

Oral Cyclosporine Treatment in Dogs: A Review of the Literature

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Cyclosporine is an immunomodulatory drug used to treat an increasing spectrum of diseases in dogs. Cyclosporine is a calcineurin inhibitor, ultimately exerting its inhibitory effects on T-lymphocytes by decreasing production of cytokines, such as interleukin-2. Although, in the United States, oral cyclosporine is approved in dogs only for treatment of atopic dermatitis, there are many other indications for its use. Cyclosporine is available in 2 oral formulations: the original oil-based formulation and the more commonly used ultramicroemulsion that facilitates oral absorption. Ultramicroemulsified cyclosporine is available as an approved animal product, and human proprietary and generic preparations are also available. Bioavailability of the different formulations in dogs is likely to vary among the preparations. Cyclosporine is associated with a large number of drug interactions that can also influence blood cyclosporine concentrations. Therapeutic drug monitoring (TDM) can be used to assist in attaining consistent plasma cyclosporine concentrations despite the effects of varying bioavailability and drug interactions. TDM can facilitate therapeutic success by guiding dose adjustments on an individualized basis, and is recommended in cases that do not respond to initial oral dosing, or during treatment of severe, life-threatening diseases for which a trial-and-error approach to dose adjustment is too risky. Pharmacodynamic assays that evaluate individual patient immune responses to cyclosporine can be used to augment information provided by TDM.

Key words: Cyclosporine; Pharmacodynamics; Pharmacokinetics; Therapeutic drug monitoring.

Cyclosporine is a potent immunosuppressive drug indicated for the treatment of autoimmune diseases and for organ transplantation. In dogs, cyclosporine is used to treat a spectrum of chronic inflammatory and immune-mediated diseases. Cyclosporins (up to 9 different molecules, A–I) are cyclic polypeptide macrolides that were originally derived from the soil fungus *Tolypocladium inflatum* (*Beauveria nivea*), but are also produced by other fungal organisms. Cyclosporine A is the molecule developed for commercial use as an immunosuppressive agent.^{1–3} Discovered by Sandoz Laboratory in 1972, the use of cyclosporine as an immunosuppressive agent was first described in humans to prevent rejection of renal allografts.^{3,4} Since that time, cyclosporine has become the cornerstone of immunosuppression for organ transplantation.^{4,5} Cyclosporine was approved by the U.S. Food and Drug Administration (FDA) in 1983 for treatment and prevention of transplant rejection in human medicine.⁶ In veterinary medicine, Novartis Animal Health received FDA approval in 2003 for oral cyclosporine capsules (Atopica) for the treatment of canine atopy. However, by this time cyclosporine had already been used in an extralabel fashion for many years for renal transplantation in dogs and

Abbreviations:

CYP	cytochrome P450
GME	granulomatous meningoencephalitis
HPLC	high-pressure liquid chromatography
IBD	inflammatory bowel disease
IFN- γ	interferon-gamma
IL-2	interleukin-2
IL-4	interleukin-4
mRNA	messenger ribonucleic acid
qRT-PCR	quantitative reverse transcriptase polymerase chain reaction
RIA	radioimmunoassay
TDM	therapeutic drug monitoring

cats,^{7–10} and for the treatment of a variety of inflammatory and immune-mediated conditions.^{11–14}

Oral Cyclosporine Formulations

Cyclosporine is a large lipophilic molecule, which must be solubilized before intestinal absorption (Fig 1).¹⁵ Commercial cyclosporine is available as 2 very different types of oral formulations. Cyclosporine initially was approved for humans as a vegetable-oil based preparation (Sandimmune), but variability in oral bioavailability caused marked intraindividual and interindividual variations in blood drug concentrations. A more recent formulation, an ultramicroemulsion preparation approved in 1996 (Neoral), forms a microemulsion upon contact with aqueous fluids, resulting in more consistent and predictable absorption.¹⁶ Oral bioavailability of the microemulsion is improved by up to 50% compared with the oil-based preparation.^{17–19} Because of the marked variability in bioavailability of the nonultramicroemulsified Sandimmune preparation, it is not recommended for oral use in dogs. Only the veterinary version of the Neoral microemulsion preparation, Atopica, is approved for use in dogs and cats in the United States. Veterinarians continue to prescribe generic ultramicroemulsified products for human use despite,

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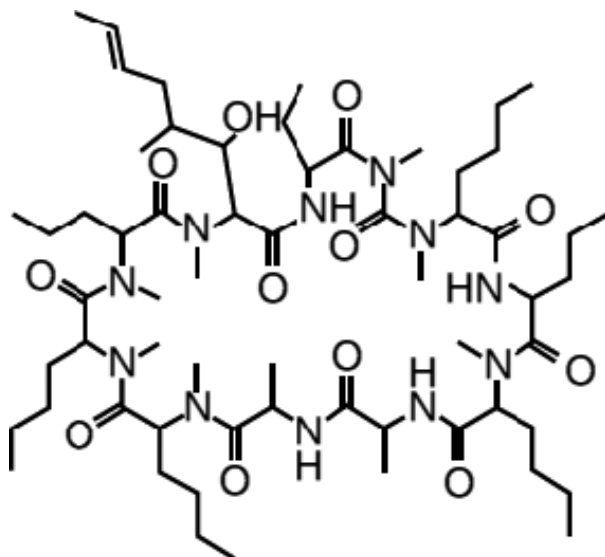


Fig 1. Chemical structure of cyclosporine.

in many countries, the availability of veterinary versions of the drug. However, only limited evidence exists that generic products for human use will be equally bioavailable in dogs, either as compared with one another, or with Atopica.

Mechanism of Action

Cyclosporine's primary immunosuppressive mechanism of action is inhibition of T-lymphocyte function. Antigen binding to CD3 receptors on the surface of T-lymphocytes causes increased intracellular calcium and activation of calcineurin. Calcineurin is an intracellular protein phosphatase that activates gene transcription factors by dephosphorylation. Cyclosporine acts to inhibit calcineurin. Calcineurin inhibitors, including cyclosporine, act by binding to intracellular cyclophilins, which are proteins that facilitate protein folding.

Cyclophilin A is the predominant cyclophilin found in T-lymphocytes. Binding of cyclosporine to cyclophilin A creates a complex with high affinity for calcineurin. Activation of T-lymphocytes results in activated calcineurin, which dephosphorylates inactive nuclear factor (NFAT). NFAT translocates into the nucleus, where it upregulates transcription of genes coding for several important cytokines. These include interleukin-2 (IL-2), interleukin-4 (IL-4), TNF- α , and INF- γ .^{6,20,21} Production of IL-2, in particular, plays a key role in the activation and proliferation of T-lymphocytes. By inhibition of calcineurin, cyclosporine specifically inhibits T-cell function, and thus cell-mediated immunity, but has little immediate impact on humoral immunity.²²⁻²⁴ Decreased IL-2 expression in CD4+ Th1 cells associated with cyclosporine treatment leads to inhibition of proliferation and activation of both T-helper and T-cytotoxic lymphocytes, and blunting of the immune response (Fig 2).

Disposition of Cyclosporine

Absorption

After oral administration, cyclosporine is absorbed across the epithelium of the small intestine. Variability in the absorption of the oil-based preparation is associated with variations in bile flow and gastrointestinal motility.²⁵ The improved predictability of absorption of the microemulsion reflects its independence from these factors, although oral bioavailability still may vary from 23 to 45%.²⁵⁻²⁷

In dogs, food may impact the oral absorption of ultramicroemulsified cyclosporine. A decrease in bioavailability and increase in pharmacokinetic variability were documented when ultramicroemulsified cyclosporine was administered with food, leading to recommendations that the drug be administered 2 hours before or after feeding.^{27,28} A later study in atopic dogs, however, did not demonstrate any impact of food on the clinical efficacy of ultramicroemulsified cyclosporine.²⁹

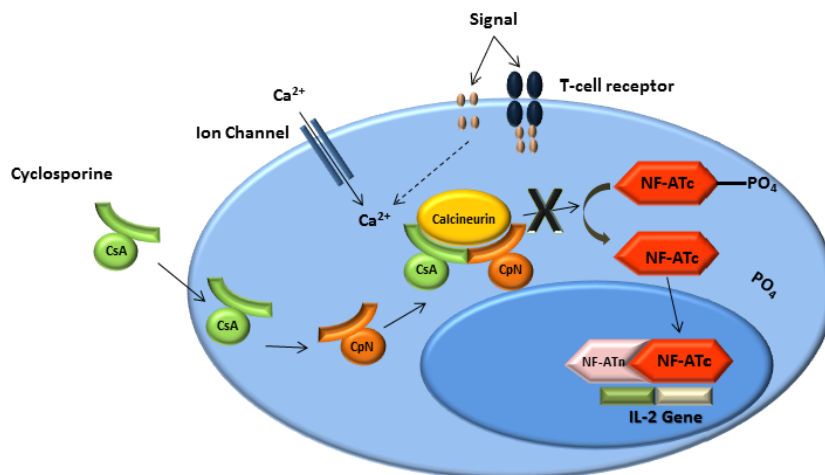


Fig 2. Cyclosporine mechanism of action.

Cyclosporine is among the many drugs that are substrates for P-glycoprotein, an efflux transporter pump located at portals of entry and sanctuaries. In intestinal epithelium, P-glycoprotein is located in the brush border of the enterocytes, where it pumps a wide variety of xenobiotics out of the cell and back into the lumen. In people, intestinal P-glycoprotein activity has been shown to influence intestinal absorption and metabolism of cyclosporine.³⁰ A study in 3 normal and 3 P-glycoprotein-deficient dogs, in contrast, failed to identify a significant difference in cyclosporine pharmacokinetics when cyclosporine was administered both PO and IV.³¹

In another study, cimetidine, an H₂-receptor antagonist, given concurrently with oral ultramicronized cyclosporine, caused a significant increase in time until maximal blood cyclosporine concentrations, an effect that may reflect either increased drug absorption or decreased clearance because of inhibition of cyclosporine metabolism.³² The overall maximum blood concentration of cyclosporine did not increase in dogs on cimetidine, suggesting that the reason was because of increased absorption.

Distribution

Cyclosporine has a high binding affinity for red blood cells and plasma lipoproteins.¹ Because up to 50% of the drug in blood is located in red cells, whole blood is recommended for therapeutic drug monitoring (TDM). Once in the circulation, cyclosporine distributes widely, accumulating in the skin, liver, kidneys, and fat of dogs, resulting in a large volume of distribution.²⁵ Tissue concentrations exceed concentrations in serum by a factor of 3 to 14.²⁵ Variability in the volume of distribution contributes to variability in magnitude and time to peak blood concentration (C_{max}) in dogs, with peak concentrations generally occurring approximately 2 hours after oral administration of ultramicronized cyclosporine.^{11,28} Blood concentrations then rapidly decrease over the remainder of the dosing interval, reflecting a relatively rapid half-life as the drug is cleared from plasma.

Metabolism

Extensive metabolism of cyclosporine by phase I microsomal (cytochrome P450 or CYP) enzymes mediates hydroxylation, *N*-demethylation, or both, yielding many different metabolites.^{1,33} Metabolism occurs in the liver, small intestine, and kidneys, with the liver being the major site of metabolism. The major cyclosporine metabolites produced by the liver in dogs include a 9 γ -hydroxylated metabolite, a 4 *N*-desmethylated metabolite, a 1- β -(8) hydroxylated metabolite, and a 1- β -1- ϵ -cyclized metabolite.³³ In dogs, hepatic metabolism is extensive and occurs quickly, with 70 to 100% of the drug being metabolized within 30 minutes.^{25,33,34} In the liver, cytochrome P-450 3A provides the key metabolic pathway. Metabolism in the small intestine is more variable and slower.³³ Variation in

Table 1. Drug interactions with cyclosporine.

Drugs that may increase cyclosporine concentrations	
Acetazolamide	Flavonoids in grapefruit juice
Allopurinol	Fluconazole
Amiodarone	Fluoxetine
Azithromycin	Ketoconazole
Bromocriptine	Imapenem
Calcium channel blockers	Itraconazole
Carvedilol	Macrolide antibiotics (Erythromycin, clarithromycin)
Chloramphenicol	Methotrexate
Cimetidine	Metoclopramide
Ciprofloxacin/Enrofloxacin	Metronidazole
Cisapride	Omeprazole
Colchicine	Sertraline
Danazol	Tacrolimus
Digoxin	Tinidazole
Estrogens	
Drugs that may decrease blood concentrations of cyclosporine	
Azathioprine	Phenobarbital
Carbamazepine	Phenytoin
Clindamycin	Rifampin
Cyclophosphamide	Sulfadiazine
Famotidine	Terbinafine
Nafcillin	Trimethoprim
Octreotide	
Drugs that may increase or decrease blood concentrations of cyclosporine	
Glucocorticoids	

the CYP 3A4 activity influences cyclosporine clearance in humans.

A number of drug interactions impacting cyclosporine involve the hepatic P-450 enzyme system. Examples of drugs for human use and use in veterinary medicine that inhibit CYP450 3A4, leading to increased cyclosporine blood concentrations, as well as drugs known to decrease concentrations of cyclosporine by either induction of the enzymes involved in cyclosporine metabolism or increased excretion of cyclosporine, are listed in Table 1.

In dogs, several drugs have been given concurrently with cyclosporine to decrease the dosage needed to maintain adequate blood concentrations of cyclosporine. Two classes of drugs have been studied for this effect: the imidazole antifungal drugs ketoconazole and fluconazole, and H-2 receptor-blocking antihistaminergic drugs, such as cimetidine.³⁵ Of these, ketoconazole has received the most attention. Ketoconazole enables a decrease in oral cyclosporine dosages in dogs by as much as 75%, although individual responses are variable.³⁶ Fluconazole also has been shown to enable reduction of oral cyclosporine dosages by between 30 and 50%.^{37,38} Cimetidine is an inhibitor of several CYP enzymes and also is a substrate for P-glycoprotein. Cimetidine significantly decreases cyclosporine clearance and prolongs elimination half-life in rabbits,³⁹ although similar effects could not be documented in rats, dogs, or humans.^{32,40,41} Interestingly, high-dose powdered whole grapefruit has been shown to increase blood cyclosporine concentrations in dogs, possibly

because grapefruit furanocoumarins inhibit intestinal P-450 3A microsomal enzymes, thus increasing oral bioavailability.⁴²

In addition to drug interactions, disease also may influence cyclosporine metabolism in dogs. Experimentally induced diabetes and pancreatectomy, for example, both increase cyclosporine clearance, whereas partial hepatectomy decreases drug clearance.^{43–45}

Excretion

Most cyclosporine metabolites are excreted predominantly through the biliary system, with minimal renal excretion.^{1,26,46}

Cyclosporine Generic Preparations

According to the FDA Orange Book (<http://www.accessdata.fda.gov/scripts/cder/ob/default.cfm>), over 20 generic cyclosporine preparations have been approved for use in humans in the United States. For FDA generic drug approval, generic drugs must demonstrate bioequivalence, with 90% confidence intervals for pivotal exposure parameters (area under the plasma drug concentration time curve [AUC] and maximum concentration in the dosing interval) falling within the range of 80–125% of the brand name (reference) product, which, for microemulsified cyclosporine, is Neoral.⁴⁷ Instead of the equivalence code “A” typically granted to equivalent generic formulations, cyclosporine generic formulations are designated by the FDA as having a therapeutic equivalence code of “AB” (http://www.fda.gov/Drugs/DevelopmentApprovalProcess/ucm079068.htm#TherapeuticEquivalence-Related_Terms), indicating that issues regarding bioequivalency to the reference compound existed, but sufficient evidence was presented to resolve these concerns, such that approval occurred as a “therapeutically equivalent” formulation in humans. Testing with generic preparations of cyclosporine has occurred primarily with young healthy human individuals.⁴⁷ With a lack of comparative data relating pharmacokinetic parameters between the Neoral formulation and generic ultramicronized formulations in diseased patients requiring immunosuppressive treatment, recommendations to physicians are to use generic formulations of cyclosporine only in low-risk human patients when additional testing can be performed to ensure attainment of desired blood concentrations.⁴⁷ One study evaluating the pharmacokinetics of both Neoral and generic ultramicronized cyclosporine in human renal transplant recipients demonstrated that absorption of generic cyclosporine was significantly less than Neoral.⁴⁸

No generic cyclosporine preparations have been approved for use in veterinary medicine. However, by virtue of the Animal Medicinal Drug Use Clarification Act (1984),^a US veterinarians are legally empowered to use any drug approved for use in any species in an extralabel fashion. The exception to this rule occurs, however, if an approved product exists for use in the

species of interest. Accordingly, this calls into question the legitimacy of US veterinarians prescribing human generic cyclosporine solely because such preparations are less expensive. Concerns regarding the use of generic cyclosporine are justified in that, although therapeutic equivalence has been demonstrated in people, data from humans do not apply to dogs. Indeed, in the authors' experience, oral bioavailability of generic preparations for human use may vary by 3-fold or more in dogs, depending on the manufacturer. Because pharmacies may change generic preparations, monitoring potentially should be implemented with each new prescription.

In addition to generic preparations, cyclosporine often is prescribed as a compounded preparation. Extreme caution is recommended with this approach. Marked variability in product quality and concentration has been demonstrated among cyclosporine preparations compounded for animals. Accordingly, prescription of compounded products is strongly discouraged, unless the needs of the patient simply cannot be met with current approved formulations.

Pharmacokinetics

Many investigators have reported the pharmacokinetics of cyclosporine in dogs (Table 2).^{11,28,31,32,38,42,43,49–57} However, comparison among studies is difficult because of differences in dosage, routes of administration, preparations used, assays of quantitation, and other factors, such as fed versus fasting state. Some crucial points, however, can be obtained from evaluating these studies. The time to maximum drug concentrations after oral administration, regardless of the preparation, occurs within 1–2 hours, suggesting that 2 hours after administration is a reasonable time for monitoring peak drug concentration. Elimination half-life is reported to be highly variable. For studies using oral cyclosporine, however, the terminal component of the drug concentration versus time curve may not necessarily reflect elimination, but may reflect prolonged absorption. Only studies using intravenous cyclosporine therefore can be used to confirm the duration of the elimination half-life. Because formation of metabolites can impact the terminal curve, studies that include only the parent compound, such as high-pressure liquid chromatography (HPLC) assays, are most informative. Based on these considerations, the elimination half-life of cyclosporine in dogs appears to range from 7 to 10 hours. Use of assays that measure more than just the parent compound can markedly affect results. For example, in 1 study using fluorescence polarization immunoassay analysis, cyclosporine half-life in plasma was recorded as 1.9 hours.⁵² Variability in half-life has been reported in other studies, being >22 hours in several studies (after intravenous dosing in 1 study,⁴³ and oral dosing in another⁵⁰). In the Auburn TDM laboratory, cyclosporine elimination half-life in dogs is markedly variable, ranging from 1 to 2 hours to over 150 hours, with longer half-lives most commonly seen in patients receiving drugs known to prolong cyclosporine elimination.

Table 2. Pharmacokinetic parameters of cyclosporine.

Species (n)	Dosage (mg/kg)	Route	Preparation	Sample	Method	CsA (ng/mL)	Parameter	T_{max} (hour)	Half-life (hour)	Duration	Status	Reference
Collie dog (3)	1	IV	NS	Plasma	HPLC	2,723 ± 229	AUC (inf)	NS	9.6 ± 3.2	Single	ABCBI-1Δ	Mealey et al ³¹
Collie dog (2)	1	IV	NS	Plasma	HPLC	2,635 (1,980–3,290)	AUC (inf)	NS	7.2 (6.9–7.5)	Single	ABCBI-WT	
Beagle (5)	5	IV	NS	WB	FPIA	3,721 ± 639	C_0	NS	9.3 ± 2.3	Single	DM	Alkharfy ⁴³
						12,110 ± 1,882	AUC (inf)					
Beagle (5)	5	IV	NS	WB	FPIA	3,681 ± 662	C_0	NS	22.6 ± 2.3	Single	H, N	
Beagle (4)	5	PO	Neoral	WB	HPLC	25,181 ± 1,514	AUC (inf)	NS	NS	Single	H, N	Fukunaga and Orito ⁴⁹
						1,188 ± 349	Peak					Radwanski et al ⁴²
Dog (8)	5	PO	Atopica	WB	HPLC-MS-MS	3,829 ± 642	AUC (inf)	1.75 ± 0.12	12.2 ± 1.4	Single	H, N	Lai ⁵⁰
						1,075 ± 105	Peak					
Beagle (6)	10–12.5 (100 mg)	PO	Neoral	WB	RP-HPLC	6,700 ± 483	AUC (inf)	1.8 ± 0.75	22.3 ± 10.5	Single	H, N	Mealey et al ³¹
						970 ± 176	Peak					
Collie dog (3)	4	PO (gavage)	NS	Plasma	HPLC	5,462 ± 991	AUC (inf)	1.3 ± 0.29	NS	Single	ABCBI-1Δ	
						992 ± 551	Peak					
Collie dog (2)	4	PO (gavage)	NS	Plasma	HPLC	4,787 ± 1,359	AUC (inf)	1.3	NS	Single	ABCBI-WT	
						895 (699–1,090)	Peak					
						4,420 (2,520–6,330)	AUC (inf)	(1.0–1.5)				
Beagle (6)	6.7–8.3 (100 mg)	PO	Neoral	WB	RIA	1,241 ± 69	Peak	1.3 ± 0.4	7.2 ± 0.7	Single	H, N	Yang ⁵⁷
Beagle (4)	25 mg	PO	Neoral	WB	HPLC	8,683 ± 1,046	AUC (inf)	0.88 ± 0.25	NS	Single	H, N	Sander and Holm ⁵¹
						331 ± 57	Peak					Katayama et al ³⁸
Beagle (4)	10	PO	Atopica	WB	FPIA	1,269 ± 256	AUC (inf)	1.75 ± 0.5	5.4 ± 0.47	Single	H, N	Allenspach et al ¹¹
						1,437 ± 246	Peak					
Dog (8)	5	PO	Atopica	WB	FPIA	6,784 ± 246	AUC (0–12)	1.5 ± 0.5	5.6 ± 1.2	Single	IBD, Severe	
						699 ± 326	Peak					
						34 ± 26	C_{min} (24 hours trough)					
Dog (16)	5	PO	Atopica	WB	FPIA	4,770 ± 2,672	AUC (0–24)	1.6 ± 0.4	7.8 ± 1.1	Single	H, N	
						878 ± 131	Peak					
						50 ± 22	C_{min} (24 hours trough)					
Dog (3)	15	PO	Neoral solution	Plasma	FPIA	6,729 ± 1,578	AUC (0–24)	1.5	1.94 ± 0.2	Single	H, N	Amatori et al ⁵²
						1,118 ± 124	Peak					Steffan et al ²⁸
Beagle (8)	5	PO, FST capsule	Microemulsion	WB	FPIA	7,465 ± 926	AUC (inf)	1.3 ± 0.5	NS	Single	H, N	
						1,059 ± 207	Peak					
						6,386 ± 2,079	AUC (0–24)	1.36 ± 2.9	NS	Single	H, N	
						845 ± 582	Peak					
						5,453 ± 1,905	AUC (0–24)					
						1,287 ± 180	Peak	1	NS	Single	H, N	
						7,533 ± 1,712	AUC (0–24)					
						949 ± 725	Peak	0.65 ± 0.26	NS	Single	H, N	

Table 2 (Continued)

Species (n)	Dosage (mg/kg)	Route	Preparation	Sample	Method	CsA (ng/mL)	Parameter	$T_{\max}$ (hour)	Half-life (hour)	Duration	Status	Reference
		PO, Fed solution				5,396 $\pm$ 2,615	AUC (0–24)					
Beagle (20)	5	PO, FST capsule	Atopica	WB	HPLC	577 $\pm$ 128 34 $\pm$ 12	Peak $C_{\min}$ (24 hours predicted)	1.4 $\pm$ 0.3	9.4 $\pm$ 1.2	Single	H, N	
Beagle (16)	5	PO, FST capsule	Atopica	WB	FPIA	3,997 $\pm$ 1,108 878 $\pm$ 131 50 $\pm$ 22	AUC (inf) Peak $C_{\min}$ (24 hours predicted)	1.6 $\pm$ 0.4	7.8 $\pm$ 1.1	Single	H, N	
Beagle (6)	6.7–8.3 (100 mg)	PO	Neoral solution	Plasma	RP-HPLC	6,729 $\pm$ 1,587 1,707 $\pm$ 72 23,091 $\pm$ 1,204	AUC (inf) Peak AUC (inf)	2.5 $\pm$ 0.55	6.82 $\pm$ 0.12	Single	H, N	El-Shabour ⁵³
Dog (4)	10	PO	Sandimmune solution	WB	HPLC	772 $\pm$ 71 4,250 $\pm$ 753	Peak AUC (inf)	1.25 $\pm$ 0.5	NS	Single	H, N	Fischer et al ⁵⁴
Dog (4)	10	PO	Neoral solution	WB	HPLC	977 $\pm$ 135 7,078 $\pm$ 1,777	Peak AUC (inf)	1.75 $\pm$ 0.5	NS	Single	H, N	
Beagle (10)	5	PO q24h	Neoral	WB	FPIA	1,088 $\pm$ 167	Peak	1.3 $\pm$ 0.36	NS	3 doses	H, N	Daigle et al ³²
Beagle (10)	6.3–9.1 (100 mg)	PO	Neoral solution	WB	RIA	1,689 (26% rel SD)	Peak	1.9	NS	Single	H, N	Ford et al ⁵⁵
German Shepherd (6)	7.5 q12h	PO	Neoral	WB	EMIT	12,790 (16% rel SD) 578 $\pm$ 459 (205–1,370)	AUC (inf) Trough	(37% rel SD) NS	NS	7 days	Anal Furunculosis	Griffiths et al ⁵⁶

AUC (ng*h/mL), area under the curve; C_0 , initial blood drug concentration; $C_{\min}$, minimum concentration after dosing; HPLC, high-performance (pressure) liquid chromatography; RP-HPLC, reverse-phase high-performance (pressure) liquid chromatography; HPLC-MS-MS, high-performance (pressure) liquid chromatography tandem mass spectrometry; FPIA, fluorescence polarization immunoassay; EMIT, enzyme multiplied immunoassay technique; RIA, radioimmunoassay; rel SD, relative standard deviation; inf, infinity; WB, whole blood; FST, fasted; H, N, healthy, normal; AD, atopic dermatitis; DM, diabetes mellitus; IBD, inflammatory bowel disease; ABCB1-1A, P-glycoprotein-deficient dogs; ABCB1-WT, normal P-glycoprotein dogs; NS, not specified.

Variability in cyclosporine half-life has therapeutic implications. The time to steady state, and thus time to evaluate therapeutic response, ranges from negligible (that is, no steady state exists) to over 18–25 days. Variability in half-life also impacts the timing of sample collection, and may impact therapeutic response. With a half-life of <2 hours, >90% of each dose is eliminated during a dosing interval, and concentrations will vary markedly with the timing of sample collection. In fact, trough concentrations are likely to be nondetectable. In contrast, when cyclosporine half-life exceeds 12 hours, at least 50% of each dose will be retained, the drug will accumulate until steady-state is reached, and less fluctuation will occur over a dosing interval. Because the drug may accumulate in some patients, peak blood concentrations also may vary. Other factors, however, also impact peak cyclosporine concentrations. Typically, peak blood concentrations are between 600 and 1,200 ng/mL after a standard 5 mg/kg microemulsified oral dose. Trough concentrations are less commonly reported, but 1 study in dogs with anal furunculosis reported a trough concentration range of 205–1,370 ng/mL after 1 week of cyclosporine treatment (Neoral) at a dosage of 7.5 mg/kg every 12 hours.⁵⁶ Concentrations are highly susceptible to individual variability, and pharmacokinetic results should be interpreted in the context of the sample submitted (plasma or whole blood), assay used, and clinical response of the patient. Variability in the dose-blood concentration relationship can be seen in Table 2. When $C_{\max}$ or AUC are adjusted for dosage among the studies, the mean $\pm$ standard deviation of $C_{\max}$ /dose is 179 ± 56 versus 863 ± 768 AUC/dose. The greater variability of the latter is likely to reflect variability in clearance as well as parameters that influence $C_{\max}$.

Adverse Effects

Although many adverse effects are associated with cyclosporine treatment in dogs, most are uncommon, with the exception of adverse gastrointestinal effects. In 1 large placebo-controlled field study safety analysis⁵⁸ in which dogs were given either placebo or ultramicroemulsified cyclosporine at a mean dosage of 5 mg/kg/day (the approved atopy dosage), the most commonly observed adverse effects in the cyclosporine group were vomiting (31%), diarrhea (20%), persistent otitis externa (7%), urinary tract infections (4%), anorexia (3%), lethargy (2%), gingival hyperplasia (2%), and lymphadenopathy (2%), and the most common serum chemistry changes included increased serum creatinine concentration (8%), hyperglobulinemia (6%), hyperphosphatemia (5%), hyperproteinemia (3%), hypercholesterolemia (3%), hypoalbuminemia (2%), hypocalcemia (2%), and increased blood urea nitrogen concentration (2%), although only changes in creatinine, cholesterol, calcium, and blood urea nitrogen were statistically significant compared with baseline concentrations.

In other published studies, the most notable adverse effects of cyclosporine in dogs were gastrointestinal in nature, including diarrhea, vomiting, and anorexia.⁵⁹

Adverse gastrointestinal effects occur across a range of dosages, with increased frequency of occurrence seen at higher dosages. Less commonly reported dermatologic adverse effects include hirsutism, coat shedding, gingival hyperplasia, gingival eruption cysts in neonatal dogs, cutaneous papillomatosis, hyperkeratosis of footpads, psoriasiform-lichenoid-like dermatitis, hyperplastic verrucous lesions, and lymphoplasmaloid dermatitis.^{60–62} Clinicopathologic abnormalities seen in conjunction with cyclosporine treatment that were not reported in the previous safety analyses include lymphopenia, eosinopenia, anemia, and leukocytosis.⁵⁹ Adverse effects common to most other immunosuppressive agents, but not reported with cyclosporine, include myelosuppression and neutropenia. Concurrent infections documented in patients receiving cyclosporine treatment include bacterial infections of the respiratory and urinary tracts, pyelonephritis, pyometra, purulent pericarditis, septic arthritis, toxoplasmosis, neosporosis, demodicosis, and pyoderma.^{63–65} Malignancies, including lymphoma, have occurred in conjunction with concurrent use of cyclosporine.^{26,66} Other potential adverse reactions uncommonly reported in the literature include hepatotoxicity, defective hepatic protein synthesis, inhibition of insulin release, increase in insulin resistance, overt diabetes mellitus, lameness, lethargy, nephropathy, transient hypoalbuminemia, anaphylactic reaction, angioedema, tremors, emergence of neoplasia, and cystic nodules in the pericardium and diaphragm.^{16,66–69}

At the FDA Center for Veterinary Medicine Adverse Event Reporting site (<http://www.fda.gov/downloads/AnimalVeterinary/SafetyHealth/ProductSafetyInformation/UCM055404.pdf>), the most common adverse events reported in dogs are vomiting (3,108), diarrhea (1,369), lethargy (1,142), anorexia (834), pruritus (790), and increased serum alkaline phosphatase (429) or alanine aminotransferase (311) activities. Gingival hyperplasia was reported in 260 animals (accessed May 2013). The FDA database provides no information regarding number of doses sold, nor does the collection of the information verify a cause-effect relationship between dose and adverse event.

Precautions

The safety and efficacy of cyclosporine have not been evaluated in dogs <6 months of age or in dogs <4 pounds in weight, and the drug should therefore either not be used or be used cautiously in such patients. Cyclosporine is not for use in breeding, pregnant, or lactating dogs. Renal damage is a relatively common adverse effect in people during cyclosporine treatment. Nephrotoxicity has not been reported in dogs receiving therapeutic dosages of cyclosporine, but experimentally has been associated with very high blood drug concentrations (>3,000 ng/mL) over prolonged periods.¹⁶

Because of the dampening of the immune system associated with cyclosporine treatment, vaccine efficacy potentially may be impacted.⁷⁰ Vaccine approval studies have documented adequate antibody titer responses in

dogs receiving a killed rabies vaccine despite administration of 4 times the recommended cyclosporine atopy dosage. Although peer-reviewed published research studies have documented vaccine efficacy in humans receiving cyclosporine,^{71,72} no such studies are available in dogs, and clear recommendations therefore are not available. Some authors discourage the use of modified live vaccines in dogs receiving cyclosporine for fear of potential reactivation of the pathogen,⁷⁰ and only killed vaccines are recommended by the drug manufacturer.

Therapeutic Drug Monitoring

Assays

The established complexities of cyclosporine disposition in normal animals, coupled with confounding factors associated with disease and differences in drug preparation, may contribute to markedly variable blood concentrations of cyclosporine both among patients and even within the same patient. Therapeutic management therefore may be facilitated by monitoring blood cyclosporine concentrations. Although traditionally considered a method for avoiding drug toxicity, TDM offers advantages beyond simple avoidance of toxic concentrations. TDM typically is considered to be most effective if a therapeutic range is available that describes a relationship between blood drug concentration and clinical response, but TDM can be equally effective in establishing the therapeutic range for an individual patient. Once a therapeutic range is established for a given patient, subsequent monitoring is designed to maintain blood drug concentrations within that range.¹⁵

Recommendations regarding target immunomodulatory blood concentrations for cyclosporine originally were based on experimental organ transplantation in dogs.^{10,73} Initial recommendations centered on achieving a minimum target trough whole blood cyclosporine concentration of 500–600 ng/mL using a monoclonal radioimmunoassay (RIA), with blood collected just before the next oral dose.^{8,16} Unfortunately, however, the process of adjusting drug doses based on monitoring cyclosporine blood concentrations is clinically complex, and not necessarily associated with the desired clinical outcome (Table 3).^{11–14,24,29,56,58,59,63,74–83}

The method used to measure cyclosporine blood concentrations must be considered when interpreting results. Past methods for measuring cyclosporine blood concentrations included HPLC and assays dependent on antibodies to the drug, including fluorescence polarization immunoassay and antibody (both monoclonal and polyclonal) RIA methods. Currently available methods include HPLC as well as a specific monoclonal RIA. HPLC has the advantage that the parent drug can be discriminated from metabolites, although most methods detect only the parent compound. RIA, in contrast, measures metabolites as well as the parent drug, and blood cyclosporine concentrations therefore will be higher by a factor of 1.5–1.7 compared to the same sample analyzed using HPLC.²⁸ Although HPLC is

considered the gold standard for measuring cyclosporine blood concentrations, HPLC is labor-intensive and not routinely offered for patient monitoring. Furthermore, with HPLC, the method of separating, detecting, and quantitating the drug (ie, mass spectrophotometry versus other methods) can influence concentrations. Fluorescence polarization immunoassay and RIA have been the methods most often employed in clinical situations, with the laboratory performing the assay typically providing recommendations regarding ideal target blood drug concentrations. Some laboratories have adjusted target blood concentrations upward to reflect the fact that fluorescence polarization immunoassay and RIA results will be approximately double HPLC assay results. Other laboratories have not made this adjustment, with the rationale that the cyclosporine metabolites measured by the fluorescence polarization immunoassay and RIA assays may arguably be pharmacologically active and contribute to the overall immunosuppressive effects. However, if the goal of monitoring is to establish the individual patient's therapeutic range, variability in drug concentrations based on differences in methods is not important, provided the same method is used each time for the patient.

Sample type also influences interpretation of cyclosporine concentration. Plasma concentrations will be lower than whole blood concentrations attributable to the concentration of cyclosporine in erythrocytes and leukocytes. Although either whole blood or plasma cyclosporine concentrations can be measured, most laboratories recommend measuring whole-blood concentrations, and some assays are validated only for whole blood.

The timing of sample collection also affects results. The short half-life of cyclosporine precludes predicting concentrations throughout a dosing interval based on a single sample. Peak concentrations are often 2- to 8-fold higher than trough concentrations in normal animals. Much study has been applied to determining the most appropriate sample collection time for TDM in patients receiving cyclosporine. In human medicine, trough blood concentrations were the initial basis for adjustment of drug dosages. However, many studies in people have since suggested that AUC or 2-hour peak drug concentrations are preferred. Although nephrotoxicity and hepatotoxicity can be predicted based on trough cyclosporine concentrations, trough concentrations have been shown to inadequately predict immunosuppression in human transplant patients.⁵ Dose adjustment based on the AUC over hours 0–12 in the dosing interval provides a much more reliable indicator of clinical immunosuppression in people, but the need to collect many blood samples adds to expense as well as patient discomfort and inconvenience. Because absorption of ultramicrocrystallized cyclosporine is virtually complete during the first 4 hours after dosing, the AUC for hours 0–4 was investigated as a simpler alternative to the AUC for hours 0–12. A close correlation was shown between the 2 methods, with fewer samples needed to calculate the AUC for 0–4 hours. Additional work in human medicine identified that the peak

Table 3. Studies in dogs in which efficacy was established for select diseases.

Investigation	Disease Investigated	Number of Dogs	Cyclosporine Preparation	Cyclosporine Dosage	Cyclosporine Concentrations	Method of Measurement	Treatment Duration	Efficacy	Reference
1	Atopy	130 (Phase 1)	Atopica	5 mg/kg q24h	N/A	N/A	4 weeks	45% had a reduction in CADESI score by >50% of baseline	Steffan et al ⁵⁸
		186 (Phase 2)					16 weeks	68% had a reduction in CADESI score by >50% of baseline	
2	Atopy	117	Atopica	5 mg/kg q24h	N/A	N/A	4 months	76% responded with excellent or good response to treatment	Steffan et al ⁷⁴
3	Atopy	30	Neoral	2.5 mg/kg q24h	N/A	N/A	6 weeks	47% had a reduction in CADESI score by >50% of baseline	Olivry et al ⁵⁹
		31		5 mg/kg q24h			6 weeks	71% had a reduction in CADESI score by >50% of baseline	
4	Atopy	14	Neoral	5 mg/kg q24h	N/A	N/A	2 weeks	93% had a marked reduction in total clinical score as compared with baseline	Fontaine and Olivry ⁷⁵
5	Atopy	15	Neoral	5 mg/kg q24h	N/A	N/A	6 weeks	69% had a reduction in CADESI score by >50% of baseline	Olivry et al ⁵⁹
6	Atopy	51	Neoral	5 mg/kg q24h	N/A	N/A	6–30 months* follow-up	78% obtained well-controlled clinical signs	Radowicz and Power ⁶³
7	Atopy	25	Atopica	5 mg/kg q24h	N/A	N/A	6 months	100% had a reduction in CADESI score of at least by >50% of baseline	Thelen et al ²⁹
8	Canine Pemphigus foliaceus	5	Neoral	5–10 mg/kg q24h	N/A	N/A	3 months	0% had a significant improvement in clinical signs	Olivry et al ¹²
9	Sebacaceous adenitis	12	Neoral	5 mg/kg q24h	N/A	N/A	12 months	83% demonstrated a significant reduction in clinical scores	Linek et al ⁷⁷
10	Perianal fistula	10	Sandimmune	7.5–10 mg/kg q12h	Adjusted to maintain trough of 400–600 ng/mL	Monoclonal RIA	20 weeks	100% of dogs showed resolution of fistulas	Mathews and Sukhiani ¹³
11	Perianal fistula	10	Sandimmune	5 mg/kg q12h	Adjusted to maintain trough of 400–600 ng/mL	Monoclonal RIA	16 weeks	100% of dogs showed complete resolution of fistulas or substantial reduction in size	Mathews and Sukhiani ¹³
12	Perianal fistula	6	Neoral	7.5 mg/kg q12h	Adjusted to maintain trough of 400–600 ng/mL Initial trough ranged from 205 to 1,370 ng/mL after 7 days	Emit assay	10–20 weeks	100% of dogs demonstrated marked improvement	Griffiths et al ⁵⁶
13	Perianal fistula	16	Neoral			HPLC	16 weeks		Mouatt ⁷⁸

Table 3 (Continued)

Investigation	Disease Investigated	Number of Dogs	Cyclosporine Preparation	Cyclosporine Dosage	Cyclosporine Concentrations	Method of Measurement	Treatment Duration	Efficacy	Reference
14	Perianal fistula	12	Neoral	Cyclosporine 1 mg/kg q12h plus ketoconazole (10 mg/kg q24h) Cyclosporine 2.5 mg/kg q12h or 4 mg/kg q24h plus ketoconazole (8 mg/kg q24h)	Adjusted to maintain trough >200 ng/mL Adjusted to maintain trough of 400–600 ng/mL Initial trough ranged from 130 to 1,398 ng/mL after 5 days Adjusted to maintain trough of 400–600 ng/mL	HPLC	4–39 weeks	100% of dogs showed marked improvement of lesions within 14 days of treatment 100% of dogs showed resolution of clinical signs by 9 weeks of treatment	Patricelli et al ²⁴
15	Perianal fistula	3	Neoral	Cyclosporine (0.5 mg/kg q12h) + Ketoconazole (7.5 mg/kg q12h) Cyclosporine (0.75 mg/kg q12h) + Ketoconazole (7.5 mg/kg q12h) Cyclosporine (1 mg/kg q12h) + Ketoconazole (7.5 mg/kg q12h) Cyclosporine (2 mg/kg q12h) + Ketoconazole (7.5 mg/kg q12h)		RIA	3–10 weeks	100% of visible lesions completely resolved	O'Neill et al ⁷⁹
16	Perianal fistula	10	Neoral	5 mg/kg q24h 4 mg/kg q12h	Mean trough after 4 weeks 39 ng/mL (25–77) Mean trough after 4 weeks 122 ng/mL (47–237) N/A	RIA	8 weeks	10% of dogs experienced resolution of external lesions 60% of dogs experienced resolution of external lesions 96% of dogs experienced complete resolution or improvement	House et al ⁸⁰
17	Perianal fistula	26	Neoral	Cyclosporine (1.5 mg/kg q24h) Cyclosporine (3 mg/kg q24h) Cyclosporine (5 mg/kg q24h)	Concentrations in all dogs ranged from 0 to 330 ng/mL	N/A	4–24 weeks	50% demonstrated improvement with resolution of CS 66% demonstrated improvement with resolution of CS 66% demonstrated improvement with resolution of CS	Hardie et al ⁸¹
18	Perianal fistula	6	Neoral			Emit assay	13 weeks		Doust et al ⁸²

Table 3 (Continued)

Investigation	Disease Investigated	Number of Dogs	Cyclosporine Preparation	Cyclosporine Dosage	Cyclosporine Concentrations	Method of Measurement	Treatment Duration	Efficacy	Reference
		6		Cyclosporine (7.5 mg/kg q24h)				100% demonstrated improvement with resolution of CS	
19	Granulomatous Meningoencephalitis	3	Atopica	Initial cyclosporine dosage of 10 mg/kg q24h	N/A	N/A	6 weeks	Complete resolution of clinical signs in all 3 dogs	Gnirs ¹⁴
20	Granulomatous Meningoencephalitis	3	Not revealed in the study	Initial cyclosporine dosage of 3 mg/kg q12h, adjusted upward in all dogs	Dog 1 at 6 mg/kg q12h dosage: trough of 235 ng/mL Dog 2 at 3 mg/kg q12h dosage: trough of 82 ng/mL Dog 3 at 3 mg/kg q12h dosage: trough of 34 ng/mL	N/A	Up to 12 months	Considered effective in 2 of 3 dogs	Adamo and O'Brien ¹⁵
21	Inflammatory Bowel Disease	14	Atopica	5 mg/kg q24h	117 ng/mL Mean trough concentration 34 ± 26 ng/mL Mean peak concentration 699 ± 326 ng/mL	FPIA	10 weeks	79% demonstrated a significant reduction in clinical score	Allenspach et al ¹¹

CADESI, canine atopic dermatitis extent and severity index; RIA, radioimmunoassay; HPLC, high-performance (pressure) liquid chromatography; CS, clinical signs; FPIA, fluorescence polarized immunoassay.

cyclosporine concentration, or the concentration in blood 2 hours postdosing, closely approximated patient outcome and 0–4 hours AUC results, and confirmed that trough concentrations correlated poorly with AUC calculations. With measurement of peak cyclosporine concentrations requiring only a single sample, adjusting drug dosages to attain target peak drug concentrations has become the single best blood concentration measurement for use during human organ transplantation. These findings apply to ultramicronized cyclosporine such as Neoral, as a 2-hour blood sample reflects peak concentrations in virtually all patients. In contrast, these findings cannot be applied to the Sandimmune formulation, because the peak blood concentration varies between 2 and 6 hours.

In veterinary medicine, measurement of trough cyclosporine concentrations prevailed for many years based on initial work carried out in renal transplant studies in dogs. For twice-daily cyclosporine dosing, trough samples are taken 12 hours postdosing, whereas, for once-daily dosing, trough samples are taken 24 hours after dosing. More recent recommendations from laboratories offering TDM in dogs typically have involved measurement of both peak and trough cyclosporine blood concentrations, although target peak concentrations have not been well established. Individual laboratory recommendations depend on the target ranges determined by each laboratory as well as the assay used to measure cyclosporine concentrations. At the Auburn University TDM laboratory, for example, typical recommended therapeutic ranges for cyclosporine concentrations based on a monoclonal immunoassay are for renal transplantation, a trough concentration of 750 ng/mL for the first 3 months and then 350–400 ng/mL; for chronic inflammatory diseases, such as inflammatory bowel disease, a trough concentration of 250 ng/mL; and, for anal furunculosis, a trough concentration of 100–600 ng/mL (Boothe, unpublished observations, 2012). For canine atopic dermatitis, Steffen et al failed to find a significant difference among atopic scores at different cyclosporine concentrations, indicating a lack of relationship between drug concentration and response, and suggesting that there was no need for drug monitoring in dogs with atopy.²⁸ However, as with other previous and comparable cyclosporine studies that failed to find significant differences using relatively small animal numbers, the risk of a type II error (failure to find a significant difference when one truly exists) was not evaluated. TDM is probably best approached as a method for determining individual patient responses rather than sample population responses and, given the complexity of cyclosporine pharmacokinetics, dose adjustments in individual patients should be made in consultation with the laboratory providing TDM.

Pharmacodynamic Assays

Pharmacokinetic measurements can provide evidence that cyclosporine blood concentrations are within the estimated target therapeutic range recommended by

reference laboratories. This therapeutic range is established to capture the majority of patients expected to respond for a given condition. For some patients, this estimation may accurately predict immunosuppression, whereas, for others, clinical efficacy may not occur despite achievement of blood cyclosporine concentrations in the target therapeutic range. This variability in response to comparable blood concentrations may be attributed to differences in individual patient pharmacologic responses to cyclosporine, specifically within target cells, such as T-lymphocytes. Wide variability in the relationship between clinical efficacy and therapeutic drug concentrations also is seen in human patients.⁸⁴ For this reason, several pharmacodynamic assays have been developed in human medicine in an attempt to better estimate the dosage of cyclosporine needed to maintain immunosuppression and prevent organ rejection in individual transplant patients while minimizing the expense and adverse effects associated with excessive drug dosages.

Pharmacodynamic assays investigate a drug's effect on target cells. Several pharmacodynamic biomarkers of the immunosuppressive effects of cyclosporine have been studied in human medicine, including lymphocyte proliferation, enzyme (calcineurin) activity, lymphocyte surface antigen expression, and intracellular cytokine quantitation.⁸⁵ Through pharmacodynamic monitoring, studies in humans have shown individually distinct degrees of calcineurin inhibitor sensitivity in patients. Selected immunologic biomarkers of immunosuppression have been validated in human medicine, suggesting their suitability for pharmacodynamic monitoring in clinical trials of human patients.⁸⁶ Pharmacodynamic monitoring shows great promise for optimizing cyclosporine treatment and delivering individualized treatment.

Few pharmacodynamic studies assessing the immunologic effects of cyclosporine are found in the veterinary literature. One study demonstrated suppression of lymphocyte proliferation by flow cytometry after use of topical cyclosporine for treatment of canine keratoconjunctivitis sicca,⁸⁷ but the findings were later refuted by another study that documented minimal cyclosporine blood concentrations and no effects on mitogen stimulation of peripheral blood lymphocytes after treatment with topical cyclosporine.⁸⁸ A separate study did, however, confirm local suppression of inflammatory markers in the conjunctival epithelium after treatment with topical cyclosporine.⁸⁹ In more recent veterinary studies utilizing cytokine analysis, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assays have been used to measure canine cellular messenger ribonucleic acid (mRNA) expression. One study investigated the effects of cyclosporine on activated canine mononuclear cells in vitro, and demonstrated a concentration-dependent decrease in IL-2, IL-4, and IFN- γ mRNA expression by qRT-PCR.⁹⁰ Another study evaluated cellular cytokine mRNA expression within lesional biopsies from dogs with anal furunculosis treated with oral cyclosporine, and demonstrated a significant reduction in IL-2

mRNA expression and a lesser decrease in IFN- γ expression with cyclosporine treatment.⁹¹

Recent pharmacodynamic studies in our laboratory have investigated ultramicronized cyclosporine (Atopica) in normal dogs. An initial in vitro investigation demonstrated cyclosporine-mediated suppression of T-lymphocyte activation-related molecules and cytokines in normal dogs.⁹² Peripheral blood mononuclear cells were isolated and activated, with half of the cells incubated while exposed to cyclosporine, and the other half not exposed to the drug. Cells then were analyzed using flow cytometry, with T-cell expression of the intracellular cytokines IL-2, IL-4, and IFN- γ evaluated after drug exposure. All cytokines demonstrated a time-dependent suppression profile. The T-cell surface molecules CD25 and CD95, which have roles in T-cell activation and development, were evaluated after drug exposure, and there was also significant suppression of expression of both biomarkers in the presence of cyclosporine. In a subsequent in vivo study, activated T-cell expression of IL-2, IL-4, and IFN- γ was investigated by flow cytometry when dogs were treated with 2 different oral cyclosporine dosages.⁹³ The dogs first were given a high dosage of cyclosporine (10 mg/kg PO q12h), with doses adjusted upward as needed to attain a target trough drug concentration >600 ng/mL as measured via HPLC, a dosing protocol known to be sufficiently immunosuppressive for organ transplantation in dogs. With high-dose cyclosporine, activated T-cell expression of IL-2 and IFN- γ was significantly suppressed, but IL-4 was not similarly affected. The dogs then were given the FDA-approved dosage of cyclosporine used to treat canine atopy (5 mg/kg PO q24h), a dosage that has been considered to be low enough to avoid predisposing to immunosuppression-associated infection.²⁷ Even with this low dosage of cyclosporine, however, T-cell expression of IFN- γ was still significantly suppressed. Mean T-cell expression of IL-2 also was decreased and, although the degree of suppression in the group of dogs as a whole was not statistically significant, expression of IL-2 in several individual dogs was markedly decreased.

Specific Disease Considerations

Atopic Dermatitis

Atopic dermatitis is a common pruritic dermatologic problem afflicting dogs, and is associated with IgE antibodies targeting environmental allergens.⁹⁴ In the United States, Atopica is FDA-approved for the treatment of atopy and, in fact, atopy is the only condition in dogs for which the systemic use of cyclosporine has been approved by the FDA. Many studies have focused on the sole use of cyclosporine for treatment of canine atopy. In an initial pilot study, 14 atopic dogs were treated with ultramicronized cyclosporine at a dosage of 5 mg/kg PO q24h for 2 weeks, and only 1 dog failed to respond to treatment.⁷⁵ In a subsequent larger scale randomized controlled trial, atopic dogs were treated with placebo or ultramicronized cyclosporine at either

2.5 or 5 mg/kg PO q24h for 6 weeks, and the dogs given the higher dosage of cyclosporine were shown to have the most marked reductions in skin lesions and pruritus scores.⁵⁹ Similar results have been seen in other studies investigating cyclosporine's utility in the treatment of atopic dermatitis.^{28,74,76,95} Most studies in atopic dogs have been performed using the proprietary forms of ultramicronized cyclosporine, Atopica or Neoral. A recent study investigating the ability of a generic ultramicronized cyclosporine preparation for human use to treat canine atopy, however, demonstrated that the generic product also was effective in decreasing the severity of clinical signs. Thirteen atopic dogs received generic cyclosporine (Equoral) at a dosage of 5 mg/kg PO q24h, and outcome was compared to outcome in 7 atopic dogs treated with prednisone. Both groups showed significant reduction in clinical signs, and there was no difference between the treatment groups.⁹⁶ Interestingly, response to cyclosporine treatment does not appear to be related to blood drug concentrations, perhaps because the drug is known to accumulate in the skin. In 1 study in atopic dogs, trough blood cyclosporine concentrations were measured in 97 patients receiving the FDA-approved dosage of approximately 5 mg/kg of cyclosporine (Atopica) PO q24h.²⁸ Although reduction in lesion scores approximated that of previous studies, no significant correlation was found between clinical improvement and blood drug concentrations. TDM of cyclosporine in dogs being treated for atopy therefore is not typically recommended initially in the course of treatment, but still may be useful in dogs that fail to respond to standard treatment with cyclosporine.

Sebaceous Adenitis

Sebaceous adenitis is an uncommon skin disease of dogs characterized by early perifollicular infiltration with inflammatory cells and later inflammation around sebaceous glands. Cyclosporine was specifically investigated in 1 study for its efficacy in treating sebaceous adenitis.⁷⁷ Twelve dogs diagnosed with sebaceous adenitis were treated with ultramicronized cyclosporine at a dosage of 5 mg/kg PO q24h for 12 months, with re-evaluations performed every 4 months. Mean clinical score was significantly decreased at all re-evaluation points compared with pre treatment assessment, and posttreatment biopsy results identified a significant decrease in inflammation compared with initial biopsies.

Pemphigus Foliaceus

Pemphigus foliaceus is a relatively common canine autoimmune skin disease characterized by scaling, crusts, pustules, alopecia, and erosions, which may remain localized or become generalized.⁹⁷ In a pilot study evaluating cyclosporine as sole treatment for treatment of pemphigus foliaceus, 5 affected dogs were given ultramicronized cyclosporine at a dosage of 5 mg/kg PO q24h for up to 3 months.¹² Complete remission of pemphigus foliaceus was not attained in

any dog, and 4 of 5 dogs had to be withdrawn from the study because of worsening signs and lack of response to cyclosporine monotherapy.

Anal Furunculosis

Anal furunculosis (or perianal fistula) is a chronic inflammatory and ulcerative disease in dogs causing focal to multifocal ulcerative tracts within perianal, anal, or perirectal tissues.⁴⁶ Oral cyclosporine has been shown in several studies to be effective in the treatment of anal furunculosis.⁴⁶ Oral cyclosporine typically is administered at a dosage between 4 and 8 mg/kg PO q24h or q12h.⁴⁶ In an initial randomized controlled trial evaluating cyclosporine for treatment of anal furunculosis, 10 dogs received cyclosporine (Sandimmune) at a dosage of 5 mg/kg PO q12h while another 10 dogs received placebo.¹³ After 4 weeks of treatment, all dogs treated with cyclosporine had shown improvement, whereas no dogs treated with placebo had improved. All affected dogs then received cyclosporine treatment longer term. After 16 weeks of treatment with cyclosporine, 85% of treated dogs showed complete resolution of disease, with the remaining dogs showing detectable improvement. Disease recurrence occurred in 41% of dogs after treatment was discontinued. Other studies have shown similar results, with a majority of dogs treated with ultramicronized cyclosporine demonstrating either complete resolution of disease or at least substantial improvement.^{24,56,79,82} Because a strong correlation between cyclosporine blood concentrations and clinical efficacy has not been recognized in dogs being treated for anal furunculosis,^{24,56,79,82} measurement of blood cyclosporine concentrations is not recommended during initial treatment.⁴⁶ Instead, treatment should be titrated based on resolution of clinical signs, with TDM reserved for those patients that fail to respond to standard treatment.⁴⁶

Cyclosporine also has been combined with ketoconazole to achieve control of anal furunculosis, with substantial reductions in the cost of cyclosporine treatment. One study combined once-daily ketoconazole (5.1–11 mg/kg) with once (2.5–5.5 mg/kg)- or twice (1.9–3.5 mg/kg)-daily ultramicronized cyclosporine, and found resolution of clinical signs in all dogs within 9 weeks, with a cost savings of 36–71% over cyclosporine monotherapy. This study targeted trough blood concentrations of 400–600 ng/mL using HPLC.²⁴ A similar study found remission in 93% of dogs treated for 16 weeks with ultramicronized cyclosporine (1 mg/kg PO q12h, initial dose) and ketoconazole (10 mg/kg PO q24h), with a target trough concentration of 200 ng/mL as measured by HPLC.⁷⁸ A third study assessing varying ultramicronized cyclosporine dosages (0.5, 0.75, 1, and 2 mg/kg PO q12h) with ketoconazole (5.3–8.9 mg/kg PO q12h) and adjusting dosage to achieve trough concentrations of at least 400–600 ng/mL by RIA reported resolution of clinical signs in 3–10 weeks for all dogs.⁷⁹ TDM is recommended when combining cyclosporine with

ketoconazole to ensure that therapeutic concentrations are being attained, and to detect potentially toxic drug concentrations. The Auburn TDM laboratory has documented a change in cyclosporine half-life from <10 to >150 hours in a dog treated simultaneously with ketoconazole and cyclosporine. On the other hand, elimination half-lives of <15 hours also have been documented in dogs receiving both drugs, indicating marked variability in response to the effects of ketoconazole. Using ketoconazole and cyclosporine concurrently is an effective method for treating anal furunculosis in large dogs, and should be considered when cost is an issue. When using this approach, appropriate monitoring for ketoconazole hepatotoxicity should be performed. The use of cyclosporine in veterinary dermatology recently was reviewed by Kovalik et al, with an emphasis on the treatment of dermatologic conditions in dogs.⁹⁸

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is recognized as one of the most common causes of chronic gastrointestinal disease in dogs, with clinical signs typically including vomiting, diarrhea, and weight loss.⁹⁹ Cyclosporine was shown to be effective as a sole drug treatment in some dogs with IBD. In a study of 14 dogs diagnosed with IBD that were refractory to glucocorticoid treatment, response to oral cyclosporine monotherapy was investigated.¹¹ All dogs were treated with ultramicronized cyclosporine at a dosage of 5 mg/kg PO q24h for 10 weeks. Clinical signs and extent of infiltration of inflammatory cells into duodenal tissues were evaluated over the treatment period. Improvement in clinical signs was observed in 12 of 14 dogs, and significant decreases in T-cell infiltration within duodenal biopsy specimens was appreciated when comparing posttreatment biopsies with pretreatment biopsies. A correlation between cyclosporine blood concentrations and resolution of clinical signs was not identified. Routine monitoring of cyclosporine concentrations in dogs being treated for IBD therefore is not recommended, except in those patients that fail to respond to standard treatment.

Myasthenia Gravis

Myasthenia gravis is a neuromuscular disorder characterized by inefficient synaptic transmission secondary to acetylcholine receptor dysfunction on the postsynaptic muscle membrane. Acquired myasthenia gravis is an immune-mediated process characterized by antibody production targeting the nicotinic acetylcholine receptors found within skeletal muscle neuromuscular junctions. Initial treatment of myasthenia gravis often involves anticholinesterase medications. If a positive response is not observed, immunosuppressive medications are considered as additional treatment options. One case report described the use of cyclosporine in 2 dogs diagnosed with acquired myasthenia gravis that initially did not respond to pyridostigmine bromide and

glucocorticoid treatment.¹⁰⁰ Ultramicronized cyclosporine at a dosage of 4 mg/kg PO q12h was initiated in both dogs, and both demonstrated improvement in clinical signs after treatment with cyclosporine.

Meningoencephalomyelitis of Unknown Etiology

Meningoencephalomyelitis of unknown etiology refers to an inflammatory condition that affects the central nervous system of dogs without a documented cause.¹⁰¹ Diseases characterized as meningoencephalomyelitis of unknown etiology include granulomatous meningoencephalitis (GME), necrotizing meningoencephalitis, and necrotizing leukoencephalitis.^{101–103} Treatment for these diseases includes aggressive immunosuppression, with cyclosporine being 1 drug used for this purpose. In an initial study evaluating treatment of GME in 3 dogs with cyclosporine, all dogs achieved complete resolution of clinical signs.¹⁴ Ultramicronized cyclosporine was administered as sole treatment at an initial dosage of 10 mg/kg PO q24h after a variable period of glucocorticoid administration.^{14,83} In a larger study evaluating 10 dogs diagnosed with meningoencephalomyelitis of unknown etiology, all dogs were treated with cyclosporine alone or in combination with steroids, ketoconazole, or both. The cyclosporine dosage was adjusted to maintain a trough blood concentration range of 200–400 ng/mL by HPLC.¹⁰⁴ All dogs had either a partial or complete initial response to treatment. In all dogs, marked improvement in inflammation was demonstrated by serial cerebrospinal fluid analyses.

Transplantation

Cyclosporine traditionally has been considered the cornerstone of immunosuppressive treatment for the prevention of organ rejection in canine transplantation medicine, although transplant procedures in dogs are not currently readily available.^{37,105} In canine transplantation, cyclosporine was administered twice daily, with blood concentrations measured and dosages adjusted as needed to meet target concentrations. Cyclosporine treatment then was continued throughout the lifetime of the recipient. In 1 recent study, 15 dogs underwent allogeneic renal transplantation and were given immunosuppressive treatment consisting of ultramicronized cyclosporine (Neoral; 10 mg/kg PO q12h), azathioprine (2–3 mg/kg PO q48h), and prednisolone (0.5 mg/kg PO q12h initially and then tapered).¹⁰⁵ Cyclosporine dosages were adjusted based on whole blood trough concentrations to maintain a target concentration of 400–500 ng/mL as measured by HPLC for the first 6 months after surgery, and then between 350 and 450 ng/mL thereafter. Nine dogs died within 1 month of surgery, 3 dogs survived between 6 and 25 months, and 3 dogs were alive at the time of publication and had survived between 22 and 48 months postsurgery. Although triple-drug immunosuppressive treatment prevented acute organ rejection in some dogs, there was a high frequency of drug-related infection, and the

authors felt that the dogs were overly immunosuppressed. The authors also stated that there was a “clear need for a pharmacodynamic assay that might achieve a balance between development of allograft rejection and infection,” as is the case in human transplantation medicine. Other older studies investigating cyclosporine treatment alone or in combination with other medications during organ transplantation in dogs have shown similar results, in that prevention of acute organ rejection is achieved in some dogs, but with a high prevalence of infection developing during treatment.^{9,10,106–109}

Other Diseases Associated with Cyclosporine Usage

Clinicians have considered using cyclosporine as part of the treatment for a wide variety of diseases that have the potential for responding to immunosuppressive treatment. Unfortunately, for most diseases, an insufficient number of cases have been published to allow objective assessment of the response to cyclosporine treatment. Diseases described in the literature for which oral cyclosporine has been used as a component of treatment include immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, and immune-mediated polyarthritis.^{110–114}

Overview of Clinical Use of Cyclosporine

Recent pharmacodynamic research evaluating T-cell responses to cyclosporine in dogs has confirmed that responses in dogs are comparable to the response profile that is well recognized in people. Individual responses to cyclosporine are extremely variable from dog to dog, both in dogs receiving the same standard oral dosage and in dogs receiving oral dosages adjusted to attain comparable blood concentrations of cyclosporine. Given that a high degree of variability in individual responsiveness to cyclosporine has been established in dogs, cyclosporine dosing protocols should be tailored to allow for this patient-to-patient variability. In our opinion, recommended dosing protocols in dogs with chronic, nonlife-threatening inflammatory skin and gastrointestinal diseases should be quite different from protocols used in dogs with more acute and life-threatening immune-mediated diseases.

In chronic inflammatory diseases that are not immediately life-threatening (eg, atopic dermatitis, anal furunculosis, mild IBD), cyclosporine often is effective at a standard, relatively low starting dosage. Although most clinical studies of these diseases have used the proprietary forms of ultramicronized cyclosporine, limited evidence indicates that generic preparations for human use may be equally effective, at least for the treatment of atopy. Generic preparations for human use probably should be considered only in nonlife-threatening diseases in which sufficient time to titrate to effect is available. Cyclosporine treatment typically is delivered long-term, with drug dosages adjusted upward if needed to effect, based predominantly on clinical signs. Most commonly, however, starting doses

do not need to be increased and, in the long-term, the cyclosporine dosage typically is tapered to the lowest effective dosage needed to maintain disease remission. Cyclosporine blood concentrations are usually not necessary in these circumstances, and remission of disease is the main criterion used to decide whether adequate cyclosporine treatment is being delivered. In fact, for many of these conditions, cyclosporine blood concentrations have been shown to have minimal correlation with disease remission, perhaps because the drug is selectively concentrated in tissues, such as the skin. TDM should be reserved for patients who fail to respond to standard treatment. Recent pharmacodynamic studies have shown that, even at standard low FDA-approved dosages, some dogs can still develop clinically relevant suppression of certain T-lymphocyte biomarkers of immunosuppression despite very low trough cyclosporine concentrations.⁹³ Specifically, IFN- γ expression can be substantially suppressed after treatment with low cyclosporine dosages, as compared with pretreatment levels of expression. This could explain the phenomenon anecdotally reported by some clinicians that individual dogs treated for atopic dermatitis can develop severe secondary infections (personal communication, Dr Amy Grooters, Louisiana State University, and Dr Juli Gunter, Mississippi State University), although the cyclosporine dosage originally chosen was not thought to cause clinically relevant immunosuppression. Therefore, even in dogs on low cyclosporine dosages, clinicians should remain vigilant for potential signs of systemic infection.

In dogs suffering from more acute and life-threatening diseases, such as severe immune-mediated hemolytic anemia and immune-mediated thrombocytopenia, however, cyclosporine treatment must be targeted to attain effective immunosuppression as rapidly as possible. These animals are somewhat comparable to patients that have recently undergone organ transplantation, in that any delay in attaining effective immunosuppression can lead to a disastrous outcome. In these patients, starting cyclosporine at a low dosage and adjusting the dosage upward to effect is not recommended. Attaining effective oral doses as rapidly and accurately as possible is essential for ensuring adequate immunosuppression while avoiding overdosage with associated adverse effects and expense. Measurement of blood cyclosporine concentrations is the best method currently available to assess adequacy of treatment, and is strongly recommended in patients with life-threatening immune-mediated diseases.

Recent pharmacodynamic studies have shown that attainment of a trough blood cyclosporine concentration of 600 ng/mL (as measured by HPLC) reliably causes substantial immune system suppression in most dogs. However, because individual dogs exhibit impaired T-cell function at much lower blood concentrations, this target blood concentration may reflect an unnecessarily high dosage for some dogs. Although, based on the human model, measurement of peak cyclosporine concentrations probably would enable far more accurate adjustment of treatment than would

measurement of trough concentrations, to date, ideal target peak concentrations in dogs have not been well established. Continued assessment of peak cyclosporine blood concentrations is recommended (in addition to traditionally measured trough concentrations) to identify target ranges for various conditions in veterinary medicine. In human medicine, patients receiving cyclosporine often have pharmacodynamic evaluation of T-cell function performed, in conjunction with the measurement of blood cyclosporine concentrations, to enable individualized adjustment of treatment for the patient. In veterinary medicine, it is likely that both measurement of blood drug concentrations and evaluation of T-cell function also will be needed before we can truly deliver individualized medicine and appropriately adjust treatment for dogs with serious diseases being treated with cyclosporine at immunosuppressive dosages.

The most common adverse effects associated with initial cyclosporine usage, especially at higher dosages, are gastrointestinal in nature, including vomiting, diarrhea, and inappetence. In a dog with a life-threatening disease and adverse gastrointestinal effects that needs ongoing cyclosporine treatment, options include administering the medication frozen (which appears to decrease nausea in some dogs) with food or both, or decreasing drug dosage. However, if cyclosporine is administered frozen or with food, it may alter drug absorption and change the amount of drug reaching the systemic circulation, thereby potentially jeopardizing the efficacy of treatment. Decreasing the cyclosporine dosage raises the same concern. Adjusting drug dosages or administration protocols to attain precisely targeted peak blood concentrations or indices of T-cell suppression would enable clinicians to minimize adverse effects by precisely administering only enough drug to cause the desired extent of immunosuppression in the individual patient.

Cyclosporine is an expensive drug, particularly at higher immunosuppressive dosages, and clinicians therefore are tempted to explore cheaper forms of the drug. In human medicine, there are many approved generic microemulsion preparations similar to the Neoral formulation, and these generic preparations have been shown to have therapeutic equivalency in people. Studies investigating the pharmacokinetic properties of these generic preparations in dogs have not been performed and, for many diseases of dogs, it is not safe to assume that a generic formulation is therapeutically equivalent to the approved product for dogs (Atopica). In our experience, there appears to be marked variability in individual dogs in the oral bioavailability of these generic products. Use of generic products can therefore place our patients at risk of either therapeutic failure or toxicity. In our opinion, the preferred cyclosporine formulation for use in dogs remains the FDA-approved veterinary product, for which extensive testing in the appropriate species has been conducted, although generic formulations may be a viable option in countries where the proprietary product is not available. Finally, the authors recommend

that compounded cyclosporine not be prescribed unless no alternative preparations exist that meet the needs of the patient.

Conclusions

Cyclosporine is a powerful immunosuppressive agent used to treat a variety of diseases in dogs. Drug absorption and clinical efficacy can vary markedly among patients. In mild diseases that are not life-threatening (eg, atopy), cyclosporine usually is administered at recommended dosages with dose adjustments based on individual patient response to treatment. In more life-threatening immune-mediated diseases, cyclosporine often is administered at higher dosages, with TDM used to ensure that adequate blood concentrations have been achieved. Trough blood drug concentrations traditionally have been used for veterinary drug monitoring, but peak concentrations may be preferable. Even with meticulous TDM, some dogs may not respond to treatment despite attainment of target blood cyclosporine concentrations. Pharmacodynamic monitoring is being investigated as an adjunctive strategy to further assess the immune system in dogs receiving cyclosporine. TDM in combination with pharmacodynamic assessment may offer a better overall evaluation of the immune response to cyclosporine treatment, providing clinically useful information for dose adjustments and allowing for better delivery of individualized medicine.

Footnote

^a Code of Federal Regulations that delineate the Animal Medicinal Drug Use Act (CFR 21)

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