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Veterinary Uses of Recombinant Human Granulocyte Colony-Stimulating Factor. Part I. Oncology

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FOCAL POINT

- Recombinant human granulocyte colony-stimulating factor (rhG-CSF) can be used to ameliorate myelosuppression associated with chemotherapy in cats and dogs and may permit more aggressive chemotherapy protocols.

KEY FACTS

- Long-term administration of rhG-CSF to normal dogs and cats may lead to the formation of antibodies to endogenous granulocyte colony-stimulating factor (G-CSF).
- A daily rhG-CSF dose of 5 to 10 µg/kg can be administered subcutaneously to reduce myelotoxicity after chemotherapy in dogs and cats.
- Pulse dosing of rhG-CSF on an as-needed basis for canine and feline cancer patients receiving chemotherapy does not lead to antibody formation.
- Because recombinant canine and feline G-CSF are currently not commercially available, rhG-CSF is the only product available for veterinary use.

Colony-stimulating factors (CSFs) are cytokines that stimulate the growth of bone marrow progenitor cells and the maturation and function of terminally differentiated blood cells. In the past decade, several CSFs, including granulocyte CSF (G-CSF), monocyte CSF, granulocyte-macrophage CSF, interleukin (IL)-3 (formerly known as multi-CSF), erythropoietin, and thrombopoietin have been purified.

Of these CSFs, granulocyte-macrophage CSF and G-CSF have been most extensively studied in veterinary patients. The indications for clinical use of G-CSF in veterinary medicine are being realized as more clients opt for chemotherapy for pets with cancer and as new uses are identified by hematopoietic growth factor research. Part I of this two-part presentation reviews the history of G-CSF use in veterinary medicine and discusses recommendations for administering G-CSF to chemotherapy patients. Part II will address the use of G-CSF in patients with infectious diseases (e.g., canine parvovirus).

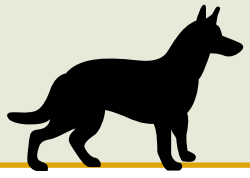
OVERVIEW

Granulocyte CSF is a cytokine produced by bone marrow stromal cells, fibroblasts, endothelial cells, monocytes, and macrophages.[1–5] G-CSF production is stimulated by IL-1, IL-3, tumor necrosis factor (TNF), interferon-γ, and bacterial endotoxin.[3,5–8] G-CSF receptors are present on granulocytes, monocytes, myeloid leukemia cells, and platelets.[9,10] G-CSF induces neutrophilia characterized by a left shift, increases the number of circulating progenitor cells, shortens the time required for neutrophil maturation and appear-

ance in the circulation, and improves neutrophil function[11–19] (see Effects of Granulocyte Colony-Stimulating Factor on Neutrophil Function).

Effects of Granulocyte Colony-Stimulating Factor on Neutrophil Function

- Enhancement of chemotaxis
- Augmentation of neutrophil-mediated antibody-dependent cellular cytotoxicity
- Stimulation of arachidonic acid release
- Enhancement of superoxide release



In human medicine, G-CSF is used to treat idiopathic and congenital neutropenia as well as to promote the growth of transplanted bone marrow after myeloablation. G-CSF is also used to enhance the collection of stem cells from peripheral blood and to treat or prevent chemotherapy-induced myelosuppression.[14] G-CSF is beneficial in the treatment of AIDS because it can increase neutrophil function, enhance the fungicidal activity of polymorphonuclear leukocytes, and permit continued antiviral therapy (which induces neutropenia in some patients).[20,21] The primary use of G-CSF in veterinary medicine has been for treatment of iatrogenic (chemotherapy-induced) and cyclic neutropenia.

Use in Dogs

The use of recombinant human G-CSF (rhG-CSF) in dogs was first reported in 1988.[22] Neutropenia associated with cyclic hematopoiesis in two gray collies was corrected by daily rhG-CSF administration. One normal dog received an identical dose (5 µg/kg subcutaneously) of rhG-CSF twice daily. The leukocyte counts had risen to 20,000/µl within 12 hours of the first dose, and marked leukocytosis (more than 50,000 leukocytes/µl) was noted in all three dogs approximately 2 weeks after the first dose.

Neutralizing antibodies directed against rhG-CSF developed in all dogs after 23 days of rhG-CSF therapy and coincided with a decline in leukocyte counts.[22] The fact that human G-CSF (hG-CSF) has 80% amino acid sequence homology with canine G-CSF (cG-CSF)[23] suggests that antibodies to hG-CSF might also cross-react with cG-CSF. In 1991, Hammond and coworkers demonstrated that normal dogs receiving rhG-CSF (10 µg/kg/day) for 30 days developed anti–rhG-CSF antibodies that cross-reacted with recombinant cG-CSF (rcG-CSF).[24] The antibodies neutralized the activity of rhG-CSF and rcG-CSF in vitro. Dogs treated with rhG-CSF developed neutropenia, which persisted for 4 months after therapy was discontinued. The neutropenia was reinduced within 1 week of rhG-CSF administration. Thus, the potential for development of cross-reacting antibodies and subsequent worsening of neutropenia has largely precluded the use of rhG-CSF in veterinary patients.

Recently, rcG-CSF has been synthesized and tested in normal and neutropenic dogs.[25,26] Although not currently commercially available for routine clinical use, immunosuppressed dogs (i.e., those receiving chemotherapy) did not develop antibodies to rhG-CSF after five rounds of chemotherapy. Dogs that received combination mitoxantrone and cyclophosphamide chemotherapy did not develop neutralizing antibodies to rhG-CSF administered at dosages of 2.5 to 10 µg/kg/day beginning 1 to 7 days after chemotherapy. One of two healthy control dogs did develop anti–rhG-CSF antibodies after 18 total doses (2.5 to 5.0 µg/kg per dose of rhG-CSF).[27] Myelosuppression in the dogs that received chemotherapy was ameliorated by rhG-CSF administration.

The results of that study suggested that rhG-CSF may be useful for treating dogs with chemotherapy-induced neutropenia and that the development of clinically significant (i.e., neutralizing) anti–rhG-CSF antibodies in these circumstances is unlikely. Because of these findings, a second study was initiated to evaluate the efficacy of rhG-CSF administration to support the bone marrow in tumor-bearing dogs undergoing intensive chemotherapy with intravenous mitoxantrone (5 mg/m^2) and cyclophosphamide (150 mg/m^2) every 21 days for four treatments.[28] Dogs evaluated after completion of four courses of chemotherapy with rhG-CSF rescue did not produce neutralizing antibodies to rhG-CSF.

Use in Cats

The use of rhG-CSF in cats has not been studied extensively. Fulton and coworkers administered dosages from 3 to 10 µg/kg to healthy cats twice daily for 21 days, demonstrating a significant increase in neutrophil counts. The counts ranged from 20,370 to 61,400 neutrophils/µl.[29] The mean of the maximum counts was 38,800 neutrophils/µl. This increase was followed by a decrease (to a mean of 18,680 neutrophils/µl) by days 17 to 21 despite continued rhG-CSF therapy.[29] As in dogs, development of cross-reactive antibodies was suspected.

Obradovich and coworkers administered rcG-CSF (5 µg/kg/day) to cats and found similar responses (rapid increases in neutrophil counts) to those in dogs and an

apparent lack of antibody formation to the rcG-CSF protein, even after 6 weeks of daily therapy.[30] These two studies suggest that the amino acid sequence may be more homologous between feline and canine G-CSF than between feline and human G-CSF. Because rcG-CSF is not commercially available, however, it cannot be used in feline patients.

Like dogs, cats might be unable to mount an immune response to the heterologous rhG-CSF protein during intensive chemotherapy. We have found that rhG-CSF (2.5 to 10.0 µg/kg/day) given subcutaneously to tumor-bearing cats ameliorates severe chemotherapy-induced myelosuppression.

To develop a practical and cost-effective protocol for feline practitioners, we have recently investigated the use of rhG-CSF in cats with myelosuppression due to high-dose (10 mg/m^2) intravenous mitoxantrone chemotherapy. The rhG-CSF dosages evaluated were 5.0, 7.5, and 10.0 µg/kg/day beginning 5 days after chemotherapy. Initial data suggest that rhG-CSF effectively reverses mitoxantrone-induced myelosuppression in cats.

No systemic toxicity associated with rhG-CSF administration was noted in our study or the previous study of normal cats.[29] Serum has been collected to test for antibody formation to rhG-CSF. However, the continued responses to rhG-CSF after 20 doses suggest that neutralizing antibodies to rhG-CSF did not develop in these cats. Although these preliminary data are encouraging, additional data are needed before appropriate recommendations can be made concerning the use of rhG-CSF in cats.

TREATMENT PROTOCOLS

The value of protocols using commercially available rhG-CSF, rather than the investigational rcG-CSF, is obvious. In addition, short pulsatile periods of G-CSF administration during leukocyte nadirs are less expensive than continuous G-CSF therapy and may be equally efficacious.

Ogilvie and coworkers compared neutrophil counts in dogs that received mitoxantrone and dogs that received a combination of mitoxantrone and daily rcG-CSF (5 µg/kg).[31] Median neutrophil counts were below normal for shorter periods in dogs that received rcG-CSF than in dogs that received mitoxantrone only. In that study, rcG-CSF administration was begun 2 days after chemotherapy. Limited evidence from investigations with human patients suggests that simultaneous administration of CSFs with chemotherapy or radiation therapy may lead to severe toxicity, including thrombocytopenia.[32,33]

Because of the potential sensitivity of rapidly dividing myeloid cells to cytotoxic chemotherapy, the manufacturers recommend that rh-CSF not be administered from 24 hours before to 24 hours after chemotherapy is given.[34] However, a recent human oncology study indicated that simultaneous infusion of G-CSF and either doxorubicin or ifosfamide permitted administration of increased doses of the chemotherapeutic agents.[35] Until further studies are completed, we recommend avoiding rhG-CSF administration within 24 hours before or after chemotherapy.

Administering rhG-CSF beginning when the neutrophil counts are below 1000 cells/µl is sufficient to lessen the severity of myelosuppression.[27,28] We have determined that short courses (three to six doses), rather than the previously reported 20-dose regimen, are sufficient to treat severe myelosuppression.[28] This confirms the report that repeated 4-day courses of rhG-CSF (10 µg/kg) are effective for treating chemotherapy-induced neutropenia in dogs.[36]

The value of G-CSF when chemotherapy has not produced complications is less clear. Is the clinical outcome in dogs and cats with chemotherapy-induced neutropenia altered by the use of rhG-CSF, or are practitioners simply playing it safe at their clients' expense? Dogs with lymphoma produce significantly less G-CSF on neutropenic days after chemotherapy than do normal dogs treated with the same chemotherapy.[37] These data suggest that tumor-bearing dogs may benefit from exogenous G-CSF to compensate for impaired G-CSF secretion.

Studies in human cancer patients suggest that rhG-CSF reduces the duration of hospitalization, neutropenia, and antibiotic use.[38,39] Current guidelines from the American Society of Clinical Oncology recommend restricting G-CSF use to febrile neutropenic patients, patients undergoing subsequent cycles of chemotherapy after a febrile neutropenic episode, or high-risk patients whose risk of febrile neutropenia is expected to be greater than 40%.[40]

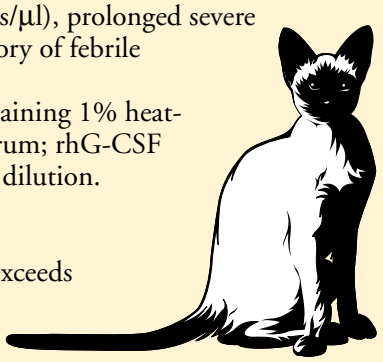
VETERINARY GUIDELINES

No similar guidelines for G-CSF use in veterinary oncology have been published. We restrict use of rhG-CSF to animals with febrile neutropenia (segmented neutrophil count less than 1000 cells/µl), prolonged severe neutropenia (less than 500 cells/µl for longer than 72 hours), or a history of febrile neutropenia with previous chemotherapy (see Administration of Recombinant Human Granulocyte Colony-Stimulating Factor to Treat Chemotherapy-Induced Neutropenia in Dogs and Cats).

The rhG-CSF is supplied as a 300-µg/ml, single-dose, preservative-free product. Manufacturer recommendations provide guidelines for diluting the product

Administration of Recombinant Human Granulocyte Colony-Stimulating Factor (rhG-CSF) to Treat Chemotherapy-Induced Neutropenia in Dogs and Cats

1. Determine differential leukocyte count and take patient's temperature. We use rhG-CSF for animals with febrile neutropenia (segmented neutrophil count below 1000 cells/µl), prolonged severe neutropenia (less than 500 cells/µl for longer than 72 hours), or a history of febrile neutropenia with previous chemotherapy.
2. Dilute rhG-CSF to a concentration of 100 µg/ml in 5% dextrose containing 1% heat-inactivated (in 56°C water bath for 30 minutes) autologous patient serum; rhG-CSF is available as a 300-µg/ml solution that may be administered without dilution.
3. Administer at a dose of 5 µg/kg subcutaneously once daily.
4. Repeat differential leukocyte count daily.
5. Discontinue administration 2 days after segmented neutrophil count exceeds 3000 cells/µl.



in 5% dextrose to concentrations of 5 to 15 µg/ml but suggest that unused drug should not be stored for later use.[34] However, the small doses required in small animal practice make it economically impractical to discard unused portions.

Commercially available rhG-CSF can be diluted to a concentration of 100 µg/ml in 5% dextrose that contains 1% sterile, heat-inactivated autologous patient serum. The unused product can be refrigerated for as long as 4 weeks with no apparent effect on efficacy. We have not tested the stability of the product in these conditions; it is thus advisable to use the product immediately and without dilution when feasible. The manufacturer specifically cautions against dilution with saline, which may cause precipitation.[34]

Changes in leukocyte number and morphology (including neutrophilia with a left shift, Döhle bodies, vacuolation, and toxic granulation) are expected after G-CSF administration.[41–43] These changes are normal sequelae to G-CSF administration and do not necessarily indicate sepsis. Increases in alkaline phosphatase activity of nonhepatic origin have been noted in human patients treated with rhG-CSF.[43]

CONCLUSION

The responsible use of rhG-CSF in veterinary practice requires careful consideration of the need to administer the drug and use of a protocol that minimizes the potential for antibody formation. Clients must be informed of the cost of therapy (approximately $0.72 per µg) versus the potential risks of withholding therapy. Until controlled randomized studies that involve tumor-bearing animals are completed, the true risks of withholding G-CSF therapy from neutropenic animals will be unclear.

What is clear is that rhG-CSF can be administered to dogs and cats cost effectively to ameliorate severe chemotherapy-induced myelosuppression and to permit more aggressive and hopefully more effective cancer treatment protocols. Part II of this article will further define the role of G-CSF in the treatment of patients with infectious diseases.

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