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Short Communication

Experimental herpes simplex virus encephalitis: A combination therapy of acyclovir and glucocorticoids reduces long-term magnetic resonance imaging abnormalities

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Despite early antiviral treatment, herpes simples virus encephalitis (HSVE) still remains a life-threatening sporadic disease with high mortality and morbidity. In patients and in experimental disease, chronic progressive magnetic resonance imaging (MRI) abnormalities have been found even after antiviral therapy. Secondary autoimmune-mediated and not directly virus-mediated mechanisms might play a key role for the outcome of disease. This study aimed to evaluate a possible beneficial effect of a therapy of acyclovir and corticosteroids versus acyclovir only. In a mouse model of HSVE (intranasal inoculation with 10^5 pfu [plaque-forming units] of HSV-1 strain F), a long-term MRI study was realized. Cranial MRI was performed serially at days 2, 7, 14, 21, 60, and 180 in different therapy groups: 1, saline; 2, acyclovir; 3, acyclovir, subsequently methylprednisolone; 4, sham-infected with saline. Brain viral load peaked at day 7 to decline thereafter to a low baseline value. Viral load in group 1 was significantly higher than in animals with antiviral therapy. In group 4, no viral DNA was detectable. Viral load did not differ significantly between acyclovir and acyclovir/corticosteroid-treated groups, suggesting that the use of corticosteroids in addition to acyclovir does not increase viral burden. MRI findings in untreated and acyclovir-treated animals revealed chronic progressive changes. In contrast, there was a significant reduction of the severity of long-term MRI abnormalities in acyclovir/corticosteroid-treated animals. With respect to abnormal MRI findings, this study demonstrates a clear beneficial effect of an acyclovir and corticosteroid therapy without influencing brain viral load. *Journal of NeuroVirology* (2003) **9,** 118–125.

Keywords: acyclovir; corticosteroids; experimental herpes simplex encephalitis; HSV-1 infection; magnetic resonance imaging

Introduction

Herpes simplex virus encephalitis (HSVE) is a severe, sporadic form of focal necrotizing encephalitis (Whitley, 1991, 1997). Without antiviral therapy, mortality rate is as high as 70%; but even after antiviral therapy, still 20% of patients die (Skoldenberg *et al*, 1984). Despite early treatment, chronic progressive tissue damage in magnetic resonance imaging (MRI) may be found up to 6 months following the onset of symptoms, in patients as well as in our experimental model (Meyding-Lamade *et al*, 1999a). These ongoing MRI findings may coincide with high morbidity and severe long-term sequelae despite early antiviral treatment (Lahat *et al*, 1999; McGrath *et al*, 1997; Skoldenberg, 1991).

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In a mouse model simulating human HSVE (Lamade *et al*, 1996), it has been shown that severity and extent of abnormalities had increased at 6 months (Meyding-Lamade *et al*, 1999a). Long-term progressive findings could be explained in two ways. First, these might be due to relapsing courses of HSVE (Kimura *et al*, 1992; VanLandingham *et al*, 1988), with the reappearance of viral genomes in the central nervous system (CNS) (Ito *et al*, 2000; Skoldenberg *et al*, 1984; Tyler *et al*, 1995; Whitley *et al*, 1986). Second, apart from direct virus-mediated tissue damage, indirect immune-mediated mechanisms, which by themselves contribute to an increasing inflammatory process, have to be taken into account (Koprowski *et al*, 1993; Meyding-Lamade *et al*, 1998a, 1999a; Pike *et al*, 1991). Specific immune responses are believed to decisively determine the course of disease (Schiff and Rosenblum, 1998; Steiner and Kennedy, 1995). Therefore, the involvement of secondary immune mechanisms in virus-independent tissue damage of neurons and glial cells is part of ongoing research (Chan *et al*, 1989; Sobel *et al*, 1986). Acyclovir only inhibits viral replication but does not influence these secondary mechanisms of tissue damage. These secondary immune mechanisms might play a key role for chronic progressive structural damage in HSVE despite low viral burden.

Cranial MRI has proven to be a sensitive diagnostic tool for the detection and monitoring of structural abnormalities in human (Demaerel *et al*, 1992; Kapur *et al*, 1994; Sartor, 1991) and experimental HSVE (Lamade *et al*, 1996; Meyding-Lamade *et al*, 1998b; Soto-Hernandez, 2000). In this study, we aimed to evaluate several therapeutic strategies targeted on the suppression of chronic progressive MRI changes. During both the acute and chronic stages of HSVE, we assessed viral burden and cranial MRI findings in animals treated with either saline, acyclovir only, or acyclovir/corticosteroids. Acyclovir was selected for our study as it is the current drug of choice for the management of HSVE (Hayden, 2001), corticosteroids because of their anti-inflammatory potency against secondary mechanisms of structural damage in general and in many autoimmune-mediated diseases and their use in HSVE in the pre-acyclovir era (Baringer *et al*, 1976; Habel and Brown, 1972; Longson and Beswick, 1971; Longson *et al*, 1975; Schimmer and Parker, 2001; Smith, 1975; Upton *et al*, 1971a, 1971b).

This study aimed to examine a possible beneficial effect of a sequence therapy of acyclovir and corticosteroids versus acyclovir monotherapy.

Results

Clinical assessment

In infected animals, the earliest clinical signs of disease were evident 2 days after infection and peaked at day 7 in groups 1 to 3. During the second week, animals were severely affected, whereas all animals scored at 60 and 180 days post inoculation (dpi) were clinically normal again (Figure 1). Mock-inoculated animals had normal findings at all times.

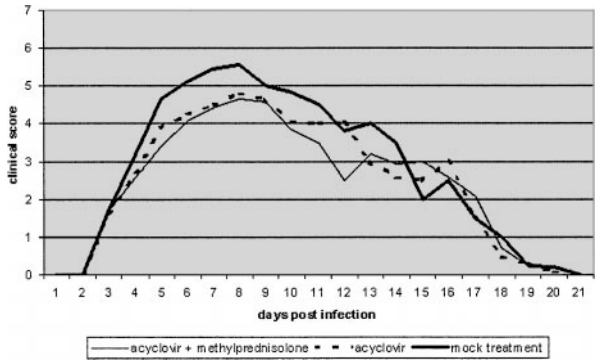

Figure 1 Clinical assessment score ranging between 0 for obviously unaffected to 7 for severely affected animals. Similar time course for all three groups, with a peak at days 7 to 8 and a decline of clinical conspicuousness thereafter. After 20 days, no more abnormalities could be registered. No significant difference between the three therapeutic groups.

MRI findings

All animals infected with HSV-1 strain F revealed MRI abnormalities at days 2, 7, 14, 21, 60, and 180 (Figure 2). Areas of abnormal signal intensity were located in the medial temporal lobe, in the frontobasal and parietal lobes, the cingulate gyrus, the thalamus, and the cerebellum. MRI findings morphologically

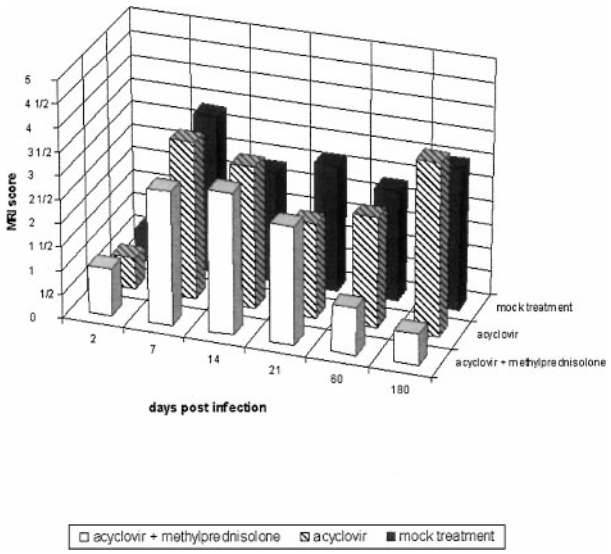

Figure 2 Effect of different treatment protocols on morphological magnetic resonance imaging findings in the course of disease. Administration of acyclovir and methylprednisolone resulted in significantly better long-term outcome as compared to untreated animals ($0.667 \approx 0.577$ versus $3.000 \approx 1.000$; $P < .05$) and to acyclovir-treated animals ($0.667 \approx 0.577$ versus $3.667 \approx 1.528$; $P < .001$).

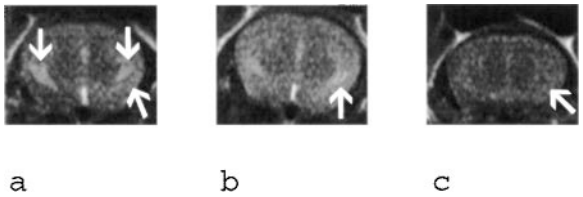

Figure 3 Example of three T2-weighted cranial magnetic resonance images of infected mice. The animal on the left (**a**) received mock treatment, the animal in the middle (**b**) had acyclovir, both animals examined at 7 days post infection (dpi). The cranial MRI of the mock treated animal shows T2-hyperintensities in the left and right periventricular area, the caudate putamen, the lateral amygdaloid, and the left temporal lobe, as indicated by *arrows*. The animal treated with acyclovir reveals just slight T2-hyperintensities in the left periventricular area, whereas after a sequence therapy of acyclovir and methylprednisolone, as shown on the right (**c**), at 21 dpi, only minor abnormalities were found in the left basal temporal lobe.

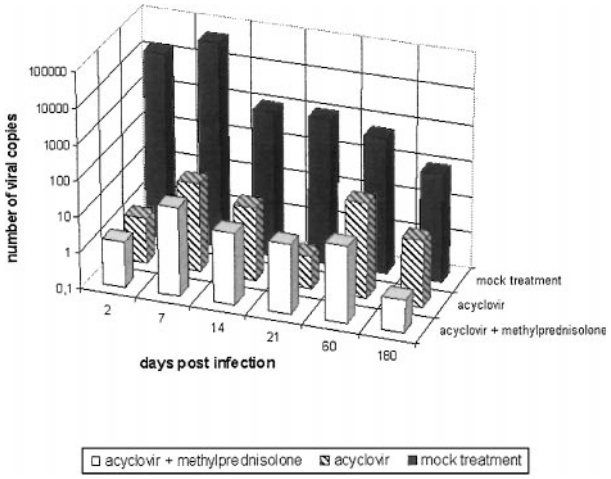

Figure 4 Time course of number of viral copies per microgram DNA extracted from animal brain tissue at days 2, 7, 14, 21, 60, and 180 following infection with HSV-1. Untreated animals had a significantly higher brain viral load compared with the groups treated with acyclovir only or with acyclovir/methylprednisolone 2 days ($P < .05$) and 7 days ($P < .001$) after inoculation. The logarithmic viral load *y*-coordinate aims to underline the non-significant development between the acyclovir-only and the acyclovir/methylprednisolone groups. Thus, the application of corticosteroids does not cause increased viral replication in the central nervous system in this study.

resembled human disease, as described recently (Lamade *et al*, 1996) (Figure 3).

At 2 dpi, only mild changes were detectable in animal brains: scores of $0.667 \approx 0.577$ in group 1, $0.667 \approx 0.577$ in group 2, and $1.000 \approx 1.000$ in group 3. The extent of pathologically affected areas in the acute phase of the disease peaked at day 7 with scores of $3.333 \approx 0.577$, $3.333 \approx 0.577$, $2.833 \approx 0.764$ in groups 1 to 3, respectively. No significance between groups was assessed at this early stage of disease. Thereafter at days 14 and 21, a slight decline of MRI abnormalities (increased T2 signal intensity) was noticed in all groups. The MRI score fell to $2.667 \approx 0.577$ in group 1, to $2.000 \approx 0.000$ in group 2, and to $2.500 \approx 0.500$ in group 3 at 21 dpi. At 60 dpi, the acyclovir/corticosteroid group displayed better results: $1.000 \approx 1.000$ versus $2.333 \approx 0.577$ each in groups 1 and 2. In the long-term course, the severity and extent of MRI findings increased again, except in group 3, revealing chronic progressive changes. After 6 months, MRI findings were most severe in the untreated ($3.000 \approx 1.000$) and acyclovir-treated group ($3.667 \approx 1.528$). Interestingly, the sequence therapy of acyclovir and corticosteroids revealed a clear beneficial effect, with a MRI score of $0.667 \approx 0.577$ at 6 months following infection, as compared to untreated animals ($P < .05$) and to the acyclovir-treated group ($P < .001$). The acyclovir/corticosteroid-treated group itself also showed a significant reduction of MRI score as compared with 14 and 180 dpi ($P < .05$). The mock-infected group 4 showed no abnormalities on cranial MRI. Thus, mice treated with acyclovir and corticosteroids revealed significantly less severe abnormalities on MRI in the chronic phase of disease.

Viral load

PCR showed the presence of HSV-1 DNA in the brain tissue of all infected animals examined. No HSV-1 DNA was detected in the brain tissue of sham-infected controls at any time. The specificity of the positive PCR reactions was verified by direct nucleotide sequence analysis of PCR products.

Two days following infection with HSV-1 strain F, $16530.7 \approx 18726.1$ viral copies were detectable in group 1, mice treated with acyclovir and acyclovir/corticosteroids revealed a significantly lower viral burden with $1.8 \approx 2.1$ ($P < .05$). Seven days after infection, viral burden peaked with $53419.0 \approx 9450.3$ viral copies/μg DNA in brain tissue of animals that received no effective therapy versus $29.7 \approx 23.0$ viral copies/μg DNA in groups 2 and 3 ($P < .001$) (Figure 4).

Decline in viral load took place between 7 and 14 days after infection. After 14 days, $1121.7 \approx 1539.5$ viral copies/μg DNA were detectable in untreated animals, $10.7 \approx 10.2$ in animals treated with acyclovir alone, and $10.1 \approx 9.8$ in the acyclovir/corticosteroid-treated group. The viral load at 21 dpi was $1145.9 \approx 696.6$ in group 1, $0.8 \approx 0.3$ in group 2, and $8.7 \approx 8.7$ in group 3, without intergroup significance. At 60 dpi, we found the following constellation: viral burden decreased to a value of $597.8 \approx 601.7$ in group 1, $42.7 \approx 8.8$ in group 2, and $13.6 \approx 10.5$ in group 3. At 180 days following infection with HSV-1 strain F, viral load decreased to $101.5 \approx 127.4$ viral copies in untreated animals versus low baseline values of $7.0 \approx 6.5$ in the acyclovir-only group and $0.8 \approx 0.2$ in the combination-therapy group. Brain viral load did not differ significantly among the treatment regimens of acyclovir only and acyclovir/corticosteroids. According to our data, the use of corticosteroids and

acyclovir does not cause an increase of HSV-1 strain F replication and brain viral load.

Discussion

The use of corticosteroids in encephalitis

In this study, we report an apparent beneficial effect of a combination therapy of acyclovir and corticosteroids in experimental HSVE with respect to cranial MRI findings. We present data from serial MRI findings and a sequential semiquantitative analysis of viral burden in brain tissue of HSV-1–infected mice subjected to different treatment protocols: untreated animals, mice treated with acyclovir only, and mice treated with acyclovir and corticosteroids were compared with respect to clinical findings, cranial MRI findings, and brain tissue level of HSV copies. Experimental HSVE was studied sequentially over 6 months following inoculation.

In human and animal studies of HSVE, it has been shown that viral load in cerebrospinal fluid or brain tissue does not correlate with the clinical severity of disease (Ando *et al*, 1993; Dennett *et al*, 1996; Meyding-Lamadé *et al*, 1998a, 1999a; Nahmias *et al*, 1982; Wildemann *et al*, 1997). In concordance with these studies, we did not find any correlation between clinical symptoms and number of viral copies in brain tissue or treatment protocols. This may be due to the insensitivity of the scoring system, which is unable to detect subtle neuropsychological deficits (Hudson *et al*, 1991). The SJL mouse model is a well-described experimental model of HSVE that simulates morphologically human disease and causes a characteristic focal, limbic encephalitis without disruption of the blood-brain barrier (Anderson and Field, 1983; Hudson *et al*, 1991; McFarland and Hotchin, 1983; McFarland *et al*, 1986; Meyding-Lamadé *et al*, 1998b). The immune system is well described and fully developped by the age of 10 to 12 weeks. At this age, we infected the animals. We started treatment of animals without delay on the day after inoculation, so long-term outcome was not influenced negatively by late implementation of therapy. Nor did we stop therapy early; total duration of acyclovir treatment was 14 days and thus in concordance with most recommendations (Gilbert *et al*, 2001; Hayden, 2001). In fact, our findings of chronic progressive MRI changes in the presence of low brain viral load support the contribution of secondary mechanisms of cell damage (Haas *et al*, 1999; Meyding-Lamade *et al*, 1999b; Plantinga and Vanneste, 2001) apart from directly virus-mediated tissue injury.

Immune pathomechanisms of cell damage should be taken into account in viral diseases of the CNS. Aurelius *et al* (1993, 1994) found intrathecal immune activity still years after completed HSVE. In brains of young HSVE patients, inflammation is still found up to 3 to 10 years after the acute disease, possibly as a consequence of a secondary neuroinflammatory reaction (Lellouch-Tubiana *et al*, 2000). Postmortem studies showed that histologic changes in HSVE not only occur at the primary sites of lesion but also at more distant sites (Esiri *et al*, 1995; Esiri and Kennedy, 1997). An *in vivo* emission computer-assisted tomography study supports these findings by the evidence of new lesions in the postacute phase. Still more impressive in this study was the *in vivo* demonstration of persisting microglia activation (Cagnin *et al*, 2001). Corticosteroids were the drugs of choice in severe HSVE in the time period before antiherpesvirus agents were available. Even corticosteroid therapy alone was reported to be successful in HSVE, possibly by suppressing these unwanted neuroinflammatory mechanisms (Habel and Brown, 1972; Longson and Beswick, 1971; Longson *et al*, 1975; Smith, 1975; Upton *et al*, 1971a, 1971b).

In our study, viral load peaked at day 7 in all treatment groups. Acyclovir-treated mice showed a significant reduction of viral load versus untreated animals. Interestingly, viral load was comparably low in the acyclovir and in the acyclovir/corticosteroid-treated groups. These results suggest that corticosteroids do not inhibit or impair the antiviral effect of acyclovir. Human and animal studies from the pre-acyclovir era indicated beneficial effects of exclusive treatment with corticosteroids though critical voices were always present (Habel and Brown, 1972; Longson and Beswick, 1971; Longson *et al*, 1975; Smith, 1975; Upton *et al*, 1971a, 1971b). Positive corticosteroid effect can be attributed to its suppressive mechanism concerning cerebral edema and local inflammation (Barthez-Carpentier *et al*, 1995; Dennett *et al*, 1996; Paillard *et al*, 1999; Straub *et al*, 1997; Yamamoto *et al*, 1997). Others found neither positive nor negative effects of corticosteroids concerning the infectious process of HSVE (Johnson, 1996). In a rabbit model, Baringer *et al* (1976) described no increase of viral load after application of corticosteroids when compared with control infected animals. Reluctance to use corticosteroids in viral diseases of the CNS results from their potent immunosuppressive effect (Gold *et al*, 2001; Schimmer and Parker, 2001). One might fear that the addition of corticosteroids leads to impaired antiviral efficacy of the antiherpesvirus treatment with acyclovir or even increased viral replication and dissemination within the CNS. So treatment of HSVE with corticosteroids remains controversial (Pike *et al*, 1991; Plantinga and Vanneste, 2001). After antiviral treatment with acyclovir was introduced in clinical practice, being superior to corticosteroid therapy without acyclovir, corticosteroids have only been used sporadically in single-case reports (Hisanaga *et al*, 1999). In a further animal model of acute HSVE, Thompson *et al* (2000) found that intraperitoneal application of dexamethasone resulted in a decrease of viral antigen expression as measured by immunohistochemistry. This study did not examine long-term abnormalities, nor did they

measure viral DNA copies in brain tissue. They did not have a surrogate marker of stuctural damage of HSVE.

Apparently unlike varicella-zoster virus (VZV), the characteristic focal HSVE occurs mainly in immunocompetent individuals, so HSV-1 reactivation is not a disorder of immune-suppressed individuals except under very specific conditions. In fact, the occurrence of HSVE in patients after irradiation and corticosteroid treatment for metastatic brain disease was reported. It was suggested that irradiation together with corticosteroids might favor the occurence of HSVE (Dragoje *et al*, 1995; Jacobs, 1999; Schiff and Rosenblum, 1998). It is commonly accepted that irradiation may reactivate latent herpesviruses (Molloy *et al*, 2000), but in the patient Dragoje *et al* (1995) reported about, one may not differentiate between a possible effect of irradiation or corticosteroid application.

Our *in vivo* MRI findings revealed chronic progressive MRI structural changes in untreated and acyclovir-treated animals. Mice treated with a combination therapy of acyclovir and methylprednisolone showed a significant reduction of these chronic progressive MRI changes, suggesting a clear beneficial effect of a combined use of acyclovir and corticosteroids upon MRI changes.

In conclusion, our present study strongly supports the hypothesis that combined treatment strategies directed against both viral replication and secondary autoimmune-mediated mechanisms of neuronal damage may beneficially affect the long-term MRI outcome of HSVE. These findings indicate that acyclovir, together with the use of corticosteroids, may succeed in a significant reduction of chronic MRI findings in HSVE. Thus, a combined therapy of acyclovir and corticosteroids might be a promising strategy to follow in this devastating disease in comparison to acyclovir only.

Materials and methods

Animals, virus, and inoculation

Female SJL mice were supplied by Bomholtgard Breeding and Research Center (DK 8680 Ry, Denmark). Mice were 12 weeks old and had a competent immune system (Hudson *et al*, 1991). Their average weight was 23 to 25 g. Animals were maintained under artificial diurnal lighting conditions (12 h each of light and darkness), with free access to standard food and water during the whole experimental period. The study was approved by the Institutional Review Board for the care of animal subjects. The care and handling of animals were in accord with German National Institutes of Health guidelines. At the age of 12 weeks, animals were intranasally inoculated with 10^5 plaque-forming units (pfu) of HSV-1 strain F, a neurovirulent wild-type strain of HSV (Darai *et al*, 1982; Ejercito *et al*, 1968), as described previously (Hudson *et al*, 1991; Lamade *et al*, 1996). In 18 control animals, mock infection with saline was performed (Table 1).

Treatment and clinical assessment

On day 0, animals were inoculated. Brains were obtained from a total of 72 animals at days 2 ($n = 12$), 7 ($n = 12$), 14 ($n = 12$), 21 ($n = 12$), 60 ($n = 12$), and 180 ($n = 12$) post infection, as shown in Table 1. Animals were randomly assigned to four treatment groups. Group 1 ($n = 18$): infected animals, sham-treated with saline; group 2 ($n = 18$): infected animals, acyclovir, 50 mg/kg/day (39); group 3 ($n = 18$): infected animals, acyclovir (see above) for 14 days and subsequently methylprednisolone, 40 mg/kg/day for further 7 days; group 4 ($n = 18$): sham-infected (saline), no treatment (Table 2). Drugs were administered intraperitoneally twice a day. The dosages of 50 mg/kg/day for acyclovir and 40 mg/kg/day for methylprednisolone were chosen to assure an effective antiviral and anti-inflammatory treatment in the CNS after intraperitoneal administration and have previously proven to be safe dosages without clinical side effects in this murine model (Meyding-Lamadé *et al*, 2002; Rau *et al*, 2002).

Clinical abnormalities were assessed daily as to appearance, condition of the eyes, posture, feeding habits and general neurological signs (Hudson *et al*, 1991). During the first 3 weeks after infection with HSV-1, mice were scored twice daily, after that period, once a week.

Cranial MRI and virus titers in brain tissue

Cranial MRI was performed on a 2.4-T MR scanner (Bruker B-C 24/40, Karlsruhe, Germany) at days 2, 7, 14, 21, 60, and 180 after infection. At each time point,

Table 1 Subdivision of animals in four different therapeutic groups

Treatment group	*Number of animals* (n)						*Number in each group*
1. Mock treatment	3	3	3	3	3	3	18
2. Acyclovir	3	3	3	3	3	3	18
3. Acyclovir + methylprednisolone	3	3	3	3	3	3	18
4. Negative-control group	3	3	3	3	3	3	18
Days post inoculation	2	7	14	21	60	180	Total number, $N = 72$

Note. Mice were scanned by magnetic resonance imaging and subsequently killed at various time points post inoculation.

Table 2 Different treatment protocols of groups 1 to 4 in experimental herpes simplex virus encephalitis

Days post infection	*Group 1—infected*	*Group 2—infected*	*Group 3—infected*	*Group 4—uninfected*
1–14	Saline	Acyclovir	Acyclovir	No treatment
15–22	No treatment	No treatment	Methylprednisolone	No treatment

MRI was obtained in three animals in each group. In a self-made bird cage coil with a diameter of 30 mm, we used the following pulse sequence parameters: coronal T1-weighted spin-echo sequence (repetition time [TR] = 470 ms, echo time [TE] = 19 ms) and a T2-weighted multi-echo sequence (TR = 3000 ms, TE = 8, 16, 24, . . . , 96 ms). Sequences were implemented with a field of view of 40 mm ≈ 40 mm. Slice thickness was 2.0 mm with an interslice distance of 2.0 mm and a total of six slices. During MRI scans, mice were anesthesized by intramuscular injection with 0.2 mg/kg atropine, with 65 mg/kg ketamine, and with 15 mg/kg xylazine. Body temperature was maintained at $37.5^{\approx} \approx 0.5^{\approx}$. T2-weighted (TE = 96 ms) and proton-density-weighted sequences were used for grading of MRI findings.

Cranial MRI findings were graded as mildly, moderately, or severely involved, according to location and extent of abnormalities. These abnormalities refer to areas with pathologically changed signal intensity as compared to the signal intensity of normal brain tissue in healthy control animals. Abnormalities were located in the frontobasal lobe, cingulate gyrus, temporal lobe, thalamus, and cerebellum (Lamadé *et al*, 1996). Grade 1 corresponded to an involvement of one location of the areas described; grade 2 to an involvement of two locations or one location on both sides with less than 50% of the described areas; grade 3, to an involvement named in grade 2, but with more than 50% of each location; grade 4, to an involvement of three locations, with less than 50% of each location; and grade 5 with more than 50% of these areas.

Viral burden was measured by nested polymerase chain reaction (PCR) using serial dilutions of target DNA extracted from brain tissue, as described previously (Meyding-Lamade *et al*, 1998a). The assay allows the detection of single HSV-1 copies. The specificity of positive PCR reactions was repeatedly verified by direct nucleotide sequence analysis of PCR products as reported elsewhere (Wildemann *et al*, 1997).

Data were analyzed by using SPSS 10.0 (SPSS, Chicago, IL). Results are expressed as mean ≈ standard deviation. After data were positively tested on normal distribution with Kolmogorov-Smirnov test, one-way analysis of variance (ANOVA) and Tukey's post hoc analysis were used to determine differences between groups. Significance was accepted at $p < .05$.

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