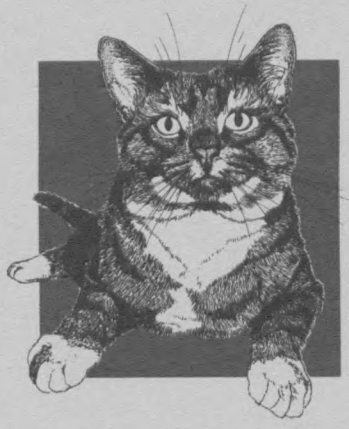

Feline Health Topics

for veterinarians

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Responding to Concerns About Anthrax in Domestic Cats

In light of recent events, it is not surprising that concern is being expressed over the health risks the causative agent of anthrax, *Bacillus anthracis*, poses to cats. The Cornell Feline Health Center and the Veterinary Diagnostic Laboratory at Cornell University have receivednumerous inquiries about the direct risk to cats, the potential role exposed or infected cats may play in transmitting infection to humans and to other animals, and methods to deal with exposed or infected cats.

It is important to note that as this issue of *Feline Health Topics* went to press, no cases of illness or exposure to domestic cats have been identified in the recent anthrax incidents. Domestic cats would not be primary targets, but it is conceivable that they could become infected from sources of the agent maliciously intended for human exposure. A cat with anthrax is incapable of transmitting the infection, but a cat's coat, once contaminated with anthrax spores, could in some cases pose a risk to health. For these reasons, it is important that companion animal veterinarians be informed about the disease, its effects on cats, its diagnosis, and its zoonotic potential.

How does anthrax affect cats?

Natural cases of anthrax in domestic cats are rarely reported. In contrast to herbivores, carnivores-including domestic cats-are relatively resistant to disease. In most reported cases, consumption of tissue from infected livestock was considered to be the likely source of infection. Because cats are efficient groomers, ingestion would be the expected route of exposure if their fur became contaminated with anthrax spores from a malicious attack. Clinical signs observed in the few reported feline cases included dyspnea and dysphagia resulting from swelling of the neck secondary to regional lymphadenopathy, hemorrhagic and ulcerative inflammation of the oral cavity and throat, enteritis, and enlargement of the kidneys, spleen, and liver. Sudden death with few premonitory signs has also been reported. The incubation period in naturally infected carnivores is often difficult to determine, but is believed to be approximately three to seven days with a range of one to 14 days.

When might I suspect that a cat has the disease?

Clinical signs associated with anthrax may provide clues, but they are signs common to other feline disorders. Sudden death in a cat without prior signs — although by no means unique to anthrax — might raise suspicion. The New York City Department of Health has compiled the following list of epidemiologic clues suggestive of a possible bioterrorist event:

- Suspected or confirmed zoonotic diseases that are not endemic to the area, especially in the absence of recent travel history, or that

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have an unusual presentation
- Simultaneous disease outbreaks in humans and animal populations
- Any unusual temporal and/or geographic clustering of illness (e.g., pets from a given area, or that recently visited a particular park)

How do I confirm that anthrax is the cause of the disease?

Laboratory confirmation of disease is absolutely essential. The Veterinary Diagnostic Laboratory at Cornell University requests the following specimens from suspected cases (required supplies are available from the laboratory):

- A pharyngeal swab submitted in Amies transport media (with or without charcoal)
- 1-2 ml of whole blood submitted in a blood culture bottle
- 1-2 ml of whole blood submitted in an EDTA (purple top) tube

Fresh specimens from infected cats are not infectious and pose no risk to human or animal health. However, once exposed to air, any vegetative forms of the organism present in the specimens will sporulate within several hours. Anthrax spores are the infectious form of the organism and are highly resistant to disinfection. Therefore, any inadvertent spills should be immediately cleaned and disinfected (disinfection instructions currently viewable in section II.1.b of the document at http://www.bt.cdc.gov/Agent/Anthrax/Anthracis20010417.pdf). **Do not open the body of a suspect anthrax case.** Blood may be collected with a needle and syringe from the heart of a deceased patient. The body should be double bagged and frozen pending results of diagnostic tests.

Veterinary practices within the Cornell University College of Veterinary Medicine referral area should contact the Diagnostic Laboratory at 607-253-3900 prior to sample submission. Specimens should be shipped overnight in a sealed, airtight, leak-proof container surrounded by absorbent material and placed inside a crush-proof mailer (mailing supplies are available from the Diagnostic Laboratory). Complete sample submission information can be found online at http://diaglab.vet.cornell.edu/issues.html or by contacting the laboratory at 607- 253-3900. Practices outside this referral area should contact their usual veterinary diagnostic laboratory prior to submitting samples.

If I'm presented with a cat with anthrax, at what risk are my staff, my clients, and I?

It is conceivable that you may be asked to examine or treat a cat with signs of disease consistent with anthrax. With the exercise of due caution, you should have no fear in doing so. Anthrax is not directly transmissible from animal to animal (e.g., cat to human or vice versa).

Will a cat with anthrax respond to treatment?

Attempts to treat cats with anthrax have never been documented. Extrapolating from treatment protocols for infected humans, administration of antimicrobi-

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The ultimate purpose of the Cornell Feline Health Center is to improve the health of cats everywhere by developing methods to prevent or cure feline diseases, and by providing continuing education to veterinarians and cat owners. All contributions are tax-deductible.

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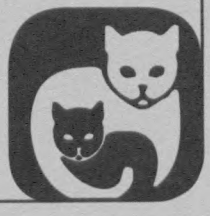

Species-Specific Recombinant Erythropoietin Preparations For Companion Animals

by James N. MacLeod, VMD, PhD, Associate Professor, James A Baker Institute for Animal Health, Cornell University College of Veterinary Medicine, and John F. Randolph, DMV, ACVIM, Professor, Cornell University College of Veterinary Medicine

Introduction

Erythropoietin is a glycosylated protein that stimulates red blood cell production. It is produced by interstitial and peritubular cells in the renal cortex and transported through the blood to the bone marrow. The hormone's biological activity involves a direct receptor-mediated stimulation of the maturation and replication of late erythroid progenitor cells, proerythroblasts, and erythroblasts. Synthesis of erythropoietin is stimulated in response to tissue hypoxia mediated by intracellular aerobic metabolism. The primary protein structure of human erythropoietin includes a 27 amino acid signal peptide and a 166 amino acid mature protein. The predicted molecular weight of 18.4 kDa based on the primary amino acid sequence is substantially less than the 32-34 kDa

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als (e.g., enrofloxacin, doxycycline, or penicillin derivatives) for 6-8 weeks in addition to supportive measures would be a reasonable course of action. Prognosis is impossible to predict.

Whom should I notify if a patient has a confirmed anthrax infection?

Local public health officials should be notified immediately if anthrax is confirmed in a feline patient.

What if a cat presents with an unidentified powdery substance on its fur?

Rational thought should prevail! In the vast majority of cases, the powder will be a benign substance (e.g., dust from the environment, or flea powder applied to the cat unbeknownst to other family members). However, if after careful consideration, anthrax spores remain highly suspect, the following should be undertaken:

- Call your local emergency response system (e.g., 911) or public health department; tests will be performed by appropriate public health officials
- Avoid handling the cat more than is absolutely necessary; disposable gloves and garments should be used and subsequently destroyed in a manner determined by public health officials if anthrax exposure is confirmed
- Confine the cat to one area to avoid spreading potentially infectious material
- Disallow movement of people and materials into and out of the area of confinement
- To avoid overwhelming the response system, use common sense in interpreting the situation. For example, cats covered with dust after rolling in the dirt should not be considered potentially exposed to a biological agent.

Where can I find additional information about anthrax?

The Cornell University Veterinary Diagnostic Laboratory webpage on emerging issues (http://diaglab.vet.cornell.edu/issues.html) is an excellent source of information which includes links to other informative sites.

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observed when erythropoietin is purified directly from blood or urine. The difference is due to glycosylation, three N-linked sugar chains at Asn 24, 38, and 83, and an O-linked mucin-like moiety at Ser 126. Compared to human, the amino acid sequences of mouse and monkey erythropoietin are 80 and 92 percent identical, respectively. The basic erythropoietin gene structure, five exons and four introns, appears to be highly conserved across different mammalian species.

Recombinant human erythropoietin (rhEPO) synthesized in Chinese Hamster Ovary (CHO) cells is produced commercially (Epogen®, Amgen, Inc. Thousand Oaks, CA) and widely used to support red blood cell production in people suffering from anemia secondary to chronic renal disease. Although the pathogenesis of the anemia is multifactorial, compensatory failure by the bone marrow to replace red blood cells primarily results from a loss of functional renal tissue and a drop in endogenous erythropoietin production. Synthesis of rhEPO for clinical use is restricted to eukaryotic cells due to the requirement of post-translational glycosylation for *in vivo* stability and bioactivity of the hormone. Devoid of sugars or even the terminal sialic acid residues, erythropoietin is rapidly cleared and metabolized by the liver.

In cats and dogs, the progressive clinical syndrome associated with chronic renal failure also includes development of a nonregenerative anemia. In parallel to the human literature, studies have documented low serum concentrations of erythropoietin despite the anemia. Therapeutic use of rhEPO in cats and dogs with anemia secondary to chronic renal failure results in a rapid and significant red blood cell response. Hematocrit and hemoglobin values rise to the normal range within several weeks depending on the dose administered and treated animals display increased alertness, physical strength, appetite, and overall attitude. These findings strongly suggest that the persistent anemia contributes significantly to some of the clinical manifestations of chronic renal failure. Unfortunately, the red blood cell status of some cats and dogs begins to decline after roughly one to four months despite continued rhEPO therapy. Therapeutic failure is believed to result from the appearance of antibodies against the human protein. Anti-rhEPO antibodies are thought not only to effectively block rhEPO's bioactivity, but also have the potential to bind and neutralize residual endogenous erythropoietin resulting in a pure red cell aplasia. This problem of immunogenicity can be life threatening and has severely limited the therapeutic potential of rhEPO for veterinary applications. The concept of erythropoietin replacement as a therapy for the anemia of chronic renal failure is appropriate for companion animals, the problem is the immunogenicity of rhEPO.

Nonregenerative anemia is also a common clinical feature in other chronic diseases including cancer. In cancer patients, anemia may be a presenting feature, or alternatively, develop during chemotherapy. Although the pathogenesis of anemia from cancer is multifactorial, three major variables identified are: 1) the inhibition of erythropoietin production and bioactivity by inflammatory cytokines and chemotherapeutic drugs, 2) direct inhibition of erythroid progenitor cells by cytokines, and 3) impaired iron metabolism. Consistent with these etiologic variables are clinical data demonstrating that anemia from cancer in 32-85 percent of human patients (depending on the cancer type) responds to pharmacologic doses of rhEPO. Furthermore, *in vitro* studies demonstrate a reversal of cytokine-mediated inhibition of erythropoiesis with increased concentrations of rhEPO. Resolution of anemia improves strength, activity, and appetite, while importantly enabling more aggressive or sustained chemotherapy to achieve a higher and prolonged rate of cancer remission.

Experimental Studies

Our research is based on the hypothesis that species-

specific erythropoietin preparations are needed to avoid the immunogenicity problems of rhEPO in veterinary applications. Full length genomic (canine) and cDNA (canine and feline) erythropoietin sequences have been cloned. Canine and feline erythropoietin exhibit 94.3 percent amino acid identity to each other, but only 81.3 percent and 83.3 percent identity respectively to human erythropoietin.

CHO cell-based expression systems have been produced to synthesize recombinant canine erythropoietin (rcEPO) and feline erythropoietin (rfEPO) in amounts sufficient for therapeutic use. Biological activity was initially demonstrated in two murine-based assays. *In vitro,* the replication of splenic erythroid progenitor cells was stimulated in a dose-dependent manner by increasing levels of erythropoietin supplementation. Similarly *in vivo,* erythropoietin administered to mice stimulated reticulocytosis in a dose-dependent fashion. With both assays, rcEPO and rfEPO displayed a profile of activity very comparable to rhEPO. A prospective study comparing rcEPO and rhEPO (Epogen®) in normal Beagles over a 28 week treatment protocol has also been conducted. Differences between the two erythropoietin preparations were dramatic and consistently observed in every dog. Every Beagle treated with rhEPO (n=6) developed red cell aplasia, four of the six dogs within four weeks. Consistent with previous studies from other groups, we did see an initial positive erythropoietic response to rhEPO. Over time, however, peripheral reticulocyte levels in rhEPO-treated dogs invariably dropped to near zero and subsequent analysis of bone marrow cytology confirmed the diagnosis of red cell aplasia. In contrast, rcEPO stimulated erythropoiesis in the dogs (n=7) without any side effects noted. Most importantly, no evidence of red cell aplasia or other immunogenicity problems developed with rcEPO.

Clinical Evaluations

Both rcEPO and rfEPO are currently being evaluated in multicenter clinical trials designed to assess safety and efficacy in privately owned patients suffering from nonregenerative anemia secondary to three conditions: 1) chronic renal failure, 2) lymphosarcoma, and 3) red blood cell aplasia due to antibodies against rhEPO. Diagnoses of these conditions are based on routine historical and clinical criteria. Lymphosarcoma patients must be medically managed with standard (i.e., cyclophosphamide, vincristine, doxorubicin, prednisone) chemotherapeutic treatment protocols and not have any evidence of myelophthisis. Red cell aplasia patients must demonstrate a failure to respond to continued rhEPO therapy at a dose of at least 100 units/kg administered thrice weekly for two weeks or longer. All patients must be one year of age or older, with no evidence that their anemia is due to iron deficiency or blood loss. Systolic blood pressure must be less than a value of 180 mm Hg. Any previous history of treatment with rhEPO (Epogen® or Procrit®) precludes entry into either the chronic renal failure or the lymphosarcoma study groups.

Therapy with rcEPO and rfEPO is initiated at 100 units/kilogram body weight, injected subcutaneously three times per week. For animals that never received Eopgen® or Procrit®, most individuals exhibit a positive erythropoietic response within two weeks of initiating rcEPO or rfEPO therapy. The dose and frequency of administration are usually adjusted at some point during the course of the study in order to achieve and maintain a target hematocrit range of 35-45 percent for dogs, and 30-40 percent for cats. For all patients, the owners are instructed to administer daily oral ferrous sulfate at a dose of 10 mg/kg body weight. Study durations are 52 weeks in the chronic renal failure group and 24 weeks in the lymphosarcoma and rhEPO-induced red blood cell aplasia groups. Serial assessment of laboratory param-

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International Symposium on Nonsurgical Contraceptive Methods for Pet Population Control

The Alliance for Contraception in Cats and Dogs (ACCD) will sponsor an International Symposium on nonsurgical contraceptive methods for dogs and cats at Callaway Gardens, Pine Mountain, Georgia (near Atlanta) on April 19-21, 2002. The Alliance is a newly formed group to promote the development of nonsurgical methods for contraception of dogs and cats. One of the specific goals of the Alliance is to sponsor an international meeting to facilitate interaction of interested individuals and to encourage exchange of ideas and research results. Organizers for the first symposium are Dr. Stephen Boyle, Chair of the ACCD, and Drs. Henry Baker and Brenda Griffin of Auburn University.

Speakers are being invited to present topics including FDA regulation of companion animal contraceptive products, gonadotropin releasing hormone (GnRH) vaccines, Zona pellucida vaccines, vaccines utilizing other antigens such as sperm or ovum specific antigens (e.g., virally vectored contraceptive vaccines), carrier antigens which induce immune response to self antigens used in contraceptive vaccines, adjuvants and formulations which enhance immune response to contraceptive vaccines, GnRH analogs, cytotoxic destruction of pituitary gonadothophs, sclerosing compounds and other contraceptive pharmaceuticals, and the role of the pharmaceutical industry in development and commercialization of contraceptive products. Keynote speakers will address the dynamics and demographics of animal population control. Experts in basic biology of reproduction and contraception in humans, wildlife, and companion animals will share their expertise and results. Poster and platform presentations of original research will be invited. Each session will be summarized by a senior scientist in the field.

The two-day symposium will begin with an evening welcome reception, followed by a full day of scientific meetings, lectures, and presentations. A luncheon and banquet will be provided to allow scientists ample opportunity to interact and network with one another. The following morning will be utilized for continuation of the scientific presentations and posters. Abstracts of all presentations, including summary sessions, will be provided to participants and be made available to those who are unable to attend. The names and contact information for all attendees will be printed in the abstract booklet to facilitate interactions following the meeting. A questionnaire will be used to solicit suggestions for the next meeting. Contributors and sponsors of the meeting will be prominently acknowledged.

Callaway Gardens is an ideal venue for a relaxed, interactive exchange of ideas and results on this important topic. The site is approximately 60 miles south of Atlanta's Hartsfield Airport which is served by most domestic and international airlines. For additional information about Callaway Gardens, visit their website at http://www.callawaygardens.com.

For additional information about the program, venue, accommodations, transportation, etc., visit the ACCD website at http:www.vetmed.vt.edu/Organization/Associations/ACCD/index.html or e-mail Dr. Baker at baker@vetmed.auburn.edu.

Research Briefs

DS Westfall, DC Twedt, PF Steyn, EB Oberhauser, JW VanCleave, "Evaluation of esophageal transit of tablets and capsules in 30 cats," ***Journal of Veterinary Internal Medicine*** **15: 467-470**

The authors have reported tablet-induced focal esophagitis and esophageal stricture formation in cats. The proposed mechanism is thought to be abnormal esophageal tablet retention resulting in focal esophagitis with subsequent stricture formation. The objective of this study was to evaluate the passage of tablets and capsules when given alone (dry swallow) and when followed by a water bolus (wet swallow) to determine if this could, in part, explain the esophageal stricture formation observed in cats. Fluoroscopy was used to evaluate tablet or capsule passage after administration. The percentage of dry tablet swallows that successfully passed into the stomach was 0.0 percent at 30 and 60 seconds, 6.7 percent at 90 seconds, 13.3 percent at 120 seconds, 26.7 percent at 180 and 240 seconds, and 36.7 percent at 300 seconds. Wet tablet swallows successfully passed 90.0 percent of the time at 30 seconds, 93.3 percent of the time at 60 seconds, and 100.0 percent of the time thereafter. The percentage of dry capsule swallows that successfully passed was 16.7 percent at each time interval. Wet capsule swallows successfully passed 96.7 percent of the time at 30 seconds and 100 percent of the time thereafter. For each time interval, wet swallows achieved significantly greater percentage passage into the stomach when compared to dry swallows ($P < .05$). This study shows that tablets or capsules given by dry swallow have prolonged retention in the esophagus compared to those given by wet swallow. On the basis of this study, the authors recommend the routine administration of a water bolus to cats receiving tablets or capsules PO to facilitate esophageal clearance. This practice may help prevent medication-associated esophagitis or stricture formation.

T.E. Carpenter, "Use of sample size for estimating efficacy of a vaccine against an infectious disease," ***American Journal of Veterinary Research*** **62:1582-1584.**

In order to determine the sample size necessary to evaluate the efficacy of a vaccine in a population, an equation was coded into a computer spreadsheet to compare the traditional sample size calculation with that needed when evaluating the efficacy of a vaccine applied in a population. It was found that the traditional approach used to conservatively estimate sample size necessary to detect a given difference in group proportions potentially greatly underestimates the number of animals needed for vaccine efficacy trials. In vaccine efficacy trials, it is necessary to estimate the effect of population-level vaccination prior to estimating sample size. In such trials, as incidence proportion in the population or herd decreases or as vaccine efficacy decreases, necessary sample size increases. The author concludes that in designing a clinical or field trial, such as one to evaluate the efficacy of a vaccine against an infectious disease in a population, one needs to approach sample size calculations in a nontraditional manner. The proportion of the population that is vaccinated, disease transmission dynamics, and vaccine efficacy will affect the incidence in the non-vaccinated and vaccinated groups and, hence, sample size. Thus, estimation of the effect of the vaccination on the population must be made prior to calculating sample size. Otherwise, sample size and the power to identify vaccine efficacy will be insufficient.

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eters (including hemogram, reticulocyte count, and biochemical profile) and blood pressure is mandatory. Although the rcEPO or rfEPO is provided free of charge to study participants, the owners of the patient are responsible for all other diagnostic and therapeutic costs. Enrollment is limited based on a careful review of the candidate and suitability for inclusion in the clinical trial.

Preliminary clinical data with rcEPO and rfEPO have demonstrated successful resolution of nonregenerative anemia in dogs and cats. For animals that never received Epogen® or Procrit®, no individuals have been encountered so far that did not exhibit a positive erythropoietic response within two weeks of initiating rcEPO or rfEPO therapy. Both of the species-specific erythropoietin preparations continue to demonstrate a good safety profile without exhibiting any evidence of immunogenicity. As with exogenous erythropoietin administration to people, hypertension and functional iron deficiencies will develop in some dogs and cats and should be monitored. Re-establishing erythropoiesis in the rhEPO-induced red blood cell aplasia group has been variable, with approximately 50 percent of treated animals responding to date.

To receive more detailed information about participation in these clinical trials, email your postal mailing address to cepo@cornell.edu (for dogs) and fepo@cornell.edu (for cats). References are available upon request.

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