Development of Caniplas®
As an Aid in the Treatment of Canine Parvoviral Enteritis

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Objectives – To determine the efficacy of Caniplas® (canine antibodies to Escherichia coli J5 lipopolysaccharide) as an aid in the treatment of naturally-occurring canine parvoviral enteritis (CPE). To develop a new prognostic for CPE for dogs presenting at veterinary clinics.

Procedure – Concentration of specific immunoglobulin G concentration (µg/mL) to E. coli J5 lipopolysaccharide (LPS or endotoxin) in Caniplas® was determined using a proprietary quantitative enzyme immunoassay (EIA). Effect of Caniplas® with an anti-LPS antibody concentration >5 µg/mL was measured in a prospective, randomized, placebo-controlled clinical study of naturally-occurring CPE. A CPE prognostic for use in veterinary clinics was developed based on clinical and blood parameters measured in this clinical study.

Results – A single dose of Caniplas® (10mL/Kg or 5mL/lb) had significant positive clinical effects, including reduced diarrhea and vomiting, increased survival and reduced time in hospital, in dogs with confirmed CPE. Dogs with CPE and clinical signs of SIRS and abnormal lymphocyte or abnormal neutrophil counts have a poor prognosis.

Conclusions and Clinical Relevance – Results from a prospective clinical trial of naturally-occurring CPE suggest that Caniplas® is a cost-effective adjunct treatment for CPE and conditions where endotoxemia or increased inflammation play a role in pathogenesis.
Introduction

It is known that septicemia and/or bacterial translocation from the gut play a key role in the pathogenesis of a number of important canine veterinary conditions including:

- Parvovirus infection (CPE)\(^1\),
- Gastric atony\(^2\),
- Gastric dilatation/volvulus\(^3\),
- Acute pancreatitis\(^4\),
- Wounds, burns and hemorrhagic shock\(^5,6\),
- Ischemia and hypoxia\(^7,8,9\), and
- Pyometra\(^10\).

Endotoxin is thought to be responsible for most, if not all, of the features of Gram-negative septicemia, which is largely mediated by cytokines and acute phase proteins\(^11\).

Prior exposure to endotoxin and subsequent development of antibodies appears to protect animals from septicemia, conditions that lead to septicemia, and adverse clinical signs associated with septicemia.

Such protection to the adverse effects of inflammation and septicemia can also be gained from passive transfer of immunoglobulin. For example, intravenous immunoglobulin (IVIG) prepared from pooled human plasma is used in human medicine in the treatment of a wide range of conditions, from replacement therapy to sepsis to neuroimmunological diseases\(^12\) and in immune mediated conditions in dogs\(^13,14\).

Plasvacc has been researching the effectiveness of anti-lipopolysaccharide antibodies in treating septicemia because it is a complicating factor in many conditions in dogs.

This white paper describes:

- Use of Caniplas\(^6\) in a prospective, randomized, placebo-controlled clinical trial in naturally-occurring CPE in the United States,
- Development of a prognostic tool for use in CPE cases.

Such work aims to provide the small animal veterinarian with professional confidence in the knowledge they are using an effective, potent, appropriately labelled and safe product for the treatment of CPE and for conditions where septicemia is a potential complicating factor.

Materials and Methods

Potency Assay (EIA)

A quantitative EIA was developed to measure specific antibodies to J5 LPS (endotoxin) in Caniplas\(^6\). Briefly, reagents used in the EIA included known quantities of J5 LPS as the antigen bound to the plate and goat anti-dog IgG (H+L) conjugated to Horse Radish Peroxidase (HRP) as the reporter molecule. Results were expressed in µg/mL as measured using a standard curve of total IgG generated using a canine plasma of known IgG concentration.

Parvoviral Enteritis Clinical Trial

The study was a prospective, randomized, placebo-controlled clinical trial of the effects of Caniplas\(^6\) (containing > 5µg/mL anti-LPS antibodies) in dogs with naturally occurring CPE from the Los Angeles area. The study was blinded but only to the extent whereby those clinicians administering Caniplas\(^6\) or saline were not the same as those recording clinical signs. Thirty (30) client-owned dogs were recruited between October 2009 and August 2010. Inclusion criteria for enrollment included; the presence and recent onset of clinical signs of vomiting and/or bloody diarrhea, lethargy and anorexia, and a positive CPV fecal antigen test. Owner consent was obtained prior to any treatment. Enrolled dogs were assigned to one of three groups; those receiving a single dose of Caniplas\(^6\) (10mL/kg) within 24 hours of admission, those receiving two doses of Caniplas\(^6\) (10mL/kg each time) within 24 hours of admission and one day following the first dose, and those receiving a single dose of 0.9% saline (10mL/kg) within 24 hours of admission and supplemental to any fluids given at admission. Dogs were successively assigned to each group upon presentation to the clinic. All dogs received initial routine supportive care including fluids and antibiotics administered intravenously. All dogs were kept in an isolation ward and in separate cages. Any additional therapies given were at the discretion of the attending clinician but when used consisted of the same brand and dose rate of drugs. Dogs were discharged when the attending clinician deemed that the animal had returned to health. Information on the length of hospital stay was obtained from hospital records of surviving dogs. Clinical parameters and blood were taken from each dog at the time of admission and daily at the same time until discharge. A clinical score was generated based on numerical values assigned to the degree of; body temperature,
The higher the clinical score the sicker the dog. Caniplas® was supplied sterile and frozen in 100mL sterile PVC disposable bags. Prior to use plasma was thawed to approximately body temperature in warm water. Dose rate of Caniplas® was 10mL per Kg bodyweight given at a rate of 5-10 mL per kg per hour administered through a 200µM blood administration set. Clinical observations were recorded at admission and then daily at the same time. Blood was collected into tubes containing ethylenediaminetetraacetic acid following enrolment, one day following the first dose of hyperimmune plasma or 0.9% saline, and just prior to discharge for haematological analysis. Hematological parameters measured included; white blood cell count and differential, platelet number, red blood cell number, hemoglobin, volume and hemoglobin concentration, total hemoglobin and hematocrit.

Results

Caniplas® Improves Clinical Outcome in CPE

Dogs affected with naturally-occurring CPE treated with one dose of Caniplas® showed significantly improved overall clinical score (lower) at both 24 (p<0.03) and 48 (p<0.05) hours following treatment (see Figure 1 next page). Clinical improvement consisted mostly of decreased scores for fecal consistency at both time points (p<0.02) and vomiting at 24 hours post-treatment (p=0.005). All dogs treated with Caniplas® survived whereas only 60% of dogs treated with only saline survived. There was a significant difference for length of hospitalization between dogs treated with one or two doses of Caniplas® (p<0.005) as a result of three one-dose dogs being treated as outpatients. Caniplas® treatment had a significant, but transitory, depressive effect on red blood cell and platelet count 24 hours following treatment.

Prognostic Indicators in CPE

Dogs that died in this trial, irrespective of treatment, had higher average heart rate, respiratory rate, lower white blood cells, lower absolute number of neutrophils and lower absolute number of lymphocytes. Of the six dogs that died in the study, five dogs presented with at least two signs of systemic inflammatory response syndrome (SIRS) and had an abnormal neutrophil or lymphocyte count.

Discussion

Caniplas® anti-J5 LPS antibody concentration is greater than 5 µg/mL. This concentration raises recipient dog plasma to >1 µg/mL when Caniplas® is given at the prescribed dose rate of 10mL/Kg or 5mL/lb. Plasma anti-LPS concentrations greater than >1 µg/mL have been demonstrated to provide protection against a lethal, live E. coli challenge. In naturally-occurring CPE, Caniplas®, in conjunction with other supportive therapies, has a profound and statistically significant positive clinical effect within 24 hours mainly through reduced vomiting and diarrhea. The average length of hospitalization for dogs treated with a single dose of Caniplas® was 1.4 days which was significantly different to dogs treated with two doses (p<0.005). This compares favorably to published data of six days of hospitalization for CPE-treated dogs. However, data in the current study were skewed by the fact that three dogs receiving a single dose of Caniplas® were treated as outpatients.

Dogs with CPE and presenting with at least two signs of SIRS combined with either an abnormal neutrophil count or abnormal lymphocyte count had a poor prognosis - five of six dogs with these clinical parameters died. For the purposes of this study SIRS was defined as; heart rate > 140/min, respiratory rate > 30/min, temperature >39.2°C or <37.8°C. For the dog, normal neutrophil counts are between 2060 and 10,600/µL and normal lymphocyte counts are between 690 and 4500/µL.

Conclusion

Results from a clinical trial using Caniplas® in the treatment of CPE suggest that Caniplas® can be used as an adjunct treatment for CPE. Dogs with naturally-occurring CPE, at least two signs of SIRS, and an abnormal neutrophil or lymphocyte count have a poor prognosis.

An accurate prognosis combined with an effective adjunct treatment for CPE should encourage veterinarians and owners to opt for treatment rather than euthanasia. In this instance, owners are likely to be rewarded with a healthy dog, and veterinarians rewarded with higher successful case throughput and increased professional standing.
Figure 1: Mean Total Clinical Scores for three treatment groups of 10 dogs per group at three time points following diagnosis of CPE. A Total Clinical Score was based on numerical values assigned to the degree of; body temperature, attitude, vomiting and diarrhea where a higher score indicates a worse condition. Those dogs receiving a single dose of Caniplas® had significantly improved Clinical Scores including those for vomiting (p<0.005) and diarrhea (p<0.02) within 24 hours of treatment. Treated dogs had a shorter stay in hospital and increased survival.