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Research Article

A Short Communication on the Potential of an Antiviral Protein, RetroMAD1™, in Treatment of Canine Parvovirus Infection in Puppies

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Abstract

Canine parvovirus infection (CPV) is a common cause of gastroenteritis in dogs which is normally associated with high mortality in puppies. The efficacy of RetroMAD1™, a novel recombinant antiviral protein, was evaluated for CPV treatment. A total of 32 naturally CPV-infected puppies up to six-month-of-age, were used for this present study. They were divided into two groups: Group A (Control) which comprised 11 puppies were given symptomatic and supportive treatment, whereas Group B (RetroMAD1™-treated) which comprised 21 puppies were given RetroMAD1™ along with the same symptomatic and supportive treatment as in Group A. RetroMAD1™ was given orally at a dose of 0.6 mg/kg, *b.i.d*. two to three hours before meal for a period of 7 days. Present results showed that the RetroMAD1™-treated group had a 26-percentage point increase in survival rate when compared to the Control group (81% vs. 55% respectively), and shorter mean recovery time of 4.9 (95% CI, 4.0 to 5.8) days *vs.* 6.5 (95% CI,4.3 to 8.7) days. The results obtained suggested that CPV treatment outcome could be improved with the concurrent use of RetroMAD1™.

***Keywords**: Canine Parvovirus; RetroMAD1™; Oral Administration; Antiviral Treatment*

Introduction

Canine parvovirus belongs to the *Parvoviridae* family, is widely known to be highly contagious and is a major causative agent of acute gastroenteritis in dogs with a high mortality rate in puppies [1]. The significant signs observed from dogs with CPV were depression/lethargy, anorexia, dehydration, vomiting and diarrhoea (haemorrhagic and non-haemorrhagic) [2].

Treatment of CPV has primarily relied on supportive and symptomatic treatment, these included intravenous fluid therapy (crystalloid and/or colloid), antibiotic treatment, antiemetic treatment and along with nutrition and supplement support [2]. The efficacy of antivirals in treating CPV has been assessed in the past and the potential of recently derived antiviral proteins or drugs in treating CPV have also been evaluated. Oseltamivir, an antiviral agent used to treat influenza in humans, was evaluated in CPV treatment [3]. However, it was ineffective in reducing morbidity or mortality [5]. Acyclovir prevented viral replication and was assessed as a prophylactic prior to experimentally infecting puppies with canine parvovirus [4]. A feline omega-interferon (IFN-ω) was shown to be an effective therapeutic agent, with field trials reporting improvement in clinical signs and reduced mortality in the treatment group compared to the placebo group [5,6]. In spite of IFN-ω receiving marketing authorisation from the European Medicines Agency (EMA), the limited availability and use of antivirals in standard treatment of CPV continues to persist, emphasizing the need for more effective antiviral options.

RetroMAD1™ is a novel 37.5kDA recombinant protein consisting of the fusion of three naturally occurring antiviral peptides derived from various organisms: Retrocyclin (rhesus monkey, *Macaca mulatta)*, MAP30 (bitter melon, *Momordica charantia*) and Dermaseptin-S1 (monkey tree frog, *Phyllomedusa sauvagii)*. Each of these peptides possesses unique antiviral properties that have been well-characterised and documented [7-9].

The present study reported the results of a field trial study conducted to evaluate the therapeutic efficacy of RetroMAD1™ in treating 32 CPV-related clinicals signs puppies aged up to 6 months old and confirmed to have CPV using Anigen® Rapid CPV Ag Test Kit. The results obtained would shed light on its potential application as part of the standard CPV treatment regimen in future.

Materials and Methods

Materials

RetroMAD1™ was obtained from Biovalence Technologies Pte Ltd. It is a commercially available product in a sterile aqueous solution containing 4mg of active principal ingredient in 1.0 ml of solution. The treatment regimen was 0.6 mg/kg, *b.i.d*., *via* oral administration.

Design of study

A total of 32 clinical-ill puppies up to 6-month-old diagnosed with CPV using the Anigen® Rapid CPV Ag Test Kit were treated at the College of Veterinary Medicine and Agricultural Sciences, De La Salle Araneta University of the Philippines, in this present study. They were divided into two groups: Group A comprised 11 puppies (patients) which served as the Control group whereas Group B comprised 21 puppies (patients) which served as the RetroMAD1™-treated group. Group A was given supportive and symptomatic treatment and Group B was given supportive and symptomatic treatment with the addition of RetroMAD1™ as shown in table 1.

Group	Number of Patients	Procedure	Duration of Treatment	Parameter
A (Control)	11	Supportive and symptomatic treatment	Until recovery or death	Percentage recovery, length of recovery
B (RetroMAD1™-treated)	21	Supportive and symptomatic treatment with the addition of RetroMAD1™	Until recovery or death	Percentage recovery, length of recovery

Table 1: *The evaluation of CPV puppies (patients).*

The present study was conducted in a period of 6 months, from September 2012 to February 2013 and was carried out at the College of Veterinary Medicine and Agricultural Sciences of De La Salle Araneta University in Manila, the Philippines. Informed consent (either verbal or written) was obtained from the owners or legal custodians of all the patients described in this work for the procedure(s) undertaken.

Treatment regimen for group B

Each Group B patient received an oral administration of RetroMAD1™ at a dose of 0.6 mg/kg. The drug was administered orally through the use of a disposable syringe without needle. It was given twice daily at least 30 minutes before food intake. Each patient in Group B would receive a total of 1.2 mg/kg/day.

Supportive/symptomatic treatment for groups A and B

Supportive/symptomatic treatment included the use of crystalloid intravenous fluid for total parenteral nutrition to address dehydration, anti-emetics (metoclopramide) and antacid (ranitidine), and antibiotics such as amoxicillin, enrofloxacin or marbofloxacin to fight against bacterial gastroenteritis and possible septicaemia. The use of anti-diarrheal drugs was omitted because retention of intestinal contents within a compromised gut increases the risk of bacterial translocation and systemic complications. The use of other antiviral drugs such as oseltamivir, recombinant human granulocyte stimulating factor (G-CSF), NSAID's such as flunixin meglumine and anti-TNF was discouraged.

Recovery and mortality

The attending veterinarian was responsible for determining recovery or mortality of the puppies. Recovery period was measured by recording time in days between the start of treatment and the mortality or the recovery, so as to determine the efficacy of RetroMAD1™. In addition, clinical signs of recovery such as activeness, return to normal body temperature range, return of appetite, reduction of diarrhoea, etc were also recorded.

Statistical analysis

T-test was used to determine if the RetroMAD1™-treated group (Group B) had significantly shorter mean recovery times compared to the Control group (Group A). Contingency analysis using Fisher's exact test was also performed to determine significant differences in the frequency of both supportive treatments used and in the onset of clinical signs between the groups. Statistical analysis was carried out using GraphPad Prism version 5.02 for Windows (GraphPad Software Inc., La Jolla, CA, USA). For all analyses, $P < 0.05$ was considered significantly different.

Results

Recovery and mortality

The present results showed that by day 8 post-treatment, 81% of puppies in the RetroMAD1™-treated group (Group B; n = 21) had recovered, compared to 55% of puppies in the Control group (Group A; n = 11) (Figure 1a). Mortality rates by day 3 post-treatment peaked to 45% for the Control group vs. 10% for the RetroMAD1™-treated group (Group B) which peaked to 19% at day 6 (Figure 1b).

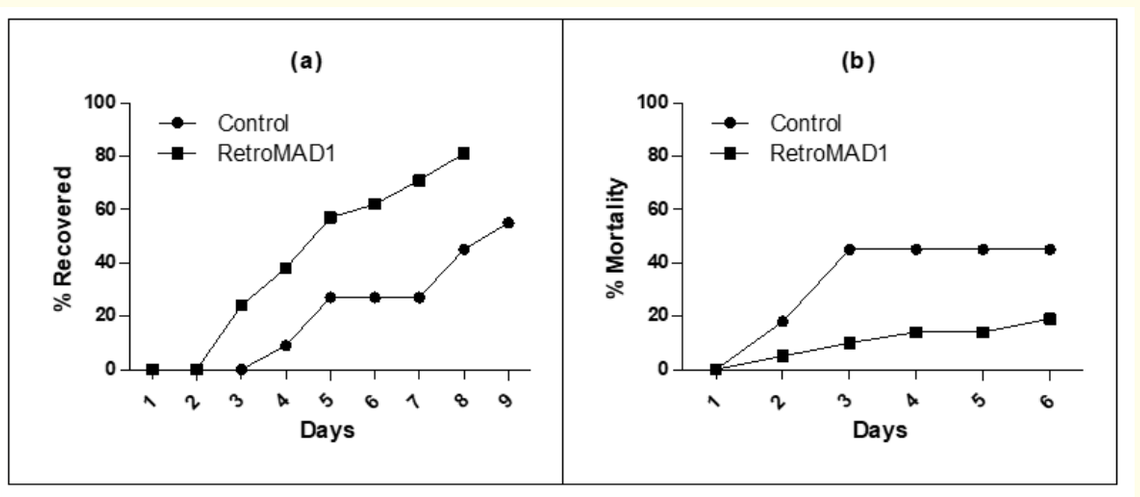

Figure 1: *Cumulative recovered rates (a) and cumulative mortality rates (b) of dogs in the RetroMAD1™-treated and control group.*

Recovery times were compared between the groups (Figure 2). The mean recovery time in the RetroMAD1™-treated group (Group B) was 4.9 days (95% CI, 4.0 to 5.8), which was significantly shorter than the 6.5 days for the control group (Group A) (95% CI, 4.3 to 8.7; P = 0.039).

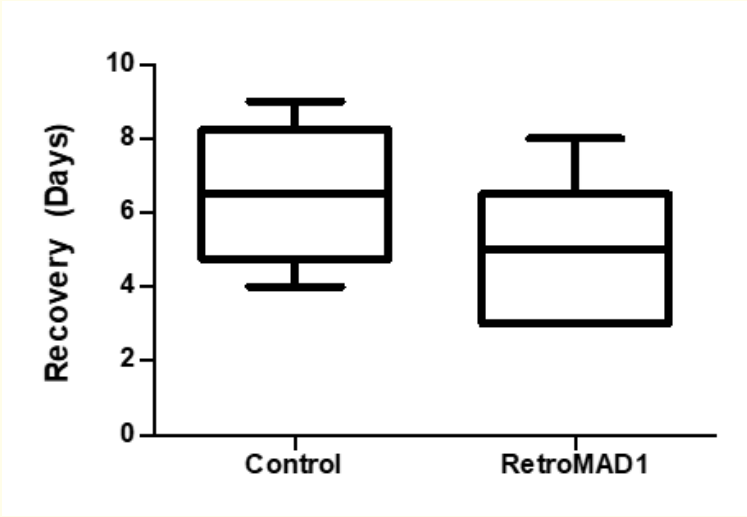

Figure 2: *Distribution of recovery times for RetroMAD1™-treated group and control group.*

Group differences in supportive/symptomatic treatment and clinical signs

Comparisons were made to identify group differences in the frequency of both supportive/symptomatic treatments received and starting clinical signs. The most commonly used supportive and symptomatic treatments for both groups were rehydration, vitamins and antibiotics. No significant difference in frequency of these treatments was found between the groups.

The most common clinical signs displayed by both groups were vomiting and bloody diarrhoea. No significant difference in starting frequency of these clinical signs was found between the groups.

Discussion

As RetroMAD1™ has been reported to successfully treat cats diagnosed with feline leukaemia, commonly referred to as FeLV [10] and is a single-stranded RNA virus.

RetroMAD1™'s promising therapeutic efficacy was demonstrated in the present study against canine parvovirus infection, a single-stranded DNA virus with a 26-percentage point increase in survival rate, i.e. 81% of recovery observed in RetroMAD1™-treated group (Group B) compared to 55% of recovery in Control group (Group A) over the course of 9 days. Shortened mean recovery time was suggestive of considerable therapeutic efficacy. In actual clinical practice, a day reduced from the usual treatment period would be considered clinically and economically important for the practitioner and for the patient. Comparisons made between the RetroMAD1™-treated and Control groups for starting frequency of both clinical signs and received supportive/symptomatic treatments revealed no significant differences which could have biased group comparison of treatment outcomes.

The poorer treatment outcome observed in the Control group (Group A) was expected. The lower survival rate (55%) was similar to typical documented rates between 64.0% to 64.1% with standard supportive/symptomatic treatment [11,12]. Additionally, the longer mean recovery time for control group with the average of 6.5 days (95% CI, 4.3 to 8.7) was comparable to standard treated cases (5.7 ± 2.5 days) [13].

In a separate study conducted by Biovalence Technologies Pte Ltd, faecal samples from CPV-infected puppies (n = 5) with clinical signs and treated with RetroMAD1™, demonstrated a significant drop in CPV viral titre (RT-PCR) of > 98% as early as 2 days from the start of treatment [14].

Conclusion

The improved survival rate (26-percentage point increase) and shortened mean recovery time (1.6 days less) associated with RetroMAD1™ suggested that current standard treatment of CPV in dogs could substantially benefit from its routine use, especially in communities with high prevalence, mortality and morbidity. Although survival rates as high as 90% have been reported [15], the level of care available in other communities might not be high due to a difference in disposable incomes for veterinary interventions. RetroMAD1™ could therefore play a critical role in such communities where CPV would be an epidemic. An added cost benefit to dog owners could come with the shortened hospitalisation/intensive care, potentially making CPV treatment more affordable in overall. As an oral administration, continuing RetroMAD1™ treatment after discharge from the veterinary clinic could further reduce time to complete viral clearance, effectively reducing the shedding period and thereby reducing risk of transmission, particularly in multiple dog households. Nevertheless, randomised blind design should be adopted in future study in order to eliminate potential bias associated with the tendency for recruitment of more clinically severe cases (desperate) inherent in a client consent design, which would have resulted in an underestimation of the actual survival rate with RetroMAD1™ use.

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Conflict of Interest

The following authors are employed by Biovalence Technologies Pte Ltd, a private-owned biotechnology company: Alfred Chua. The author declares no competing interests.

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