

## Cyclosporine

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Cyclosporine is a potent immunosuppressive drug indicated for the treatment of inflammatory and immune-mediated diseases, and for organ transplantation. Cyclosporins are cyclic polypeptide macrolides originally derived from the soil fungus *Beauveria nivea* (*Tolypocladium inflatum*), but are also produced by other fungal organisms. Cyclosporine A is the molecule developed for commercial use as an immunosuppressive agent. Discovered in the 1970s, the use of cyclosporine as an immunosuppressive agent was first described in humans to prevent rejection of renal allografts. Within a decade, cyclosporine had become the cornerstone of immunosuppression for organ transplantation. In veterinary medicine, oral cyclosporine capsules received FDA approval in 2003 for the treatment of canine atopy, and were more recently also approved for allergic skin disease in cats. Cyclosporine has been used in an extra-label fashion for many years for renal transplantation in dogs and cats, and for the treatment of a variety of inflammatory and immune-mediated conditions.

Cyclosporine's primary immunosuppressive mechanism of action is inhibition of T lymphocyte function. Cyclosporine acts to inhibit calcineurin, an intracellular protein phosphatase that activates gene transcription factors through dephosphorylation. In the untreated patient, activation of T cells results in activation of calcineurin, which dephosphorylates inactive nuclear factor (NFAT). NFAT translocates into the nucleus, where it upregulates transcription of genes coding for several important cytokines, including IL-2, IL-4, TNF- $\alpha$ , and INF- $\gamma$ . Production of IL-2 in particular plays a key role in the activation and proliferation of T cells. Calcineurin inhibitors, including cyclosporine, act by binding to intracellular cyclophilins, which are proteins that facilitate protein folding. Binding of cyclosporine to cyclophilin A creates a complex with high affinity for calcineurin. Through inhibition of calcineurin, cyclosporine specifically inhibits T cell function and thus, cell-mediated immunity, but has little immediate impact on humoral immunity. Decreased IL-2 expression in CD4+ Th1 cells associated with cyclosporine therapy leads to inhibition of proliferation and activation of both T-helper and T-cytotoxic lymphocytes, and blunting of the immune response. Cyclosporine has also been shown to have many other local anti-inflammatory and immunosuppressive effects, especially in the skin.

Cyclosporine is a large lipophilic molecule which must be solubilized prior to intestinal absorption. Commercial cyclosporine is available as two very different types of oral formulations. Cyclosporine was initially approved in humans as a vegetable-oil based preparation (Sandimmune®), but variability in oral bioavailability caused marked variability in blood drug concentrations. A more recent formulation, an ultramicroemulsified ("modified") preparation approved in 1996 (Neoral®), forms a microemulsion upon contact with aqueous fluids, resulting in more consistent and predictable absorption. Oral bioavailability of the microemulsion is improved by up to 50% compared to the oil-based preparation. Because of the marked variability in bioavailability of the non-ultramicroemulsified (Sandimmune®) preparation, it is not recommended for oral use in small animals.

Cyclosporine has a high binding affinity for red blood cells and plasma lipoproteins. 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. Tissue levels exceed levels in serum by a factor of 3 to 14. Peak blood concentrations generally occurring approximately 2 hours after oral administration of cyclosporine. 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.

Extensive metabolism of cyclosporine by the hepatic cytochrome P-450 system yields many different metabolites, some of which may retain therapeutic efficacy. In dogs, several drugs that inhibit P-450 enzymes have been given concurrently with cyclosporine in order to decrease the dose needed to maintain adequate blood drug concentrations. Ketoconazole, in particular, has been used to decrease in oral cyclosporine dosages in dogs by as much as 75 percent, although individual responses are variable. Like vincristine, cyclosporine is a drug that is handled by the P-glycoprotein efflux pump, a pump coded for by the ABCB1-1 $\Delta$  (MDR1) gene. Dogs with the

MDR1 gene mutation, however, have been shown to have relatively unchanged cyclosporine pharmacokinetics compared to dogs with normal P-glycoprotein function. However, P-glycoprotein, as well as having a potential effect on drug bioavailability, metabolism, and excretion (functions usually evaluated pharmacokinetically), may also impact the effect that the drug has on target cells. For cyclosporine, in particular, P-glycoprotein is responsible for pumping the drug out of target T cells, and a lack of this pump may lead to very high intracellular T cell drug levels. Certainly, our laboratory has seen individual dogs with the MDR1 gene mutation that had very markedly suppressed T cell function despite blood cyclosporine levels that were within target ranges.

The 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 drug concentrations both between patients and even within the same patient. Therapeutic management may therefore be facilitated by monitoring blood cyclosporine concentrations. 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. Currently available methods for TDM include HPLC, a specific monoclonal RIA, and a dimension cyclosporine immunoassay. HPLC has the advantage that the parent drug can be discriminated from metabolites, although most methods detect only the parent compound. Both RIA and dimension cyclosporine immunoassay, in contrast, measure metabolites as well as the parent drug, and blood cyclosporine concentrations will therefore be higher by a factor of 1.5 to 1.7 compared to the same sample analyzed using HPLC. Although HPLC is considered the gold standard for cyclosporine assays, HPLC is labor intensive and not routinely offered for patient monitoring. TDx 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 TDx 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 TDx and RIA assays may arguably be pharmacologically active and contribute to overall immunosuppressive effects. Much study has gone into determining the most appropriate sample collection time in patients receiving cyclosporine. In human medicine, trough blood concentrations were the initial basis for adjustment of drug dosages. However, multiple studies in people have since suggested that area under the plasma drug concentration time curve (AUC) or 2 hour peak drug concentrations are preferred. With measurement of peak cyclosporine concentrations requiring only a single sample, adjusting drug doses to attain target peak drug levels has become the single best blood concentration measurement for use during human organ transplantation. In veterinary medicine, measurement of trough cyclosporine concentrations also prevailed for many years based on initial work done in canine and feline renal transplant studies. Recommendations from laboratories offering TDM have often involved measurement of both peak and trough cyclosporine blood levels, although target peak concentrations have not been well established. Individual laboratory recommendations depended on the target ranges determined by each laboratory as well as the assay used to measure cyclosporine concentrations. Currently, the Auburn University Clinical Pharmacology Laboratory is the only veterinary pharmacology laboratory routinely offering cyclosporine blood level assays.

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, calcineurin enzyme activity, lymphocyte surface antigen expression, and intracellular cytokine quantification. Through pharmacodynamic monitoring, human studies have shown individually distinct degrees of calcineurin inhibitor sensitivity in patients. Pharmacodynamic monitoring shows great promise for optimizing cyclosporine therapy and delivering individualized therapy. At Mississippi State University, we have conducted extensive investigations into the pharmacodynamic evaluation of cyclosporine in dogs. We initially measured activated T cell expression of IL-2, IL-4, and IFN- $\gamma$  via flow cytometry in dogs administered two different oral cyclosporine dosages. The dogs were first administered a high dose of cyclosporine (10 mg/kg orally twice daily), with doses adjusted upwards as needed to attain a target trough drug concentration greater than 600 ng/mL as measured via HPLC, a dosing protocol known to be sufficiently immunosuppressive for canine organ transplantation. With high dose cyclosporine, activated T cell expression of IL-2 and IFN- $\gamma$  was significantly

suppressed. The dogs were then administered the FDA-approved dose of cyclosporine used to treat canine atopy (5 mg/kg orally once a day), a dose which has been considered to be low enough to avoid predisposing to immunosuppression-associated infection. Even with this low dose of cyclosporine, however, T cell expression of IFN- $\gamma$  and IL-2 was still markedly suppressed in some dogs. Subsequent studies evaluating activated T cell mRNA IL-2 and IFN- $\gamma$  expression utilizing molecular methods have demonstrated that results using a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay are comparable to flow cytometry, and that the technique shows promise as a pharmacodynamic assay in dogs. One advantage of the qRT-PCR assay compared to flow cytometry is that it can be performed on blood samples mailed in by practitioners. This assay has now been offered to practitioners for several years through our Mississippi State University Pharmacodynamic Laboratory, and assists veterinarians in adjusting oral cyclosporine doses in dogs to optimize systemic immunosuppressive effects. Cyclosporine has been shown to have much the same effect on T cell cytokine production in cats as it does in dogs. A pharmacodynamic assay based on this effect in cats is not yet commercially available, although MSU is currently working on developing such an assay.

Cyclosporine is FDA-approved for the treatment of canine atopic dermatitis and feline allergic skin disease, and has also been used to prevent transplant rejection and to treat sebaceous adenitis, pemphigus foliaceus, anal furunculosis, feline stomatitis, inflammatory bowel disease (IBD), myasthenia gravis, non-infectious inflammatory meningoencephalitis, pure red cell aplasia, immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia (IMT), and immune-mediated polyarthritis in dogs and cats. Pharmacodynamic research evaluating T cell responses to cyclosporine in dogs has confirmed that canine responses are comparable to the response profile that is well recognized in people: that individual responses to cyclosporine are extremely variable from dog-to-dog, both in dogs receiving the same standard oral dose, and in dogs with oral doses adjusted to attain comparable blood levels. Given that a high degree of variability of individual responsiveness to cyclosporine has been established in dogs, cyclosporine dosing protocols should be tailored to allow for this patient-to-patient variability. In my opinion, recommended dosing protocols in dogs with chronic, non-life-threatening inflammatory skin and gastrointestinal diseases should be quite different from the protocols used in dogs with more acute and life-threatening immune-mediated diseases.

In chronic inflammatory diseases that are typically not immediately life-threatening, such as skin conditions, anal furunculosis, and mild IBD, cyclosporine is often effective at a standard, relatively low starting dose. Cyclosporine therapy is typically delivered long term, with drug doses adjusted upwards 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 is typically tapered to the lowest effective dosage needed to maintain disease remission. Currently recommended starting cyclosporine doses in dogs are 5 mg/kg once daily for most skin diseases and IBD, and 5 mg/kg once to twice daily for anal furunculosis. In cats with skin conditions such as allergic skin disease, eosinophilic granuloma complex and pemphigus foliaceus, a starting cyclosporine dose of around 5-8 mg/kg daily is recommended. Cyclosporine blood concentrations are usually not necessary for treatment of these conditions, as remission of disease is the main criterion used to decide whether adequate cyclosporine therapy 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. Recent pharmacodynamic studies, however, have shown that, even at standard low FDA-approved doses, some dogs can still develop significant suppression of certain T-lymphocyte biomarkers of immunosuppression despite very low trough cyclosporine concentrations. This could explain the phenomenon reported by dermatologists, that individual dogs treated for atopic dermatitis can develop severe secondary infections, although the “atopy” cyclosporine dose was originally not thought to cause clinically significant immunosuppression. Therefore, even in dogs on low cyclosporine doses, clinicians should remain vigilant for potential signs of systemic infection.

In canine patients suffering from more acute and immediately life-threatening diseases such as severe IMHA and IMT, in contrast, cyclosporine 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 dose and adjusting doses upwards “to effect” is not recommended. Attaining effective oral doses as rapidly and accurately as possible is essential for ensuring adequate immunosuppression whilst avoiding overdosage with associated adverse effects and expense. Currently recommended starting cyclosporine doses for life-threatening diseases range from 5 mg/kg to 10 mg/kg twice daily. Subsequent measurement of blood cyclosporine concentrations and/or assessment of activated T cell mRNA IL-2 expression using qRT-PCR within one week of commencement of treatment, with dose adjustments as needed, are the best methods that are currently routinely available to assess adequacy of therapy, and are strongly recommended in patients with life-threatening diseases.

Side effects are uncommon with cyclosporine therapy in dogs and cats, with the exception of gastrointestinal side effects such as vomiting, diarrhea, anorexia and nausea. Administering the medication frozen and/or with food can reduce gastrointestinal side effects, although there is a risk that such measures will also alter drug absorption profiles. Uncommonly, cyclosporine can cause an idiosyncratic hepatotoxicity, which does not seem to be dose dependent. Gingival hyperplasia and hypertrichosis have also occasionally been reported with cyclosporine therapy. Chronic cyclosporine therapy may also predispose to neoplasia such as lymphoma. One advantage of cyclosporine as an immunosuppressive agent is that it is not myelosuppressive. Experimentally, oral cyclosporine has been shown to increase platelet thromboxane production, which may be a concern in patients with IMHA, where hypercoagulability and resultant pulmonary thromboembolism can be a major contributor to patient mortality. However, to date, it has not been demonstrated whether this phenomenon is clinically relevant in IMHA patients with naturally occurring disease. Furthermore, recent work in our laboratory has shown that, when cyclosporine and low-dose aspirin are given concurrently, the aspirin nullifies the surge in thromboxane seen in dogs that are receiving cyclosporine alone.

Cyclosporine is an expensive drug, particularly at higher immunosuppressive doses, and clinicians are therefore tempted to explore cheaper forms of the drug. In human medicine, there are many approved human generic microemulsion (“modified”) 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 it is not safe to assume that a generic formulation is therapeutically equivalent to the approved canine product (Atopica®). Clinically, there appears to be some variability seen in individual dogs in the oral bioavailability of these generic products. Use of generic products may therefore have the potential place our patients at risk of either therapeutic failure or toxicity although, if blood drug levels or pharmacodynamics assays are used to monitor therapy, this risk is minimized. The proprietary human microemulsified cyclosporine product, Neoral®, currently costs around \$2 for a 25 mg capsule and \$8 for a 100 mg capsule, while the generic equivalents cost less than \$1 for the 25 mg capsule, and around \$2 for the 100 mg capsule. The veterinary product, Atopica®, tends to be priced comparably to the human proprietary products, but has the advantage of being FDA-approved and available in a range of capsule sizes that are convenient for dosing accuracy in our small animal patients (10 mg, 25 mg, 50 mg and 100 mg), as well as a 100 mg/ml oral suspension and, currently, is price-supported by a manufacturer’s coupon. Non-modified (Sandimmune® or equivalent) cyclosporine has highly unreliable bioavailability, and should not be used. Nor should compounded cyclosporine, because compounders usually do not specify if the product is modified or non-modified. Unfortunately, transdermal cyclosporine has been shown to be inadequately absorbed in cats.