

## Cyclophosphamide

*Pharmacodynamic Laboratory, Mississippi State University, 2017*

Cyclophosphamide, a cell-cycle nonspecific nitrogen mustard derivative alkylating agent, was one of the first major chemotherapeutic agents approved by the FDA over 50 years ago, and has since become very well-established in human medicine as both an antineoplastic drug and as an immunosuppressive agent. Within a few years of FDA approval in the late 1950s, the use of cyclophosphamide for the prevention of transplant rejection in experimental models and for the treatment of both neoplasia and immune-mediated diseases was described in both dogs and cats. Cyclophosphamide has persisted to this day as one of the core drugs used in many small animal cancer chemotherapeutic protocols. In contrast, after many years as one of the most commonly immunosuppressive drugs utilized to treat immune-mediated diseases in cats and dogs, the use of cyclophosphamide as an immunosuppressive agent in small animal patients has in the past two decades essentially faded away. The reasons for the steadily diminishing popularity of cyclophosphamide as an immunosuppressive agent are myriad, and include a high incidence of unacceptable side effects, the development of other immunosuppressive agents that are generally safer and more convenient to administer, and the publication of a number of papers a decade or so ago that suggested that cyclophosphamide was associated with poor outcomes when used to treat conditions such as immune-mediated hemolytic anemia.

Cyclophosphamide is a prodrug that is metabolized by the hepatic cytochrome P450 enzyme system to eventually form active metabolites such as 4-hydroxycyclophosphamide, 4-hydroperoxycyclophosphamide, and aldophosphamide. These metabolites can enter cell cytoplasm, where they are ultimately metabolized to phosphoramidate mustard and acrolein. Phosphoramidate mustard is an alkylating agent that replaces a hydrogen atom with an alkyl group on the guanine base of DNA, which interferes with nuclear DNA replication and cytoplasmic RNA transcription by forming crosslinks both within and between nucleotide strands. Cyclophosphamide has long been reported to be a potent immunosuppressive that inhibits humoral and cell-mediated immunity, including inhibition of primary and secondary immune responses, reduction of antigen trapping in lymph nodes, and inhibition of local inflammatory responses, although in a number of experimental studies in dogs, cyclophosphamide often appears to be relatively mildly immunosuppressive compared to other drugs.

Cyclophosphamide shares the toxicity profile of most alkylating agents, with common side effects including gastrointestinal signs, myelosuppression, and hair loss. Gastrointestinal signs are relatively common, and include nausea, anorexia, vomiting and diarrhea. Dose reduction and antiemetic agents are often enough to control gastrointestinal signs, but occasionally persistent gastrointestinal side effects will prevent ongoing use of the drug, especially in cats. Myelosuppression appears to be dose dependent, and is associated with both the use of high drug doses and the use of lower doses over a sustained period of time. Moderate to severe neutropenia is the most potentially life-threatening feature of cyclophosphamide myelosuppression, but moderate thrombocytopenia and mild anemia may also occur. Neutropenia is typically reversible with drug dose reduction or discontinuance, but can occasionally persist for weeks or even months, particularly after chronic cyclophosphamide therapy. Recombinant granulocyte colony-stimulating factor can be used to hasten recovery in dogs with severe cyclophosphamide-induced neutropenia. Alopecia is most common in susceptible breeds such as poodles and old English sheepdogs. Interestingly, cyclophosphamide has been used in the past to “chemically shear” sheep.

A major side effect of cyclophosphamide that is (to a large extent) unique to this drug is the development sterile hemorrhagic cystitis (common in dogs, and uncommon but reported in cats). Cystitis is mediated by urinary excretion of the cyclophosphamide metabolite acrolein. Cystitis is often severe and debilitating to the patient, and will not resolve until the drug is discontinued. Unfortunately, distressing signs of cystitis can sometimes persist for days or even weeks after drug discontinuation. Cystitis is more likely to develop after long-term therapy with cyclophosphamide, which can present a particular problem for patients with immune-mediated diseases because such diseases are often persistent, and tend to relapse if immunosuppressive therapy is discontinued prematurely. The incidence of cystitis can be reduced significantly by the concurrent administration of furosemide or sodium 2-

mercaptoethane sulfonate (mesna), a sulfhydryl donor that binds acrolein, by ensuring ready access to water, by giving concurrent low doses of furosemide, and by taking canine patients for regular walks. The chronic local bladder inflammatory effects of cyclophosphamide have also been reported to predispose to the development of irreversible bladder wall fibrosis and even transitional cell carcinoma.

Over the years, a number of immune-mediated and inflammatory diseases in dogs and cats have been treated with cyclophosphamide, including immune-mediated hemolytic anemia (IMHA), immune-mediated thrombocytopenia, megakaryocyte hypoplasia; pure red cell aplasia, systemic lupus erythematosus, immune-mediated polyarthritis, inflammatory bowel disease, glomerulonephritis, noninfectious inflammatory meningoencephalitis, immune-mediated vasculitis and pemphigus. For many years, cyclophosphamide was considered a “big gun” to be used in dogs with severe or life-threatening IMHA. However, in the late 1990s and early 2000s, a number of case studies were published that suggested that, at best, cyclophosphamide was not better than glucocorticoids alone for the treatment of IMHA and, at worst, associated with a higher than expected mortality rate. Given the known limitations of retrospective studies, including the associated potential for “case selection bias” (that is, the dogs with the most severe IMHA may have been given cyclophosphamide because it was the drug perceived to be most potent), it is hard to know with any real certainty whether cyclophosphamide actually worsens prognosis in dogs with IMHA. Nevertheless, there is no doubt that, since publication of these papers, the use of cyclophosphamide to treat conditions such as canine IMHA has markedly decreased.

Compared to many immunosuppressive agents, cyclophosphamide has been relatively cheap, with a generic 25 mg tablet costing under \$2, and a 50 mg tablet costing under \$4, although there has been a recent (hopefully temporary) marked surge in drug prices, with a 50 mg tablet now costing over \$10. One of the major problems associated with using cyclophosphamide as an immunosuppressive agent is that it is difficult to dose accurately, and even more difficult to taper, especially in smaller patients. Cyclophosphamide is available as 25 or 50 mg tablets that composed of an active inner tablet surrounded by an inert outer flecked tablet. Because of uneven distribution of the drug through the tablet, cyclophosphamide tablets cannot be split or crushed without a risk of major dosing inaccuracies. Without drug compounding, cyclophosphamide doses must therefore be in multiples of 25 or 50. Compounded cyclophosphamide can solve dosing issues, and is cheaper, but a recent paper showed a worrying variation in dose accuracy with cyclophosphamide from various veterinary compounding pharmacies. Published immunosuppressive doses for cyclophosphamide in dogs include 50 mg/m<sup>2</sup> or 1.5 to 2.5 mg/kg every second day or daily on a “4 days on, 3 days off” weekly protocol. In cats and small dogs, similar total weekly doses can be used, but “pulsed” at infrequent dosing intervals that ensure that the total weekly dose is equivalent to seven times the calculated daily dose. Since myelosuppression can occur at any time during chronic cyclophosphamide therapy, complete blood counts must be performed regularly throughout the course of drug treatment. Cyclophosphamide is available in an intravenous form as well as an oral form, and a recent study in dogs confirmed that equivalent oral and intravenous doses of cyclophosphamide achieved comparable blood levels of the active metabolite 4-hydroxycyclophosphamide. Intravenous cyclophosphamide may therefore be a viable treatment option in vomiting animals that are unable to tolerate oral immunosuppressive agents.