assay.
[b] Rats were infected with 1×10^5 PFU in the subcutis of the hind paw.
[c] Compound given intraperitoneally at a dose of 100 mg/kg twice a day.
[d] Control rats received 0.2 ml PBS twice a day intraperitoneally.
[e] Expressed as number of rats harbouring infectious virus per total number of rats.
[f] Significant ($P < 0.05$) when compared to control rats.

9–12) is the plateau phase in which loss of functionality and oedema of the leg is the most eminent phenomenon. In this period the inflamed hind paw starts to loose its hair indicating tissue destruction. The last phase (day 13–15) is the healing period in which paw thickness and inflammation gradually decreases. In this period also functionality returns. The observed effects are all RCMV induced, since mock infection in the left paw did not show any sign of inflammation or increase in paw thickness. Treatment with DTPA or DFO did not lead to a significant reduction in paw thickness increase (Fig. 3).

3.4.2. Viral dissemination from the hind paw infection locus

Subcutaneous injection of RCMV led to a generalised infection as shown by the presence of infectious virus in spleen, liver and salivary gland at 15 days p.i. (Table 2). At 4 and 8 days p.i. no infectious virus was detected in all organ samples except in the spleen of one animal. At day 15 p.i. all liver, spleen and salivary gland samples of the infected (non-treated) rats harboured RCMV. Treatment with DFO or DTPA reduced the number rats harbouring infectious virus in both the spleen and liver at 15 days p.i..

Plaque assays of the salivary glands extracted at 4 and 8 days p.i. remained negative. However, at day 15 p.i. the salivary glands yielded high amounts of infectious virus, and in contrast to the spleen and liver samples, salivary gland samples did not show virus reduction upon treatment with DFO or DTPA.

In spleens of control and DFO-treated rats viral DNA was found in 10^{-2} dilutions whereas spleen samples of DTPA-treated rats remained negative. In liver and salivary glands no differences in RCMV DNA content were found between DFO-treated, DTPA-treated and control rats. Viral DNA was present in 10^{-2} dilutions for liver samples and in 10^{-9} dilutions for salivary gland samples, respectively. Although infectious virus and viral DNA could be detected in liver and spleen, no RCMV induced antigens were found in these organs by immunohistochemistry. Only the salivary glands at 15 days p.i. yielded positive staining. DFO or DTPA treatment did not result in a decline in the number of cells expressing viral antigens.

3.4.3. Viral antigen detection in the hind paw

After staining with MoAb 8 and 35, the amount of viral antigens was determined. In the hind paw of RCMV-infected rats cells with a cytoplasmic staining were observed in the hypodermis and the reticular layer of the dermis. In control, i.e. non-treated, animals the amount of viral antigens could be correlated to the macroscopic findings in the hind paw as described above. In the phase preceeding increase of paw thickness, the amount of RCMV-positive cells was low ($-/+$). Maximal increase in paw thickness correlated with a large number amount of

infected cells (+ + +) at day 8 p.i. In the last phase (i.e. 15 days p.i.) when paw thickness decreased, also the presence of virus decreased, leading to a low level (+) of RCMV-positive cells. Treatment with DFO or DTPA did not reduce the number of viral antigen-positive cells when compared to control rats.

3.4.4. Inflammatory response

Influx of T-lymphocytes and monocytes/macrophages at the inoculation site gradually increased and the number of both cell types reached a maximum at 15 days p.i.. Also the expression of IL-2R increased over time until day 15 p.i.. No differences were found in the amount of T-lymphocytes, monocytes/macrophages and expression of IL-2R between DFO-treated, DTPA-treated and control rats (Table 3).

4. Discussion

Recently it has been reported that DFO (Cinatl et al., 1994) and DTPA (Cinatl et al., 1995a) are able to reduce CMV replication in human cells. In one case (Gumbel et al., 1995) an antiviral effect was also observed in vivo. In order to further study this effect in vivo, a well defined animal model with the appropriate virus (RCMV) was used. Our in vitro data are in agreement with the previous reports on human cells and indicate that both DFO and DTPA are able to lower viral replication. However, only DTPA reduced the amount of infected cells completely. Bearing in mind previous results (Cinatl et al., 1994, 1995b) one could state that the magnitude of the decrease of infection index by DFO and DTPA is dependent on the viral load.

In vivo, two infection models, i.e. the intraperitoneal and the subcutaneous infection model were used. With the intraperitoneal infection model characterized by a fast spread of the virus into internal organs, the effect of both compounds on dissemination of virus was studied. By using the local, i.e. subcutaneous infection model, it was possible to study not only the spread of the infection in the host but also to follow the local inflammatory response. The latter is important in the case of DFO and DTPA since both compounds would have anti-inflammatory effects (Scholz et al., 1996, 1998).

In the generalized (intraperitoneal) infection model no effect of DFO and DTPA was observed either on morbidity and mortality or on the presence of virus in internal organs. The salivary gland is the preferred organ for this virus and contained high amounts of infectious virus. In this organ, no effect of both compounds was observed in all animals tested. In contrast, in the local infection model an antiviral effect, as shown by a decrease in the number of rats harbouring infectious virus in liver and spleen at day 15, was found. Also no viral DNA in spleens of DTPA-treated rats could be detected. The difference in

Table 3
Table showing the inflammatory response at 4, 8 and 15 days after RCMV[a] injection in the rat hind paw

Treatment	8 and 35[b]			W3-13[c]			ED-1[d]			IL-2R[e]		
(Days p.i.)	4	8	15	4	8	15	4	8	15	4	8	15
DFO[f]	+[h]	+++	++	+	+/++	+++	+	++	++++	−/+	++	+/++
DTPA[f]	+	+++	+	+	+/++	++	+	++	+++	−/+	+++	+/++
None[g]	−/+	+++	+	+	+/++	++	+	++	+++	−/+	+++	+/++

[a] Rats were subcutaneously infected with 1×10^5 PFU.
[b] Cells reactive with MoAb 8 and 35, specific for RCMV early antigens.
[c] Cells reactive with MoAb W3-13, specific for T-lymphocytes.
[d] Cells reactive with MoAb ED-1, specific for monocytes/macrophages.
[e] Cells expressing the interleukin-2 receptor.
[f] Compound given intraperitoneally at a dose of 100 mg/kg twice a day.
[g] Control rats received 0.2 ml PBS twice a day intraperitoneally.
[h] The presence of cells was semiquantitatively analysed and expressed as median (− through ++++).

effect in both models could be due to differences in viral load: in spleen samples of control (i.e. non-treated) rats, viral DNA present in the intraperitoneally infected rats exceeded the amount of viral DNA detected in the subcutaneously infected animals.

The mechanism by which both compounds act is not fully elucidated but it is thought that the inhibition of an iron-dependent ribonucleotide reductase may play an important role in inhibiting RCMV replication, as was shown for other herpes viruses (Spector et al., 1991).

In order to evaluate the effect of DTPA and DFO on the inflammatory response, paw thickness was measured daily and the number of inflammatory cells (T-lymphocytes and monocytes and macrophages) and the IL-2R expression reflecting T-cell mediated immune activation in the hind paw was determined. In control (i.e. non-treated) animals the observed inflammatory response was consistent with the previous report of Persoons et al. (1998). Treatment of DFO or DTPA did not diminish the amount of T-lymphocytes, monocytes/macrophages and expression of IL-2R. Also macroscopically, both compounds were not able to reduce the increase in paw thickness. The latter is in contrast to the report of Blake et al. (1983)) in which a bolus of 30 mg DFO i.p. (instead of 100 mg/kg i.p. twice a day used in our experiments) was able to reduce foot pad swelling after monosodium urate monohydrate cristal or carrageenan injection in the rat foot pad. In addition to the inhibitory actions of iron depletion on viral replication as discussed above, iron itself has been associated with an increase in free radicals resulting in an increase in oxidative stress (Kadiiska et al., 1995). In autoimmune uveitis this increase in oxidative stress will lead to retinal tissue destruction. The magnitude of retinal cell loss can be diminished by DFO treatment (Wu et al., 1993). It is not known why both DFO and DTPA failed to have an effect on the inflammatory response in the hind paw. However, one should bare in mind that immunologically induced damage and direct virally induced cell lysis are not totally separable. It is known that CMV infection not only increases intracellular reactive oxygen intermediates (ROIs), but also benefits from a ROI-mediated enhancement of viral gene expression and thus viral replication (Speir et al., 1996). Both the viral replication and the inflammatory process could result in a vicious circle. This could account for the differences in the observed immunomodulary capacities of DFO and DTPA between our data and those of Wu et al. (1993) and Kadiiska et al. (1995). Recently, Gumbel et al. (1995) reported that addition of DFO to conventional treatment was able to reduce CMV induced retinitis refractory to ganciclovir and foscarnet in an AIDS patient. It is unclear whether the improvement of visual acuity was the result of the immunomodulatory or the CMV-inhibiting capacities of the drug or a combination of both.

In conclusion, our data indicate that despite previous in vitro reports, in vivo usage of DFO or DTPA as monotherapy for acute CMV infection is unwanted. The use of both compounds in combination therapy with either ganciclovir, foscarnet or cidoforin could be the subject of further investigation.

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