PAPER

Ciclosporin use in multi-drug therapy for meningoencephalomyelitis of unknown aetiology in dogs

OBJECTIVE: To evaluate the efficacy and safety of ciclosporin therapy alone or in combination with corticosteroids and/or ketoconazole in dogs with diagnosis of meningoencephalomyelitis of unknown aetiology.

METHODS: Medical records of 10 dogs diagnosed with meningoencephalomyelitis of unknown aetiology and treated with ciclosporin therapy alone or in combination with corticosteroids and/or ketoconazole were reviewed at the Veterinary Medical Teaching Hospital, University of Wisconsin-Madison. Laboratory abnormalities, side effects, clinical and cerebrospinal fluid responses to treatment and association between blood ciclosporin level and response to treatment were evaluated. Histopathological diagnosis was available in three patients.

RESULTS: No significant abnormalities were detected on serial complete blood count and serum chemistry panel in any of the dogs. Side effects of ciclosporin therapy included excessive shedding, gingival hyperplasia and hypertrichosis. Overall median survival time for all dogs in the study was 930 days (range, 60 to more than 1290 days). In all dogs, serial cerebrospinal fluid analysis showed a marked improvement in the inflammation.

CLINICAL SIGNIFICANCE: Results suggest that ciclosporin either alone or in combination with ketoconazole may be a safe and effective treatment for meningoencephalomyelitis of unknown aetiology in dogs.

P. F. ADAMO, H. RYLANDER AND W. M. ADAMS*

Journal of Small Animal Practice (2007) **48**, 486–496
DOI: 10.1111/j.1748-5827.2006.00303.x

Department of Medical Sciences, and *Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706, USA

INTRODUCTION

Non-suppurative encephalitides of unknown aetiology are relatively common in dogs, and among these granulomatous meningoencephalomyelitis (GME), necrotising meningoencephalitis (NME) and necrotising leucoencephalitis (NLE) are frequently reported (Jull and others 1997, Matsuki and others 2004, Braund 2005, Schatzberg 2005). Ante-mortem diagnosis is clinically challenging as these encephalitides present with similar clinical signs, cerebrospinal fluid (CSF), magnetic resonance imaging (MRI) or computed tomography (CT) abnormalities, and histological confirmation is required for a definitive diagnosis (Schatzberg 2005). In the case of presumptive clinical diagnosis of GME, NME or NLE without ante-mortem histopathological study, the term meningoencephalomyelitis of unknown aetiology (MUE) has been suggested (Schatzberg 2005).

GME is characterised histologically by large perivascular cuffs of mononuclear cells in the parenchyma and meninges of the brain and the spinal cord (Vandevelde and others 1981, Cuddon and Smith-Maxie 1984, Thomas and Eger 1989, Sorjonen 1990, Summers and others 1995a, Braund 2005, Schatzberg and others 2005). GME has been described more commonly in small breed dogs, especially in toy breeds and terriers; however, many breeds can be affected (Cordy 1979, Muñana and Luttgen 1998, Braund 2005). This disease most commonly affects young to middle-aged dogs, and a female predisposition has been observed (Bailey and Higgins 1986, Sorjonen 1990, Summers and others 1995a, Kipar and others 1998, Muñana and Luttgen 1998). Three morphological forms of GME have been described: ocular, focal and disseminated, and typical clinical signs have been described for each disease form (Cuddon and Smith-Maxie 1984, Sorjonen 1990, Braund 1994, Summers and others 1995a).

NME has been described mainly in the pug; however, Maltese terrier, Pomeranian, Yorkshire terrier and shih-tzu may also be affected (Cordy and Holliday 1989, Stalis and others 1995, Summers and others 1995b, Uchida and others 1999, Matsuki and others 2004). This disease is characterised histopathologically by inflammatory changes with lymphocytic, plasmacytic and histiocytic infiltrations and extensive parenchymal necrosis restricted to the cerebral hemispheres (Cordy and Holliday 1989, Stalis and others 1995, Summers and others 1995b). In this disease, the profound

inflammation extending from the leptomeninges through the cerebral cortex into the corona radiata usually leads to a loss of demarcation between cortical and grey matter (Summers and others 1995b, Schatzberg 2005).

NLE has also been described in small breed dogs, mainly in Yorkshire terrier and Chihuahua, and it is characterised predominantly by multi-focal white matter lesions commonly located in the brainstem and perivascular regions (Tipold and others 1993, Summers and others 1995b, Jull and others 1997). In this disease, cortex and meninges are relatively spared, but degree of necrosis in the white matter and brainstem is considerably more severe than that in NME and GME (Tipold and others 1993, Summers and others 1995b, Schatzberg 2005).

Magnetic resonance abnormalities, particularly when associated with CSF analysis, may give an indication of GME, NME or NLE. In GME, lesions are mostly confined to white matter with single or multiple hyperintense lesions on T2-weighted (T2W) images and fluid-attenuated inversion recovery (FLAIR) images and mildly to moderately hypointense on T1-weighted (T1W) images relative to adjacent parenchyma, with variable parenchymal or meningeal enhancement after administration of gadolinium (Lobetti and Oearson 1996, Cherubini and others 2004, Lamb and others 2005). In NME, lesions are mostly in the telencephalon, with involvement of both cortex and subcortical white matter, and in NLE, lesions tend to affect both forebrain and brainstem and are confined to the periventricular white matter (Jull and others 1997, Schatzberg 2005) However, as these MRI abnormalities may also be reported in other infectious and inflammatory encephalitides (Tiches and others 1998, Lamb and others 2005), final diagnosis requires histopathology.

GME, NME and NLE usually have an abrupt onset with a rapidly progressive course, and if untreated, they cause death within days or weeks after the onset of clinical signs (Muñana and Luttgen 1998, Cuddon and others 2002, Coates and others 2005). Aggressive immunosuppression is the current mainstay therapy for these diseases, and the prognosis is usually worse in NME and NLE (Schatzberg 2005).

In GME, an immune-mediated pathogenesis is the most favoured theory at this time (Kipar and others 1998). In both GME and NME, anti-astrocytes antibodies have also been identified, further supporting the contribution of the humoral immune response in these two diseases (Uchida and others 1999, Matsuki and others 2004). Furthermore, it is speculated that GME, NME and NLE are diverse manifestations associated with a single disease (Matsuki and others 2004, Schatzberg 2005).

The term MUE was used in this study to include presumptive diagnoses of GME, NME, NLE, other idiopathic inflammatory central nervous system (CNS) diseases and possible infectious aetiologies that may have been missed by routine serological and PCR tests. The purpose of this retrospective study was to evaluate the efficacy and safety of ciclosporin (CiA) therapy alone or in combination with corticosteroids and/or ketoconazole (KZ) in dogs with MUE and to compare the results with historical literature data. Three of the cases in this study had a definitive diagnosis of GME but still were included in the MUE group as a whole for the purpose of the study. The clinical and treatment responses of three of the 10 dogs reported here have been described elsewhere (Adamo and O'Brien 2004).

MATERIALS AND METHODS

Case selection

Medical records of dogs that were presented to the Veterinary Medical Teaching Hospital of the University of Wisconsin-Madison, between December 2002 and December 2005, with a presumptive diagnosis of MUE treated with CiA therapy alone or in combination with corticosteroids and/or KZ were reviewed.

Inclusion criteria

Ten dogs met the criteria for inclusion in this study. Inclusion criteria included physical and neurological examinations, CT or a complete MRI series of the brain and CSF analysis (cell count, cytological examination and total protein (TP) concentration determination). For the MRI series, T1W, T2W and FLAIR images in transverse, sagittal and dorsal image planes, as well as T1W postcontrast (intravenous injection of 0·1 mmol/kg of gadopentetate dimeglumine [Magnevist; Berlex Laboratories]) were evaluated.

Diagnosis of MUE

Dogs fulfilling the following criteria were included in the study: focal or multi-focal anatomic diagnosis, CSF mononuclear pleocytosis (total nucleated cell count [TNCC] less then 3 cells/μl and a TP content less then 25 mg/dl were considered normal CSF parameters [Chrisman 1992, Bailey and Vernau 1997]), MRI changes characterised by single or multiple T2W and FLAIR hyperintense lesions within the brain and/or spinal cord and focal parenchymal or meningeal contrast enhancement, CT changes characterised by single or multiple areas of brain contrast enhancement or histopathologically confirmed GME or NME/NLE.

In all cases, diagnosis of MUE was made based on signalment, history, clinical signs, CT (one case), MRI (nine cases), CSF analysis obtained from the cerebellomedullary cistern and neurological and ophthalmic examination. *Toxoplasma gondii*, *Cryptococcus neoformans*, *Neospora caninum*, *Ehrlichia canis*, *E. platis* and *E. equi* and *Richettsia richettsii* were ruled out in six cases by serology tests. Canine distemper virus encephalitis was ruled out in six cases by negative CSF neutralising antibody titre and negative urine reverse transcriptase-polymerase chain reaction assay. The diagnosis of GME was made histologically from a CT-guided needle biopsy in one dog and was confirmed in two other dogs at post-mortem examination.

Assessment of CiA response

The response to CiA therapy was evaluated in terms of clinical response and CSF response.

(i) Clinical response was assessed by comparing the neurological status of the patient before and during CiA treatment, and it was graded as unchanged, worse, partial or complete resolution of clinical signs. In dogs with ocular involvement, clinical response was considered partial if the optic neuritis resolved and vision partially returned or returned only in one eye.

(ii) CSF response was assessed comparing CSF results before and during CiA treatment, and it was graded as worse, improved or normal.

Statistical analysis

Overall median survival time was determined for all dogs, from Kaplan-Meier analysis, and the result was compared with previously published results (Mathews and others 1994, Muñana and Luttgen 1998, Coates and others 2005, Schatzberg 2005). Finally, association between blood CiA levels and response to treatment was analysed.

RESULTS

Signalment and clinical signs

Affected breeds were dachshund (three dogs), Chihuahua (three dogs), West Highland white terrier (two dogs), Brittany spaniel (one dog) and terrier mix (one dog). Age at presentation varied from 18 month to 13 years (mean/median age: 7/7·5 years). Nine dogs were female, and among these, three were spayed. Clinical signs on presentation included circling, pacing, central vestibular dysfunction, unilateral or bilateral acute blindness, cervical pain and generalised seizures (Tables 1 and 2).

Treatment

CiA alone or in combination with KZ. Seven dogs (dogs 1, 2 and 6 to 10) were treated with either CiA alone or in combination with KZ. Among these, four dogs (dogs 1, 2, 7 and 9) were treated with CiA alone. Two dogs (dogs 2 and 6) had previously been treated with corticosteroids, and side effects from that treatment was the cause of the change of therapy to CiA. In two dogs (dogs 6 and 8), KZ was added after an initial 30 and 12 months of CiA therapy, respectively; in these dogs, KZ was added to reduce the cost of the therapy. One dog (dog 10) was treated with a combination of CiA and KZ from the onset of treatment.

CiA and corticosteroids. Three dogs (dogs 3, 4 and 5) were treated with both CiA and corticosteroids. In these dogs, CiA was added to the initial corticosteroid treatment because of poor response to the corticosteroids therapy alone. In one of these dogs (dog 5), KZ was added four months later to reduce the cost of the therapy.

Anticonvulsant therapy. Two dogs (dogs 1 and 5) were treated with potassium bromide (KBr) and one dog (dog 8) with phenobarbital combined with KBr for seizure control. Both drugs were given following standard recommended protocols.

Dosage of CiA

The dosage of CiA was adjusted to obtain a set target blood CiA level of 200 to 400 ng/ml. The target range for blood CiA concentration used in this study was selected on the basis of the reported therapeutic range for trough blood CiA when it was used as part of the immunosuppressive protocol for dogs with perianal fistula (100 to 300 ng/ml) (Mathews and Sukhiani 1997) and renal allograft (400 to 600 ng/ml) (Mathews and others 1994). In some dogs, blood was drawn between four and six hours after CiA administration, and in other dogs, trough blood CiA levels were evaluated. The dose of CiA varied from 3 to 15 mg/kg orally (PO), every 12 hours, when used alone or with corticosteroids, and from 5 to 12 mg/kg PO, every 24 hours, when used in combination with 8 mg/kg PO, every 24 hours, of KZ (Tables 1 and 2). Blood CiA levels were tested by high-performance liquid chromatography.

Follow-up

The first re-evaluation was done between two days and two weeks after beginning of the treatment, then approximately every two months during the first seven months, every three to six months thereafter and at any time when worsening of clinical signs was noted. Laboratory evaluation included urinalysis, complete blood count (CBC), serum chemistry panel, whole-blood CiA level and CSF analysis. Serum phenobarbital and/or KBr were also tested in dogs receiving anticonvulsant therapy.

Histopathological diagnosis, blood abnormalities and side effects

Disseminated GME was confirmed histologically in three dogs (dog 2, 4 and 5).

No significant abnormalities were detected on the serial CBC and serum chemistry panels in any of the dogs. Side effects included excessive shedding (one dog), gingival hyperplasia (four dogs) and hypertrichosis (three dogs). Gingival hyperplasia was observed between eight months and one year from the beginning of CiA therapy. Anorexia, vomiting and diarrhoea were observed in two dogs (dogs 5 and 10) treated with CiA and KZ, which resolved with reduction of CiA dose. Chronic urinary tract infection (UTI) developed in two dogs (dogs 4 and 5) treated with concurrent corticosteroids and CiA therapy and persisted in one dog (dog 1) when CiA therapy replaced four months of corticosteroids therapy.

Clinical response and survival time

At the time of writing, five dogs were alive and five dogs had died from progression of MUE. Overall median survival time for all dogs in the study was 930 days (range, 60 to more than 1290 days) (Fig 1). One dog (dog 2) had a complete response before dying from a recurrence of GME 19 months later. Four dogs (dogs 1, 3, 4 and 5) had a partial improvement and died after 11, 14, two and 17 months, respectively. Five dogs had a complete clinical response and are still alive after 12 months (dogs 7 and 10), 16 months (dog 8), 32 months (dog 6) and 35 months (dog 9), respectively.

CSF response

In all but one dog (dog 3), serial CSF analysis during therapy showed a marked improvement compared with the analysis before therapy. TNCC and TP became normal in all five surviving dogs (dogs 6 to 10).

DISCUSSION

CiA has profound immunosuppressive properties in vitro and in vivo and clinically has been used to inhibit rejection of transplanted organs (Daigle 2002). CiA suppresses T-cell-mediated immune responses through inhibition of synthesis of interleukin-2 and other cytokines (Gorman 1995). CiA has poor brain barrier

Table 1. Response to therapy in the succumbed dogs

Dog	Signalment	Clinical signs on presentation	Therapy	CiA dose	Follow-up	Blood CiA level (ng/ml)	CSF before and after CiA treatment	Clinical response	Infections and side effects	Histological diagnosis	Survival time (months)/ cause of death
1	Terrier cross F, eight years	Vestibular ataxia, generalised seizures	On prednisone during previous four months				TNCC: 410/μl (78% lymph, 10% neutr, 12% macr), TP: 67·1‡		UTI		11/euthanasia because severe UTI and recurrence of neurological signs
			CiA (+KBr)	5 mg/kg twice a day	Two weeks		TNCC: 74/μl (80% lymph, 16% macr, 4% neutr), TP: 37	Partial (persistent mild ataxia)			
			CiA (+KBr)	5 mg/kg twice a day	Two months	1311*	TNCC: 8/μl (90% lymph, 10% macr), TP: 29·6	Partial (persistent mild ataxia)			
			CiA (+KBr)	3 mg/kg twice a day	Three months	518*	TNCC: 27/μl (84% lymph, 14% macr, 2% neutr), TP: 41·53	Partial (persistent mild ataxia)			
			CiA (+KBr)	5 mg/kg twice a day	Five months	1221*	TNCC: 1/μl (82% lymph, 18% macr), TP: 27·9	Partial (persistent mild ataxia)			
2	Dachshund FS, seven years	Central vestibular signs					TNCC: 8/μl (69% lymph, 14% neutr, 17% macr), TP: 29·6‡			GME	19/succumbed because of cluster seizures
			CiA	3 mg/kg twice a day	One week	117*	TNCC: 4/μl (95% lymph, 5% mono), TP: 13·7	Complete			
			CiA	6 mg/kg twice a day	One month	370*		Complete			
			CiA	3 mg/kg twice a day	Seven months	215*	TNCC: 7/μl (91% lymph, 9% mono), TP: 16·1	Complete			
			CiA	3 mg/kg once a day	Nine months	247*		Complete			
			CiA	3 mg/kg EOD	12 months	88*	TNCC: 8/μl (92% lymph, 8% macr), TP: 13·8	Complete			
			CiA	3 mg/kg EOD	16 months	78*	TNCC: 14/μl (97% lymph, 3% macr), TP: 15·6	Complete			
			CiA	3 mg/kg EOD	19 months	87*		—			
3	Chihuahua, longhair F, 18 months (on steroids EOD during previous two months)	Vestibular taxia, circling, pacing					TNCC: 2/μl (38% lymph, 62% macr), TP: 9·9‡				14/euthanasia because of severity of clinical signs
			CiA+C	5 mg/kg twice a day	Three weeks	40*	TNCC: 2/μl (13% lymph, 87% macr), TP: 8·3	Partial (mild ataxia)			
			CiA+C	15 mg/kg twice a day	Two months	560*		Partial (mild ataxia)			
			CiA+C	10 mg/kg twice a day	Four months	296*	TNCC: 2/μl (36% lymph, 64% macr), TP: 11·3	Partial (mild ataxia)			
			CiA+C	15 mg/kg twice a day	Six months	424*		Partial (mild ataxia)			
			CiA+C	15 mg/kg twice a day	Nine months	218*		Partial (mild ataxia)			

(continued)

Table 1. (continued)

Dog	Signalment	Clinical signs on presentation	Therapy	CiA dose	Follow-up	Blood CiA level (ng/ml)	CSF before and after CiA treatment	Clinical response	Infections and side effects	Histological diagnosis	Survival time (months)/ cause of death
4	West Highland white terrier FS, six years	Central vestibular signs					TNCC: 225/μl (88% lymph, 12% macr), TP: 157·3‡		UTI	GME	Two/euthanasia because of severe UTI, kidney failure and lack of substantial neurological improvement
			CiA+C	3 mg/kg twice a day	One week	82*	TNCC: 22/μl (86% lymph, 14% mono), TP: 36·4	Partial (mild ataxia)			
5	Dachshund FS, 13 years	Non-ambulatory tetraparetic, monolateral central blindness history of idiopathic epilepsy					TNCC: 48/μl (60% lymph, 9% neutr, 31% macr), TP: 41·3‡		UTI	GME	17/euthanasia because of severity of clinical signs
			CiA+C (+Br)	6 mg/kg twice a day	One week, two months	26*, 176*		Partial (ambulatory tetraparetic, monolateral blindness)			
			CiA+C (+Br)	12 mg/kg twice a day	Four months	272*		Partial (ambulatory tetraparetic, monolateral blindness)			
			CiA+C+KZ (+Br)	6 mg/kg once a day+ KZ 8 mg/kg once a day	Five months	173†	TNCC: 5/μl (79% lymph, 21% mono), TP: 32·6	Partial (ambulatory tetraparetic, monolateral blindness)			
			CiA+C+KZ (+Br)	12 mg/kg once a day+ KZ 8 mg/kg once a day	10 months	460†		Partial (ambulatory tetraparetic, monolateral blindness)			

CiA Ciclosporin, CSF Cerebrospinal fluid, TNCC Total nucleated cell count, lymph Lymphocytes, neutr Neutrophils, macr Macrophages, TP Total protein, UTI Urinary tract infection, KBr Potassium bromide, mono Mononuclear cells, C Corticosteroids, KZ Ketoconazole, F Female, FS Female spayed, EOD Every other day

*Four to six hours after dose

†Twenty-four hours trough

‡CSF before CiA treatment

Table 2. Response to therapy in the dogs still alive

Dog	Signalment	Clinical signs on presentation	Therapy	CiA dose/KZ dose	Follow-up	Blood CiA level (ng/ml)	CSF before and after CiA treatment	Clinical response	Side effects	Survival time (months)
6	Brittany spaniel F, six years	Unilateral blindness (optic neuritis), cervical pain					TNCC: 456/μl (10% lymph, 73% neutr, 17% macr), TP: 110·3§μ		Gingival hyperplasia	35
			CiA	5 mg/kg twice a day	2 months	1329*	TNCC: 6/μl (82% lymph, 13% neutr, 5% macr), TP: 13·2	Complete		
			CiA	2·5 mg/kg twice a day	4 months	300*		Complete		
			CiA	2·5 mg/kg twice a day	8 months	148*	TNCC: 42/μl (99% lymph, 1% macr), TP: 20·8	Complete		
			CiA	5 mg/kg twice a day	14 months	555*	TNCC: 7/μl (93% lymph, 2% neutr, 5% macr), TP: 12·8	Complete		
			CiA	5 mg/kg twice a day	20 months	389*; 277†	TNCC: 5/μl (88% lymph, 7% neutr, 5% macr), TP: 20·3	Complete		
			CiA	5 mg/kg twice a day	29 months	230†	TNCC: 167/μl (92% lymph, 3% neutr, 5% macr), TP: 72·1	Complete		
			CiA+KZ	5 mg/kg or 8 mg/kg once a day	30 months	1368‡				
			CiA	2·5 mg/kg or 8 mg/kg once a day	30·5 months	461‡	TNCC: 48/μl (90% lymph, 4% neutr, 6% macr), TP: 42·9	Complete		
			CiA	3·7 mg/kg or 8 mg/kg once a day	32 months	923‡	TNCC: 0/μl (90% lymph, 10% macr), TP: 20·3	Complete		
7	West Highland white terrier FS, eight years	Two months of bilateral blindness with absent PLR both eyes (bilateral optic neuritis)					TNCC: 42·8/μl (53% lymph, 22% neutr, 23% macr), TP: 42·8§		Gingival hyperplasia	15
			CiA	5 mg/kg twice a day	1 month	94†	TNCC: 2/μl (77% lymph, 33% macr), TP: 25·7	Partial (resolution of bilateral optic neuritis), vision improved in one eye		
			CiA	7·5 mg/kg twice a day	4 month	363†	TNCC: 0/μl (60% mono, 40% lymph), TP: 21·9	Partial (resolution of bilateral optic neuritis), vision improved in one eye		
			CiA	6 mg/kg twice a day	8 months	207†	TNCC: 0/μl (53% macr, 47% lymph), TP: 20·3	Partial (resolution of bilateral optic neuritis), vision improved in one eye		
			CiA	6 mg/kg twice a day	12 month	180†	TNCC: 0/μl (83% macr, 17 lymph), TP: 20·5	Partial (resolution of bilateral optic neuritis), vision improved in one eye		

(continued)

Table 2. (continued)

Dog	Signalment	Clinical signs on presentation	Therapy	CiA dose/KZ dose	Follow-up	Blood CiA level (ng/ml)	CSF before and after CiA treatment	Clinical response	Side effects	Survival time (months)
8	Chihuahua FS, four years	Generalised cluster seizures					TNCC: 47/μl (78% lymph, 1% neutr, 20% macr), TP: 43·4§		Gingival hyperplasia, hypertrichosis	19
			CiA (+Pb)	6 mg/kg twice a day	2 days	162*	TNCC: 17/μl (84% lymph, 1% neutr, 15% macr), TP: 27·7	Complete		
			CiA (+Pb)	6 mg/kg twice a day	2 weeks	158*	TNCC: 6/μl (98% lymph, 2% macr), TP: 17·9	Complete		
			CiA (+Pb)	6 mg/kg twice a day	1 month	161*	TNCC: 10/μl (84% lymph, 22% neutr, 7% macr, 9% mono), TP: 19·5	Complete		
			CiA (+Pb)	10 mg/kg twice a day	2 months	205*	TNCC: 3/μl (65% lymph, 1% neutr, 34% macr), TP: 18·2	Complete		
			CiA (+Pb)	10 mg/kg twice a day	5 months	100†	TNCC: 6/μl (74% lymph, 1% neutr, 2% eosin, 23% macr), TP: 19·1	Complete		
			CiA (+Pb)	12 mg/kg twice a day	6 months	176†		Complete		
			CiA (+Pb)	12 mg/kg twice a day	7 months	93†	TNCC: 3/μl (69% lymph, 31% macr), TP: 18·2	Complete		
			CiA+KZ (Pb+KBr)	12 mg/kg or 8 mg/kg once a day	12 months	85‡	TNCC: 0/μl (91% lymph, 9% macr), TP: 17·3	Complete		
			CiA+KZ (Pb+KBr)	12 mg/kg or 8 mg/kg once a day	16 months	157‡		Complete		
9	Chihuahua F, eight years	Circling, decreased vision, unilateral blindness with contralateral CP deficits	CiA				TNCC: 360/μl (74% lymph, 7% neutr, 19% macr), TP: 99·1§		Gingival hyperplasia, hypertrichosis, shedding	35
			CiA	3 mg/kg twice a day	One week	216*	TNCC: 42/μl (74% lymph, 2% neutr, 24% macr), TP: 61·7	Partial, decreased circling, improved vision		
			CiA	6 mg/kg twice a day	Two months	235*	TNCC: 1/μl (78% lymph, 4% neutr, 18% macr), TP: 21	Partial, persistent monolateral blindness		
			CiA	6 mg/kg twice a day	Seven months	337*	TNCC: 10/μl (90% lymph, 10% mono), TP: 22·8	Partial, persistent monolateral blindness		
			CiA	6 mg/kg twice a day	10 months	592*	TNCC: 14/μl (80% lymph, 14% mono, 6% neutr), TP: 24	Partial, persistent monolateral blindness		
			CiA	6 mg/kg twice a day	12 months	404*	TNCC: 9/μl (91% lymph, 9% macr), TP: 18·5	Partial, persistent monolateral blindness		
			CiA	3 mg/kg once a day	21 months			Partial, persistent monolateral blindness		
			CiA	3 mg/kg EOD	26 months	27‡	TNCC: 2/μl (63% lymph, 13% neutr, 24% mono), TP: 17·1	Partial, persistent monolateral blindness		
			CiA	Treatment discontinued	27 months			Partial, persistent monolateral blindness (based on owner phone conversation)		

(continued)

Table 2. (continued)

Dog	Signalment	Clinical signs on presentation	Therapy	CiA dose/KZ dose	Follow-up	Blood CiA level (ng/ml)	CSF before and after CiA treatment	Clinical response	Side effects	Survival time (months)
10	Dachshund FS, nine years	Bilateral blindness with absent PLR both eyes (bilateral optic neuritis)	CiA+KZ	5 mg/kg or KZ 8 mg/kg once a day	Two weeks	311‡	TNCC: 38/μl (67% lymph, 33% macr), TP: 28·7§ TNCC: 44/μl (62% lymph, 23% neutr, 15% macr), TP: 22·2	Partial resolution of bilateral optic neuritis, partial return of vision with persistent PLR abnormalities in both eyes	Hypertrichosis	15
			CiA+KZ	5 mg/kg or KZ 8 mg/kg once a day	Three months	421‡	TNCC: 1/μl (90% lymph, 10% mono), TP: 15·8	Partial resolution of bilateral optic neuritis, partial return of vision with persistent PLR abnormalities in both eyes		
				5 mg/kg or KZ 8 mg/kg once a day	Six months	449‡	TNCC: 3/μl (87% lymph, 8% neutr, 5% mono), TP 15·8 mg/dl	Partial resolution of bilateral optic neuritis, partial return of vision with persistent PLR abnormalities in both eyes		
				5 mg/kg or KZ 8 mg/kg once a day	12 months	491‡	TNCC: 2/μl (lymphocytes, not enough cells after centrifugation for differential count) TP 13·8 mg/dl	Partial resolution of bilateral optic neuritis, partial return of vision with persistent PLR abnormalities in both eyes		

CiA Ciclosporin, KZ Ketoconazole, CSF Cerebrospinal fluid, TNCC Total nucleated cell count, lymph Lymphocytes, neutr Neutrophils, macr Macrophages, Eosin Eosinophils, TP Total protein, mono Mononuclear cells, Pb Phenobarbital, KBr Potassium bromide, F Female, FS Female spayed, PLR Pupillary light reflux
*Four to six hours after dose
†Twelve hours trough
‡Twenty-four hours trough
§CSF before CiA treatment

permeability and tends to be concentrated mainly in the intracellular compartment (Begley and others 1990, Steffan and others 2004). One study suggested that CiA may be effectively trapped in the cerebral endothelial cells and the choroid plexus (Begley and others 1990, Okonkwo and others 2003). Because in MUE the blood-brain barrier (BBB) during inflammation is disrupted, therapeutic CiA concentration may be most probably present in affected areas of the CNS; in addition, as T-cell response is initiated in the peripheral lymphoid organs in autoimmune diseases (Janeway and others 2005), there may not be a need for CiA to cross the BBB to suppress the pathological immune response to the CNS.

Signalment and clinical signs of affected dogs in the present study were similar to that described in the literature for GME (Cuddon and Smith-Maxie 1984, Sorjonen 1990, Braund 1994, Kipar and others 1998, Muñana and Luttgen 1998, Suzuki and others 2003), NME and NLE (Cordy and Holliday 1989, Tipold and others 1993, Stalis and others 1995, Summers and others 1995b, Vandevelde 1998).

In the present study, an unusually high incidence of optic nerve involvement was seen associated with disseminated signs of encephalitis (dogs 6, 7, 9 and 10). In these dogs, the concurrent optic nerve and brain involvement was particularly evident on MRI study. The combination of these two neuroanatomic localisations made GME the most likely presumptive diagnosis in these cases. Dogs with ocular GME have been reported to subsequently develop CNS involvement; however, the ocular form is the least reported (Braund 1985, Myre and others 1991, Coates and others 2005). The likely high occurrence of the ocular form in this report may be a reflection of the use of MRI to diagnose optic neuritis in dogs, which enabled discovery of brain lesions consistent with GME or other MUE.

Treatment with CiA was considered safe because there were few adverse effects, similar to those reported in literature (Guaguere and others 2003, Robson 2003). Gingival hyperplasia may be the result of decreased transglutaminase activity. Transglutaminase is a calcium-dependent enzyme involved in

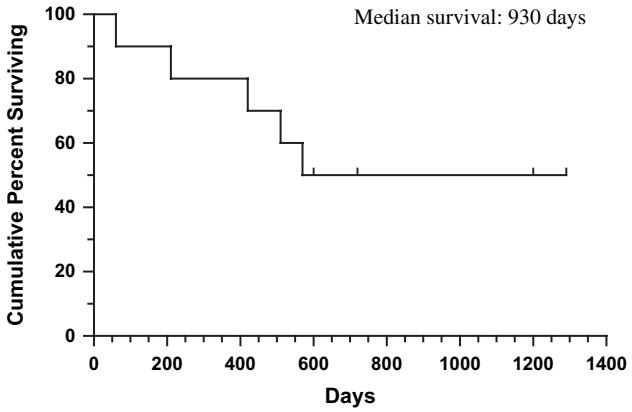

FIG 1. Kaplan-Meier graph of outcome for dogs with meningoencephalitis of unknown aetiology, treated with ciclosporin A (either alone or with corticosteroid and/or ketoconazole)

apoptosis and is highly concentrated in the gingival tissue. CiA, as calcium antagonist, may decrease normal transglutaminase activity, with consequent gingival overgrowth (Nishikawa and others 1996, Lewis and Reiter 2005). Chronic UTI that developed in three dogs (dogs 1, 4 and 5) treated with corticosteroids and CiA therapy may suggest that these two drugs in combination may increase the risk of this complication.

All dogs in this study had either a partial or complete clinical response to the treatment. In the deceased group (Table 1), four dogs had a partial clinical response and one dog had a complete clinical response before succumbing to GME or MUE. In this group, clinical improvement was seen after CiA was added to the corticosteroid treatment in three dogs (dogs 3, 4 and 5). In the other two dogs (dogs 1 and 2), the treatment was initially partially effective in one dog (dog 1) and completely effective in the other dog (dog 2) when CiA replaced corticosteroids. In these two dogs, even though the final outcomes were fatal, owners were pleased with the CiA treatment because the dogs did not have the adverse effects of corticosteroid therapy. In this group of dogs, three dogs were presented with the disseminated form of GME, which was diagnosed both histopathologically from a CT-guided needle biopsy and at post-mortem examination in one dog (dog 5) and at post-mortem examination in two other dogs (dogs 2 and 4). None of these dogs had any evidence of optic neuritis, in contrast to the surviving group where it was present in four of the five dogs.

In the surviving group (Table 2), two dogs (dogs 6 and 8) had complete clinical and CSF responses to the treatment and three dogs (dogs 7, 9 and 10) had a partial clinical response with normal CSF results at the last re-evaluation. In four dogs in this group, the concurrent optic nerve and brain involvement made GME the most likely presumptive diagnosis. Ocular and disseminated forms of GME are usually associated with the shortest survival periods (Vite 2005). Although our survival findings are in contrast with the literature, this study is inadequate to consider a concurrent optic nerve involvement a positive prognostic factor.

Because blood CiA levels were evaluated at different times after CiA dose, a definitive association between blood CiA level and response to treatment was difficult to determine. However, trough blood CiA level was considered more appropriate for a tentative evaluation of the clinical efficacy of the treatment. Trough blood CiA levels, ranging between 93 ng/ml and 363 ng/ml (mean: 191 ng/ml) at 12 hours after treatment in dogs treated with or without steroids and between 85 ng/ml and 461 ng/ml (mean: 373ng/mL) at 24 hours after treatment in dogs treated with CiA and KZ, were effective for long-term remission of the clinical signs in six dogs and for normal CSF in the surviving dogs.

A direct comparison of treatment efficacy with historical literature data is not possible because in the present study 70 per cent of dogs did not have a histopathological diagnosis. Also, all dogs in the Schatzberg study (Schatzberg and others 2005) and most cases in the Coates study (Coates and others 2005) lacked histopathological confirmation. The Muñana study (Muñana and Luttgen 1998) may be biased towards the most severe cases because those animals succumbed acutely to disseminated GME, so perhaps were not representative of the true prognosis when steroids are used as monotherapy. In the present study, the median survival time was 930 days. This is higher than the 531 days survival time in the study in which 10 dogs with MUE were treated with cytosar and prednisone (Schatzberg and others 2005), the 450 days survival time in the study of 20 dogs with MUE treated with procarbazine and corticosteroids (Coates and others 2005) and the 404 days survival time in a group of seven dogs with histopathological diagnosis of GME treated with radiation plus corticosteroids (Muñana and Luttgen 1998). The survival time of dogs with MUE treated in this study is longer when compared with the 41 days survival time in a group of 15 dogs with focal signs of GME treated with corticosteroids alone (Muñana and Luttgen 1998). Also, as five dogs in this report were censored in survival analysis, as they were still alive at the time of writing, a longer median survival time for this group is anticipated.

It appears that CiA is altering the immune response in a positive way in these cases of MUE. It may also be that prednisone, CiA, cytosar and procarbazine are complementary therapies that can be substituted for one another when response is incomplete. When histopathological confirmation is available, several treatment groups might be considered, including prednisone, CiA, cytosar and procarbazine. This may be the best method to determine which treatment is superior.

Three dogs in this study (dogs 1, 5 and 8) received anticonvulsant treatment for seizure control. It is likely that phenobarbital was responsible for the low blood CiA level in dog 8. Phenobarbital decreases blood CiA levels by increasing cytochrome P450 3A and P-glycoprotein effect (Fireman and others 2004). This was not observed in the other two dogs (dogs

1 and 5) receiving KBr. Based on these limited observations, it may be advisable to choose KBr instead of phenobarbital as anticonvulsant therapy in dogs treated with CiA.

In three dogs (dogs 5, 8 and 10) treated with KZ and CiA, the treatment was found clinically effective and the dose of CiA was reduced by 50 per cent (net cost saving of 46 per cent). This combined treatment also had the advantage of being given only once daily. No significant side effects from KZ were observed in these dogs after seven months (dog 8) and 12 months (dogs 5 and 10), respectively, of receiving this combined therapy. KZ has been shown to decrease the systemic clearance of CiA in dogs through inhibition of hepatic cytochrome P450 3A microsomal enzymes (D'mello and others 1989).

Limitations of this study include the small number of cases, the short follow-up of some survivors and the limited number with histopathological confirmation. Prospective evaluation of a larger treatment group with a longer follow-up and a histopathological diagnosis of GME is warranted to substantiate these initial findings.

CiA appears to be an alternative treatment for MUE in dogs and has the benefit of avoiding side effects caused by corticosteroid treatment.

Acknowledgements

The authors thank Dr Ilene Kurzman for providing assistance with the statistical analysis. The study in part was supported by the Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA.

References

Adamo, P. & O'Brien, R. (2004) Use of Cyclosporine to treat Granuloma Meningoencephalitis in three dogs. *Journal of the American Veterinary Medical Association* **225**, 1211-1126

Bailey, C. & Vernau, W. (1997) Cerebrospinal fluid. In: Clinical Biochemistry of Domestic Animals. 5th edn. San Diego Academic Press, San Diego, CA, USA. pp 785-827

Bailey, C. S. & Higgins, R. J. (1986) Characteristic of cerebrospinal fluid associated with canine granulomatous meningoencephalomyelitis: a retrospective study. *Journal of the American Veterinary Medical Association* **188**, 418-421

Begley, D. J., Squires, L. K., Zlokovic, B. V, Mitrovic, D. M, Hughes, C. C, Revest, P. A. & Greenwood, J. (1990) Permeability of the blood-brain barrier to the immunosuppressive cyclic peptide cyclosporin A. *Journal of Neurochemistry* **55**, 1222-1230

Braund, K. (2005) Granulomatous meningoencephalomyelitis. In: Clinical Neurology in Small Animals – Localization, Diagnosis and Treatment. Ed C. Vite. International Veterinary Information Service, Ithaca, NY, USA. http://www.ivis.org. B0227.0203 (accessed 15 May 2006)

Braund, K. G. (1985) Granulomatous meningoencephalomyelitis. *Journal of the American Veterinary Medical Association* **186**, 138-141

Braund, K. G. (1994) Granulomatous meningoencephalomyelitis. In: Clinical Syndrome in Veterinary Neurology. 2nd edn. Ed K. G. Braund. Mosby, St Louis, MO, USA. pp 135-139

Cherubini, G., Anderson, T., Rusbridge, C., Mantis, P. & Cappello, R. (2004) MRI findings in 7 dogs with confirmed GME. Annual Symposium ECVN proceeding. September 24 to 25, Glasgow, UK

Chrisman, C. (1992) Cerebrospinal fluid analysis. In: Diseases of the Spine. Ed M. Moore. W. B. Saunders Co, Philadelphia, PA, USA pp 781-809

Coates, J., Barone, G., Dewey, C., Hallowey-Azene, N., Vitale, C. & Session, J. (2005) Procarbazine for treatment of suspected Granulomatous Meningo-encephalomyelitis: 20 cases (1998-2004). 23rd ACVIM. June 1 to 4, Baltimore, MD, USA. p 912

Cordy, D. R. (1979) Canine granulomatous meningoencephalomyelitis. *Veterinary Pathology* **16**, 325-333

Cordy, D. R. & Holliday, T. (1989) A necrotizing meningoencephalitis of Pug dog. *Veterinary Pathology* **26**, 191-194

Cuddon, P., Coates, J. & Murray, M. (2002) New treatment for granulomatous meningoencephalitis. 20th American College of Veterinary Internal Medicine Forum. 29 May to 1 June, Dallas, TX, USA. pp 319-321

Cuddon, P. A. & Smith-Maxie, L. (1984) Reticulosis of the central nervous system in the dog. *Compendium on Continuing Education for the Practicing Veterinarian* **6**, 23-32

Daigle, J. C. (2002) More economical use of cyclosporine trough combination drug therapy. *Journal of American Animal Hospital Association* **38**, 205-208

D'Mello, A., Venkataramanan, R., Satake, M., Todo, S., Takaya, S., Ptachcinski, R. J., Burckart, G. J. & Starzl, T. E. (1989) Pharmacokinetics of the cyclosporine-ketoconazole interaction in dogs. *Research Communications in Chemical Pathology and Pharmacology* **64**, 441-454

Fireman, M., DiMartin, A. F., Armstrong, S. C., Cozza, K. L. (2004) Med-psych drug-drug interactions. *Psychosomatics* **45**, 354-360

Gorman, N. T. (1995) Immunology. In: Textbook of Veterinary Internal Medicine. Ed E. Feldman. W. B. Saunders Co, Philadelphia, PA, USA. pp 1978-2002

Guaguere, E., Steffan, J. & Olivry, T. (2003) Cyclosporin A: a new drug in the field of canine dermatology. *Veterinary Dermatology* **15**, 61-74

Janeway, C., Travers, P., Walport, M. & Shlomchik, M. (2005) T cell-mediated immunity. In: Immunobiology. 6th edn. Ed C. Janeway. Garland Science, New York, NY, USA. pp 319-366

Jull, B., Merryman, J., Thomas, W. & McArthur, A. (1997) Necrotizing encephalitis in a Yorkshire terrier. *Journal of the American Veterinary Medical Association* **211**, 1005–1007

Kipar, A., Baumgartner, W., Vogl, C., Gaedek, K. & Wellman, M. (1998) Immunohistochemical Characterization of inflammatory cells in brains of dogs with granulomatous meningoencephalomyelitis. *Veterinary Pathology* **35**, 45-52

Lamb, C., Croson, P., Cappello, R. & Cherubini, G. (2005) Magnetic resonance imaging findings in 25 dogs with inflammatory cerebrospinal fluid. *Veterinary Radiology & Ultrasound* **46**, 17-22

Lewis, J. & Reiter, A. (2005) Management of generalized gingival enlargement in a dog – case report and literature review. *Journal of Veterinary Dentistry* **22**, 160–169

Lobetti, R. G. & Oearson, J. (1996) Magnetic resonance imaging in the diagnosis of focal granulomatous meningoencephalomyelitis in two dogs. *Veterinary Radiology & Ultrasound* **37**, 424-427

Mathews, K., Holmberg, D. & Johnston, K. (1994) Renal allograft survival in out bred mongrel dogs utilizing combination immunosuppressive drug therapy and donor bone marrow. *Veterinary Surgery* **23**, 347-357

Mathews, K. & Sukhiani, H. (1997) Randomized controlled trial of cyclosporine for treatment of perianal fistula in dogs. *Journal of the American Veterinary Medical Association* **211**, 1249-1253

Matsuki, N., Fujiwara, K., Tamahara, S., Uchida, K., Matsunaga, S., Nakayama, H., Doi, K., Ogawa, H. & Ono, K. (2004) Prevalence of autoantibodies in cerebrospinal fluids from dogs with various CNS diseases. *Journal of Veterinary Medical Science* **66**, 295-297

Muñana, K. R. & Luttgen, P. J. (1998) Prognostic factors for dogs with granulomatous meningoencephalomyelitis: 42 cases (1982-1996). *Journal of the American Veterinary Medical Association* **212**, 1902-1906

Myre, S., Schoder, T., Grund, V., Wandstrat, T., Nicely, P., Pesce, A. & First, M. (1991) Critical ketoconazole dosage range for cyclosporin clearance inhibition in the dog. *Pharmacology* **43**, 233-241

Nishikawa, S., Nagata, T., Morisaki, I., Oka, T. & Ishida, H. (1996) Pathogenesis of drugs induced gingival overgrowth: a review of studies in the rat model. *Journal of Periodeontology* **67**, 463-471

Okonkwo, D. O., Melon, D. E., Pellicani, A. J, Mutlu, L. K., Rubin, D. C., Stone, H. R. & Helm, G. A. (2003) Dose-response of cyclosporine A in attenuating traumatic axonal injury in rat. *Neuroreport* **14**, 463-466

Robson, D. (2003) Review of the pharmacokinetics, interactions and adverse reactions of cyclosporine in people, dogs and cats. *Veterinary Record* **152**, 739-748

Schatzberg, S. J., Haley, N., Barr, S., de Lahunta, A. & Sharp, N. J. H. (2005) Polymerase chain reaction screening for DNA viruses in paraffin embedded brains from dogs with necrotizing meningoencephalitis, necrotizing leukoencephalitis, and granulomatous meningoencephalitis. *Journal of Veterinary Internal Medicine* **19**, 553-559

Schatzberg, S. J. (2005) An update on granulomatous meningoencephalomyelitis, necrotizing meningoencephalomyelitis and necrotizing leukoencephalitis. 23rd ACVIM Forum. 1 to 4 June, Baltimore, MD, USA. pp 351-353

Sorjonen, D. C. (1990) Clinical and histopathological features of granulomatous meningoencephalomyelitis in dogs. *Journal of American Animal Hospital Association* **26**, 141-147

Stalis, I., Chadwick, B., Dayrell-Hart, B., Summer, B. & van Winkle, T. (1995) Necrotizing meningoencephalitis of Maltese dogs. *Veterinary Pathology* **32**, 230-235

Steffan, J., Strehlau, G., Maurer, M. & Rolphs, A. (2004) Cyclosporin A pharmacokinetics and efficacy in the treatment of atopic dermatitis in dogs. *Journal of Veterinary Pharmacology and therapeutics* **27**, 231-238

Summers, B. A., Cummings, J. F. & de Lahunta, A. (1995a) Granulomatous meningoencephalomyelitis. In: Veterinary Neuropathology. Eds B. A. Summers and A. de Lahunta. Mosby, St Louis, MO, USA. pp 110-114

Summers, B. A., Cummings, J. F. & de Lahunta, A. (1995b) Inflammatory diseases of the central nervous system. In: Veterinary Neuropathology. Ed B. A. Summer. Mosby-Year Book, St Louis, MO, USA. pp 402-501

Suzuki, M., Uchida, K., Morozumi, M., Hasegawa, T., Yanai, T., Nakayama, H. & Tateyama, S. (2003) A comparative pathological study on canine necrotizing meningoencephalitis and granulomatous meningoencephalitis. *Journal of Veterinary Medical Science* **65**, 1233-1239

Thomas, J. B. & Eger, C. (1989) Granulomatous meningoencephalomyelitis in 21 dogs. *Journal of Small Animal Practice* **30**, 287-293

Tiches, D., Vite, C., Dayrell-Hart, B., Steinberg, S., Gross, S. & Lexa, F. (1998) A case of canine central nervous system cryptococcosis management with fluconazole. *Journal of the American Animal Hospital Association* **34**, 145-151

Tipold, A., Fatzer, R., Jaggy, A., Zurbriggen, A. & Vandevelde, M. (1993) Necrotizing encephalitis in Yorkshire Terriers. *Journal of Small Animal Practice* **34**, 623-628

Uchida, K., Hasegawa, T., Ikeda, M., Yamaguchi, R. & Tateyama, S. (1999) Detection of an autoantibody from pug dogs with necrotizing encephalitis (pug dog encephalitis). *Veterinary Pathology* **36**, 301-307

Vandevelde, E. (1998) Neurologic diseases of suspected infectious origin. In: Infectious Diseases of the Dog and Cat. 2nd edn. Ed M. Vandevelde. W. B. Saunders Co, Philadelphia, PA, USA pp 530-539

Vandevelde, M., Fatzer, R. & Fankhauser, R. (1981) Immunohistological studies on primary reticulosis of the canine brain. *Veterinary Pathology* **18**, 577-588

Vite, C. (2005) Inflammatory diseases of the central nervous system (last updated: 17 February 2005). In: Braund's Clinical Neurology in Small Animals: Localization, Diagnosis and Treatment. Ed K. Braund. International Veterinary Information Service, Ithaca, NY, USA. http://www.ivis.org (accessed 15 May 2006)