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HPMPC therapy of MCMV-induced retinal disease in the SCID mouse measured by electroretinography, a non-invasive technique

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Abstract

The purpose of these studies was to investigate the use of non-invasive electroretinography for the evaluation of retinal disease and its treatment in an ocular murine cytomegalovirus (MCMV) disease model. While under anesthesia, $10^{2.6}$ plaque forming units (pfu) of salivary gland passaged, Smith strain MCMV was injected in the anterior chamber of 6- to 8-week-old severe combined immunodeficiency (SCID) mice. At various times post-inoculation, bright-flash scotopic electroretinogram, viral titer, and histology were obtained from the injected eye. Antiviral therapy was tested using 0.1 and 5 mg/kg/day subcutaneous injections of HPMPC (Cidofovir) once daily for 5 consecutive days. In infected animals, the a- and b-waves of the electroretinographic (ERG) signal were significantly reduced as of 10 days post-inoculation when compared to control animals. Therapy with HPMPC 0.1 mg/kg/day subcutaneously (s.c.) once daily for 5 consecutive days was able to delay the decrease in ERG wave amplitude and inhibit viral replication, whereas 5 mg/kg/day s.c. significantly protected the ERG, completely inhibited viral replication, and maintained ocular viral titer below the limit of detection for up to 17 days post-infection. The reduction of ERG activity during progression of retinal disease correlated well with reduction of disease pathology. ERG recording represents a valuable non-invasive technique to measure the progression of the retinal disease induced by MCMV and the efficacy of antiviral treatment in the ocular MCMV disease model.

Keywords: Cytomegalovirus; Electroretinography; Cidofovir; Retinal disease; SCID mouse

1. Introduction

Cytomegalovirus (CMV)-associated retinitis is a devastating complication that occurs in the immunocompromised patient. Prior to the introduction of effective antiretroviral regimens, up to 40% of patients with acquired immunodeficiency syndrome (AIDS) were at risk to develop progressive retinal destruction leading to partial or complete loss of vision (Drew, 1992).

Since CMV is a host-specific virus, the development of animal models of human CMV disease necessitates the use of surrogate viruses, among them, murine CMV (MCMV). As observed with humans, the immunological status of the host plays a critical role in the development of retinal disease following MCMV inoculation. Through the use of specific antibodies and/or methylprednisolone (corticosteroid), it was demonstrated that $CD8^+$ T cells were responsible for mediating the pathology associated with retinitis in BALB/c mice infected with MCMV (Atherton et al., 1992). Immunosuppression induced by methylprednisolone has been shown to be responsible for increased replication and dissemination of MCMV following supraciliary inoculation in BALB/c mice (Duan et al., 1994), while retrovirally induced murine AIDS (MAIDS) led to a higher incidence of necrotizing retinopathy in C57BL/6 mice following subretinal injection of MCMV (Dix et al., 1994).

Different aspects of the immune control of MCMV retinitis have been investigated. T and NK cells were shown to play a significant role in preventing retinitis (Inoue et al., 1993; Lu et al., 1997; Bigger et al., 1998). Antibody immunotherapy did not prove to be effective therapeutically, failing to reduce the MCMV titers, the frequency or the severity of retinitis in a MAIDS retinitis model (Dix et al., 1997a) whereas with cytokine immunotherapy, IL-2, but not IL-12, was able to prevent retinitis (Dix et al., 1997b).

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Although, the effect of systemic administration of antivirals on CMV diseases has been extensively studied (Neyts et al., 1992; Smee et al., 1992; Bolger et al., 1999), their effect on the progression of CMV retinal disease in mice has not been fully investigated.

Several endpoints for measuring ocular MCMV disease have been used, of which histopathology and viral titration were the most frequently employed (Schawrtz et al., 1974; Holland et al., 1990; Bale et al., 1990; Atherton et al., 1992; Inoue et al., 1993; Duan et al., 1994; Dix et al., 1994, 1997a,b; Lu et al., 1997; Bigger et al., 1998). Non-invasive methods of evaluating the retinal status in murine models have not been frequently used. Mizota et al. (1991) studied the effect of MCMV infection on the electroretinographic (ERG) signal, a functional measurement of the retinal activity, obtained from immunocompetent and immunodeficient animals. Following anterior chamber inoculation with high MCMV titer (10^5 plaque forming units (pfu)/eye), a transient decrease in the maximal b-wave amplitude was obtained in BALB/c mice whereas a rapid and permanent loss was obtained in severe combined immunodeficiency (SCID) mice. Retinal disease was characterized by variable inflammation and necrosis involving either the outer nuclear layer or full thickness of the retina. Using a low titer inoculum of MCMV in the SCID mouse, we have utilized the ERG technique as an endpoint to monitor retinal disease caused by MCMV and its therapy with systemically administered HPMPC (Cidofovir). Viral titration and histology were also employed to monitor the effects of treatment on viral replication and disease.

2. Materials and methods

2.1. Drugs and chemicals

HPMPC was synthesized in-house according to literature methods. All other reagents were of the highest purity possible and obtained from standard commercial sources.

2.2. Animals

Female SCID mice (Charles River, St. Constant, Quebec) 6–8 weeks old were used for these studies. Animals were maintained in a sterile environment in a 12 h light–dark cycle with free access to food and water. Animals were removed from their sterile environment for the duration of the ERG recordings. All experimental procedures were designed to conform to the guidelines of the Canadian Council on Animal Research and our own institutional animal care committee guidelines.

2.3. Virus

The Smith strain MCMV propagated in Nu/Nu mice and isolated from salivary gland homogenates (Duan et al., 1998) was used in all experiments, at an inoculum of $10^{2.6}$ pfu/1.9 μl Dulbecco's Modified Eagle Medium (DMEM). Inoculums varying between $10^{1.6}$ and $10^{3.6}$ pfu were also initially assessed.

2.4. Anterior chamber inoculation, treatment, and viral titration

Mice were anesthetized using Nembutal (65 mg/kg i.p.). The right anterior chamber was perforated with a sterile 30G needle and the aqueous humor was blotted. A 1.9 μl volume of either viral suspension or virus-free control salivary gland homogenate was injected in the anterior chamber via a blunted 30G needle connected to an automated microinjector (Hamilton Company, Reno, NA). Following inoculation, animals were randomly assigned to different treatment groups.

Groups of animals were treated subcutaneously (s.c.) once daily with either vehicle control (phosphate-buffered saline, PBS), HPMPC 0.1 or 5 mg/kg/day for 5 consecutive days, starting 3 h post-infection (p.i.). The total number of mice used in each group was for PBS, $n = 17$, HPMPC 0.1 mg/kg/day, $n = 16$, and HPMPC 5 mg/kg/day, $n = 17$. At various times p.i., the inoculated eyes from some animals in each treatment group were collected, individually added to 1 ml of DMEM, and frozen at −80 °C. After completion of the study, the eyes were thawed and homogenized using a Polytron (Brinkman Instruments, Mississauga, Ont.). Viral titers were determined by plaque assay on primary mouse embryo fibroblast (MEF) cells (Nedrud et al., 1979).

2.5. ERG recordings

Following a 60-min dark adaptation, SCID mice were anesthetized using xylazine/ketamine (50 mg/kg:50 mg/kg, intramuscular) and maintained at 37 °C. The pupil was dilated with 1% phenylephrine in saline.

A platinum subdermal electrode (Grass Instruments, Quincy, MA, USA) was inserted at the base of the nose (reference) and a gold cup electrode was inserted in the mouth (ground). A silver-impregnated nylon fiber (Sauquoit Industry, Scranton, USA) was laid down on the corneal surface to act as the active electrode. The recorded signal was amplified 2000× through a Grass P511 amplifier (Grass Instruments) with a band pass frequency of 0.1–1000 Hz, and digitized for 250 ms at a sampling rate of 15,000 samples/s (AcqKnowledge Version 3.2, BIOPAC Systems Inc.). ERG waves were obtained by averaging the signal obtained by at least five stimuli, separated in their presentation by 20 s. A Ganzfeld flash stimulation was obtained using a light intensity of 9 cd/m^2 (Grass PS-33 photic stimulator) attenuated by a 0.3 log unit neutral density filter.

The a-wave amplitude of the ERG signal (the negative potential reflecting photoreceptor hyperpolarization) was measured from the baseline to the first negative trough whilst the b-wave (the positive potential reflecting bipolar and/or

Müller cell depolarization) was calculated from the a-wave trough to the maximum positivity that followed it, on a signal that had previously gone through low pass filter (low pass FIR, cutoff frequency of 50 Hz, −80 dB) to reduce the contamination of the overlying oscillatory potentials. ERG changes measured in these experiments were evaluated against a separate control group of unmanipulated animals (control ERG, $n = 30$).

2.6. Histology

At various times p.i., the inoculated eyes from some animals in each treatment group were carefully dissected out and placed in 1 ml of 10% neutral formalin, and serial sections prepared and stained with hematoxylin–eosin. Blinded scoring of MCMV-induced retinal pathology was performed with the following scores: 0, normal or injection artifact; 0.5, mild atypical retinopathy with some folding (<75% of the retina); 1, moderate atypical retinopathy, more extensive folding (>75% of the retina), evidence of lymphocyte infiltration, photoreceptor atrophy; 2–4, mild to severe necrotizing retinitis (necrotizing retinitis, increasing involvement of the retina extending from the ciliary body, and characterized by lymphocyte infiltration extensive folding of the retina, retinal detachment, and cytomegaly).

2.7. Statistics

ANOVA followed by Student–Newman–Keuls multiple comparison was used for statistical analysis (Release 6.08, SAS Institute Inc., Cary, NC); $P < 0.05$ was considered to be statistically significant.

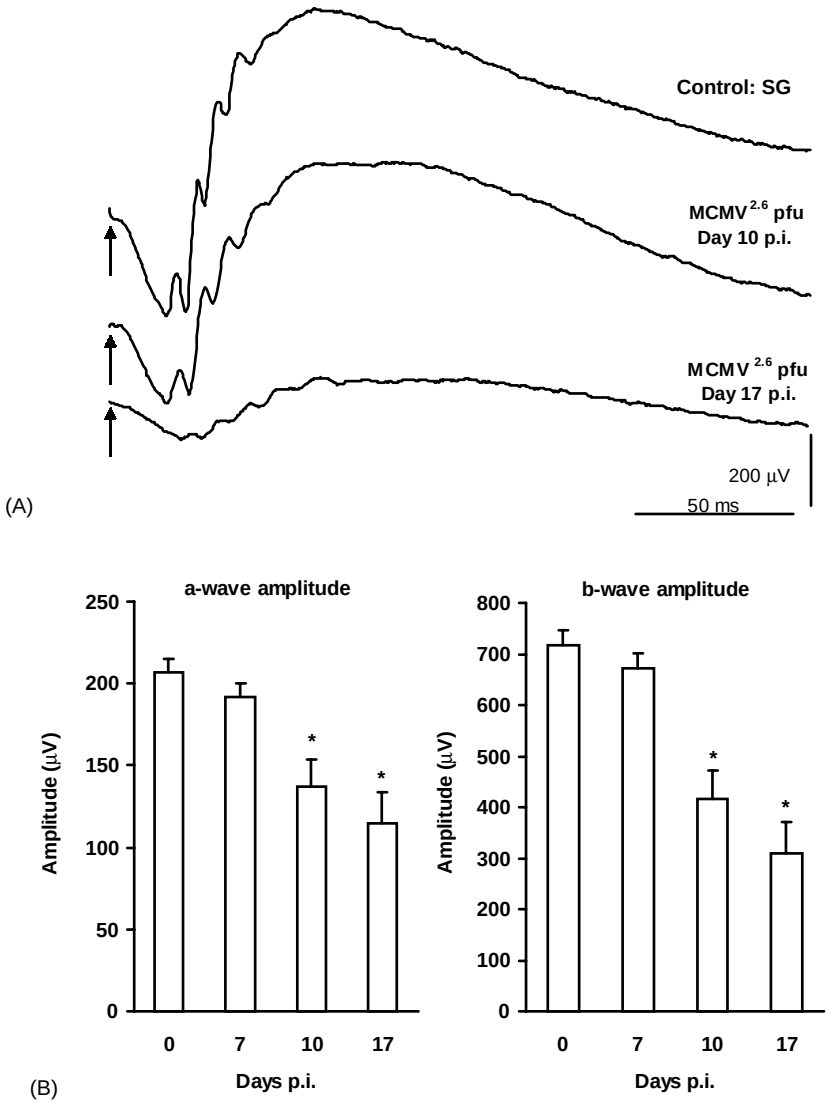

Fig. 1. Changes induced in the amplitude of ERG waves following anterior chamber inoculation with MCMV. (A) Representative ERG recordings obtained from a mouse inoculated with control salivary gland (top trace) and at different days p.i. in a mouse inoculated with $10^{2.6}$ pfu MCMV (middle and bottom traces). ↑= indicates stimulation. (B) Histograms of amplitude loss over time in mice inoculated with $10^{2.6}$ pfu MCMV via the anterior chamber. Each bar is the mean a- or b-wave amplitude in µV ± S.E.M. of either 16 or 17 determinations. *Significantly different ($P < 0.05$) from day 0.

3. Results

When salivary gland homogenate prepared from non-infected mice was injected in the right anterior chamber of SCID mice, no significant changes in the a- or b-wave amplitude were observed throughout the observation period when compared to the pre-inoculation values (data not shown). Furthermore, microscopic examination of the eye did not reveal any inflammation at the injection site.

When salivary gland homogenate containing $10^{2.6}$ pfu of Smith strain MCMV was injected in the right anterior of SCID mice, both the a- and the b-wave amplitudes decreased in a time-dependent manner (Fig. 1A). When compared to pre-inoculation values, the a- and b-wave amplitudes were significantly reduced on days 10 and 17 p.i. (Fig. 1B). The b-/a-wave ratio was significantly lower at days 10 and 17 p.i. (Table 1). Beyond this day the animal's health started deteriorating due to disseminated MCMV infection, as evidenced by ruffled fur, reduced motility, and weight loss. No attempt was made to record ERG past day 18 p.i.

Antiviral therapy using HPMPC at doses of 0.1 and 5 mg/kg s.c. was evaluated. These doses of HPMPC were chosen based on the inoculum of MCMV used in these studies and the potency of HPMPC against MCMV hepatitis (Bolger et al., 1999). Administration of HPMPC 3 h p.i. at a dose of 0.1 mg/kg/day s.c. once daily for 5 consecutive days was able to delay the loss in ERG a- and b-wave amplitudes upon infection with $10^{2.6}$ pfu MCMV. On day 10 p.i., the net decrease in the a- and b-wave amplitudes was significantly less than that observed in the vehicle-treated group (Fig. 2) and the b-/a-wave ratio remained close to the pre-inoculation value (Table 1). On day 17 p.i., HPMPC at a dose of 0.1 mg/kg/day failed to prevent the decrease in wave amplitude with a net loss of $\approx$90 μV for the a-wave and $\approx$ 400 μV for the b-wave, corresponding to a 42 $\pm$ 5% and 56 $\pm$ 5% decrease, respectively. The b-wave was reduced to a greater extent as demonstrated by a decrease in the b-/a-wave ratio. These losses were similar to those observed in the PBS-treated group. At a dose of 5 mg/kg/day, HPMPC protected the function of the retina as measured by ERG. The loss in ERG amplitude remained significantly smaller than that observed in the PBS-treated group or the group treated with HPMPC 0.1 mg/kg/day, whether measured on day 10 or 17 p.i. (Fig. 2 and Table 2). The maximal a- and b-waves net decrease were 26 $\pm$ 11 μV and 123 $\pm$ 37 μV, respectively, corresponding to losses of 13 $\pm$ 5% and 17 $\pm$ 5%. At 17 days, the b-/a-wave ratio was still comparable to that prior to viral inoculation (Table 1).

Viral titers were measured at various times p.i. Virus was never detected in the injected eye of mice receiving virus-free salivary gland homogenate. In the eyes of animals which were injected with $10^{2.6}$ pfu MCMV, the amount of virus that was recovered on day 7 p.i. was $10^{1.9\pm0.2}$ pfu/eye and increased with time reaching a maximal value of $10^{2.8\pm0.2}$ pfu on day 17 p.i. (Fig. 3 and Table 2). Viral titers were measured in the HPMPC-treated groups and compared to those obtained in the PBS-treated group. When infected animals were treated with HPMPC 0.1 mg/kg/day, a significant reduction of the viral titer was observed on days 7 and 10 compared to that found in the PBS group (Fig. 3). The viral titer had declined below detection limit on day 7 before rebounding as of day 10 p.i. On day 17 p.i., viral titer had increased and was not different from that observed in the control group. Treatment with HPMPC 5 mg/kg/day was able to significantly reduce viral titer compared to the PBS-treated group up to day 17 p.i. (Fig. 3 and Table 2).

Histopathological evaluation of the MCMV- and salivary gland-injected eyes revealed the development of progressive retinal abnormalities following MCMV infection only.

Table 1
Effects of HPMPC on ERG b-/a-wave ratio 7, 10, and 17 days p.i. following anterior chamber inoculation with $10^{2.6}$ pfu MCMV

Days p.i.	Treatment[a]		
	PBS	HPMPC 0.1 mg/kg/day s.c.	HPMPC 5 mg/kg/day s.c.
7	3.71 $\pm$ 0.30	3.38 $\pm$ 0.12	3.73 $\pm$ 0.09
10	2.95 $\pm$ 0.20†	3.28 $\pm$ 0.14	3.36 $\pm$ 0.12
17	2.41 $\pm$ 0.21†	2.68 $\pm$ 0.11†	3.30 $\pm$ 0.11

[a] SCID mice were treated subcutaneously once daily for 5 consecutive days with either PBS or HPMPC starting 3 h p.i. The number of animals in each group ranged from $n = 6$–15. The b-/a-wave ratio in non-inoculated untreated mice was 3.44$\pm$0.06 (control, $n = 30$). The results are presented as the mean $\pm$ S.E.M.

† Significantly lower from non-inoculated untreated mice ($P < 0.05$).

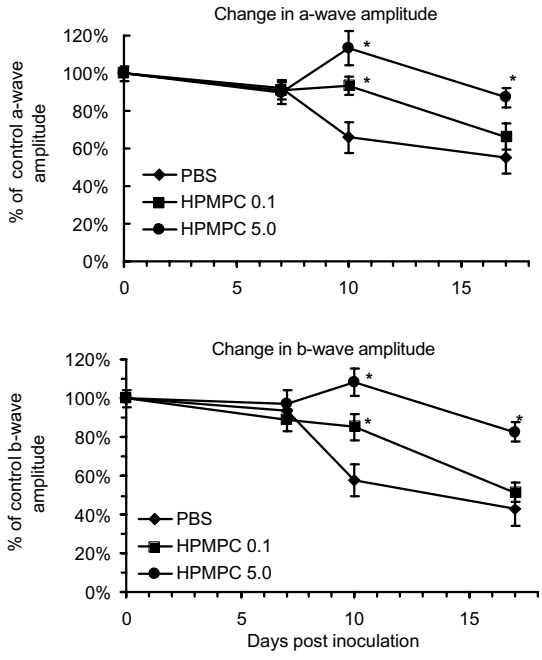

Fig. 2. Effect of PBS (◆), HPMPC 0.1 mg/kg/day (■), and HPMPC 5 mg/kg/day (●) s.c. treatment on the ERG a-wave amplitude (upper panel) and b-wave amplitude (lower panel) following anterior chamber inoculation of $10^{2.6}$ pfu MCMV. Values are expressed as percent of control a- and b-wave amplitudes $\pm$ S.E.M. *Significantly different ($P < 0.05$) from PBS-treated group.

Table 2
Effect of HPMPC on the ERG, viral titers, and histology score on day 17 p.i. following anterior chamber inoculation with $10^{2.6}$ pfu MCMV

Inoculation	Treatment[a]	a-wave[b] amplitude (μV)	b-wave[b] amplitude (μV)	Titer[c] (log pfu/eye)	Histology[d] score
$10^{2.6}$ pfu MCMV	PBS s.c.	115 ± 19† (45 ± 9)†	308 ± 63† (57 ± 12)†	2.8 ± 0.2†	2.0 ± 0.4†
$10^{2.6}$ pfu MCMV	HPMPC 0.1 mg/kg/day s.c.	138 ± 14† (34 ± 6)†	370 ± 39† (48 ± 6)†	2.3 ± 0.07†	1.3 ± 0.4†
$10^{2.6}$ pfu MCMV	HPMPC 5 mg/kg/day s.c.	181 ± 11 (13 ± 5)	594 ± 34 (17 ± 5)	<0.7	0

Values are presented as the mean ± S.E.M.

[a] SCID mice were treated subcutaneously once daily for 5 consecutive days with either PBS or HPMPC starting 3 h p.i.

[b] The a-wave and b-wave amplitude in non-inoculated, untreated controls was 207 ± 8 μ volts and 717 ± 31 μ volts, respectively (control, $n = 30$). Values in parenthesis represent the percent reduction from the non-inoculated animals. The number of MCMV-infected mice used for ERG recording ranged from 10 to 15.

[c] Viral titers were determined on the MCMV-infected right eye with the number of determinations ranging from 9 to 10.

[d] Histology scores were determined by examination of serial sections of the eye, the number of eyes examined ranging from 4 to 6. % loss = % reduction from the non-inoculated treated animals.

† Significantly different from non-inoculated, untreated controls ($P < 0.05$).

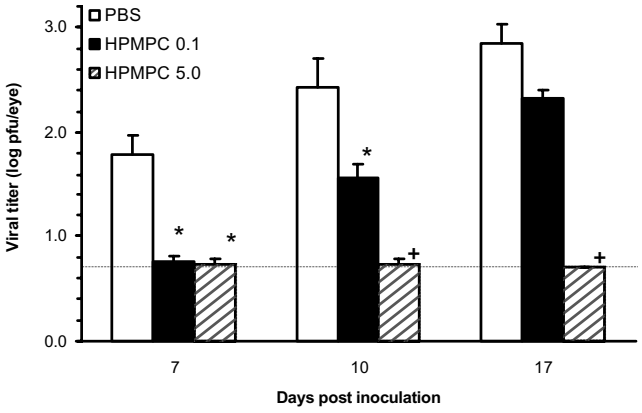

Fig. 3. Effects of HPMPC on viral titers following anterior chamber inoculation with $10^{2.6}$ pfu MCMV. Each bar is the viral titer (log pfu/injected eye) ± S.E.M. of 3–10 determinations. Dotted line represents limit of detection at $10^{0.7}$ pfu/eye. *Significantly different ($P < 0.05$) from PBS-treated group. †Significantly different ($P < 0.05$) from PBS-treated group and HPMPC 0.1 mg/kg/day group.

Although severe disease (full thickness retinitis) was observed only on occasion, milder disease consisting of retinal inflammation and folding, photoreceptor outer segment shortening; inflammation of ciliary processes, and congestion of anterior chamber (Fig. 4) as well as retinal detachment and cytomegaly were observed more frequently. The histology score observed on day 17 p.i. for the PBS group was 2.0 ± 0.4 (Table 2). Treatment with HPMPC for 5 consecutive days at a dose of 0.1 mg/kg/day s.c. reduced the score to 1.3 ± 0.4, while a dose of 5 mg/kg/day s.c. completely prevented the development of retinal pathology associated with MCMV anterior chamber inoculation (Fig. 4). Changes in ERG b-wave amplitude (expressed as a percent of the pre-inoculation b-wave amplitude) were linearly correlated with the extent of ocular disease (Fig. 5).

4. Discussion

We have evaluated the use of ERG in comparison to virology and histology as a non-invasive technique for the assessment of MCMV retinal disease and the efficacy of systemic antiviral therapy in an ocular MCMV disease model. Following anterior chamber inoculation of SCID mice with $10^{2.6}$ pfu MCMV, we were able to monitor the decrease of retinal function through the progressive loss of components of the ERG (a- and b-wave amplitudes). Anterior chamber inoculation was chosen as the route of inoculation over supracilliary inoculation. While supracilliary inoculation is known to produce full thickness necrotizing retinitis in immunodeficient (Atherton et al., 1991) and immunosuppressed (Atherton et al., 1992; Duan et al., 1994) mice, we had concerns that this route of inoculation might mechanically disrupt the retina resulting in inoculation-dependent artifacts which might alter recording of the ERG. Furthermore, the aggressive retinal disease produced by supracilliary inoculation might be a less sensitive model to measure a progressive loss of retinal function and antiviral efficacy. Salivary gland-inoculated eyes neither displayed any sign of inflammation upon microscopic examination nor alteration of the ERG compared to non-inoculated eyes. Mizota et al. (1991) had previously shown that anterior chamber inoculation of MCMV with 10^5 pfu resulted in a rapid and permanent loss of the ERG b-wave amplitude in SCID mice. When comparing the results obtained in both experiments, the ERG loss that these investigators observed was more rapid and pronounced than what was observed in these studies. This can be easily explained by the difference in viral titer used. In these experiments, we used a 250-fold lower inoculum to induce retinal disease. It has been demonstrated that the survival of SCID mice following MCMV infection was inversely proportional to the inoculum (Smee et al., 1992; Neyts et al., 1992, 1993; Bolger et al., 1999). Also, in preliminary experiments, we observed a titer-dependent loss of ERG b-wave amplitude. The ability to measure the ERG in animals not yet displaying signs of disseminated MCMV infection would permit assessment of the impact of MCMV replication on retinal disease without the possible interference of systemic disease. In the late stage of disseminated CMV infection, animals become dehydrated and hypothermic, factors that could influence recording of the ERG. Thus, the use of a low titer would provide for

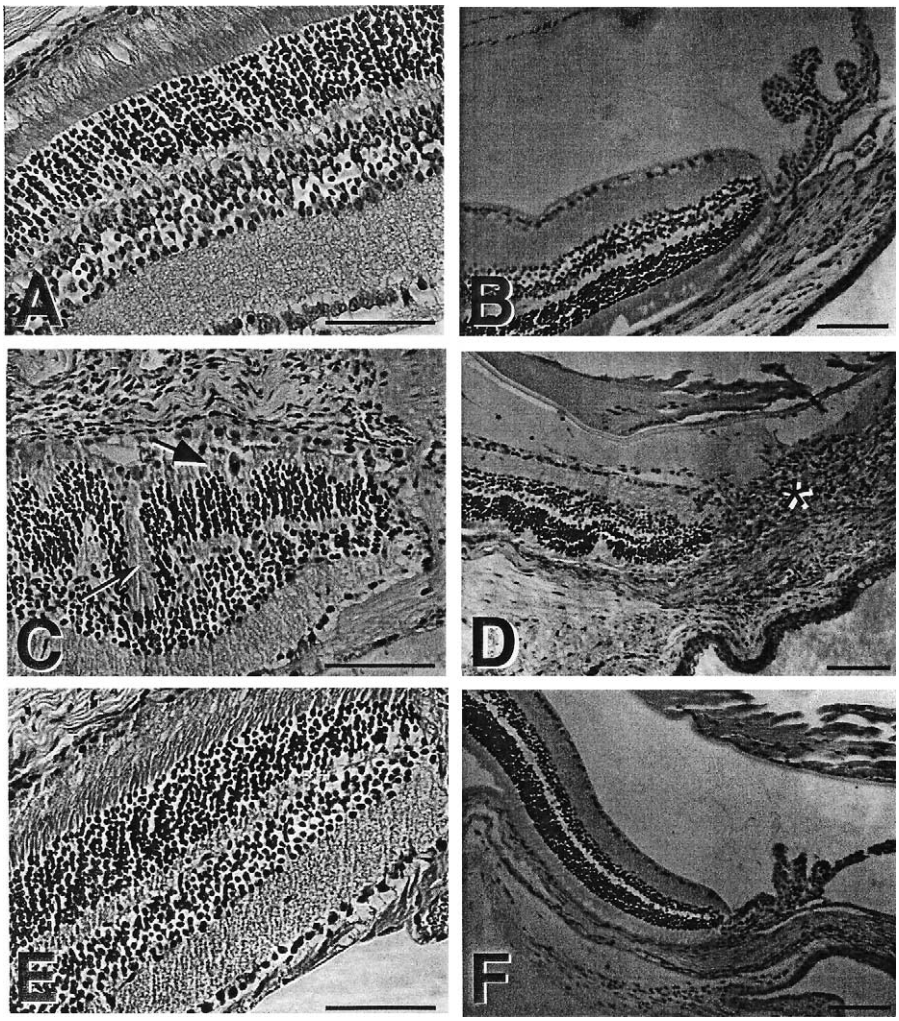

Fig. 4. Photographs of the SCID mouse retina on day 17 post anterior chamber inoculation with: (A) and (B) control salivary gland homogenate (histopathological score of 0); (C) and (D) $10^{2.6}$ pfu MCMV (score of 2.5); (E) and (F) and after HPMPC 5 mg/kg/day treatment for 5 days (score of 0). Following ERG measurement at various time p.i., eyes from animals in treatment group were carefully dissected out and placed in 1 ml of 10% neutral formalin, processed, and stained with hematoxylin–eosin. Retinal sections near the optic nerve head (A, C, E) and the ora serrata (B, D, F). Small arrow: retinal fold; large arrow: photoreceptor outer segment shortening; asterisk: inflammation of ciliary processes and congestion of anterior chamber. Calibration bars: 50 μm (A, C, E); 100 μm (B, D, F).

a sensitive model for measuring both the reduction of the retinal function and antiviral efficacy. Consistent with this premise, it was demonstrated that the ED_{50} of HPMPC for reduction of MCMV-induced hepatitis in BALB/c mice was increased at higher inoculums (Bolger et al., 1999).

The pattern of loss of ERG components we observed is similar to that obtained by Mizota et al. (1991). Both the a-wave (photoreceptor response) and b-wave (inner retina) are reduced in amplitude over time, the a-wave reflecting outer retinal function while the b-wave reflects inner retinal function. These observations suggest that the photoreceptor cells that are driving the ERG are affected by the infection, a common finding in animal models with retinal degenerative disease (Smith and Hamasaki, 1994). Although in this study the inflammatory disease produced by anterior chamber inoculation was mild, the pathological changes mediated by MCMV infection, namely destruction of the outer retina by way of shortening of the photoreceptor outer segment, retinal folding and detachment, are in good agreement with this observation. These histopathological changes are also compatible with the cell-to-cell propagation of the viral infection from the ciliary body to the peripheral and then the posterior retina. However, computation of the b-/a-wave ratio also revealed that the b-wave is substantially more reduced than what could be predicted from the loss of the a-wave. This observation suggests that despite the absence of neuronal loss, the inner retinal cells responsible for generation of the b-wave may be physiologically affected by the progressive retinopathy. A more rational explanation would be that Müller cells that form and extend from the outer to the inner limiting membrane may be affected by an imbalance between potassium sources and sinks in a process that

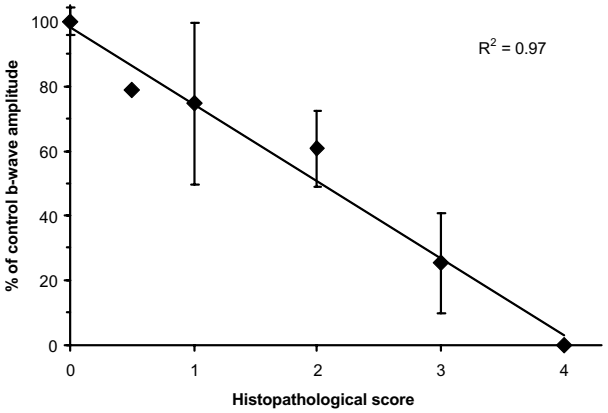

Fig. 5. Correlation between the histology score in the eyes of SCID mice inoculated with $10^{2.6}$ pfu MCMV and the ERG signal (measured as the b-wave amplitude expressed in percent of control). Serial sections of eyes were examined in a blinded fashion and a histology score assigned as described in Section 2. The number of samples at each score were for 0, $n = 22$; 0.5, $n = 1$; 1, $n = 2$; 2, $n = 9$; 3, $n = 3$; 4, $n = 1$. For the score of 1, the average value is given ± range. Other error bars are the S.E.M.

does not affect inner retinal cell survival but affects b-wave generation.

Systemic administration of the antiviral HPMPC 0.1 mg/kg/day s.c. once daily for 5 consecutive days was able to delay the MCMV-induced ERG changes that were identified as of day 10 p.i. in the control-infected group. This finding is consistent with the recovery of virus from the eyes of the HPMPC 0.1 mg treated group. In this group virus recovery was close to the detection limit on day 7 p.i. and progressively increased. On day 10 p.i., the amount of virus that was recovered from the eyes was still significantly reduced and was associated with no decrease of the ERG signal. Following sufficient time without treatment, viral replication was established and was associated with a net decrease in the ERG as observed on day 17 p.i. Consistent with our observation of a reduced titer in the inoculated eyes as a consequence of HPMPC treatment, Neyts et al. (1993) had previously reported that systemic HPMPC treatment was capable of blocking viral replication in the brain of intracerebrally inoculated SCID mice, an indication in their model of its ability to cross the blood–brain barrier and in our case the blood–retinal barrier. HPMPC reduced viral titers in the brain to below the limit of detection on day 8 p.i. and that reduction occurred for a prolonged period of time. They also found that after cessation of treatment, infection would eventually rebound and that mortality would ensue.

When the highest dose (5 mg/kg/day) of HPMPC was used, no significant decrease of the ERG components was observed, virus was never recovered from injected eyes even on day 17 p.i. and histology revealed no significant disease pathology. Thus, continued suppression of viral replication is required to prevent loss of the ERG and prevent disease-induced changes of retinal morphology. Increases of survival time in MCMV-infected SCID mice associated with larger doses of HPMPC have also been reported (Smee et al., 1992; Neyts et al., 1992, 1993; Bolger et al., 1999).

In summary, ERG was evaluated as a technique to monitor the progression of retinal disease following anterior chamber inoculation of MCMV and its response to antivirals in the SCID mouse. The relationship between MCMV-induced changes in the ERG, viral replication, and retinal pathology were studied as well as the therapeutic efficacy of systemic HPMPC to prevent these changes. We were able to demonstrate that: (I) ocular infection with $10^{2.6}$ pfu MCMV lead to a progressive reduction of the ERG a-and b-wave amplitudes which was associated with viral replication and the development of disease pathology and (II) systemic treatment with HPMPC prevented loss of the ERG signal, limited viral replication, and reduced disease pathology. These observations suggest that monitoring the ERG in the SCID mouse represents a valid non-invasive technique to monitor both the progression and effective therapy of MCMV retinal disease.

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