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Clinico-Therapeutic Trial of Acyclovir against Canine Parvovirus Infection

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Abstract

The main goal of this study was to investigate the safety and efficacy of acyclovir, as an antiviral agent on the clinical recovery of puppies with acute hemorrhagic diarrhea. The dogs were naturally infected by CPV-2. Out of the total 30 dogs, 20 dogs were naturally infected with CPV-2, and 10 healthy dogs less than 6 months of age were selected. The animals were grouped into 3 groups, each containing 10 puppies. Group A was treated with Acyclovir along with supportive treatment, group B was treated with only supportive treatment, while group C was kept as a negative control group and without treatment. Blood samples and fecal samples were collected before treatment and after treatment from all groups. In parvo-infected dogs, marked hemoconcentration, leucopenia, neutropenia, and alterations in the biochemical profile were noticed as compared to healthy dogs. The Acyclovir along with supportive treatment progressively improved the leukocyte, neutrophil counts over time in parvovirus-infected dogs compared to dogs that received only supportive treatment. Our obtained results show that Acyclovir has the best effect in the prevention of CPV2 replication in puppies through the absence of a clinical sign of CPV-2 and hematological recovery.

Keywords: Acyclovir; Canine parvovirus; Supportive treatment

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Introduction

Canine parvovirus enteritis is considered one of the most devastating contagious canine diseases and it is still progressing around the world [1]. Canine Parvovirus has two types, known as CPV1 and CPV2. Patients infected with CPV-1, are mostly asymptomatic. CPV2 causes the most serious infection and affects wild canids and domestic dogs. CPV2 has two strains known as CPV-2a and CPV-2b [2].Canine Parvovirus 2 (CPV-2) is a single-stranded, non-enveloped, DNA virus that belongs to the *Parvoviridae* family. It has icosahedral symmetry and ranges to 25 nm in diameter [3]. It is believed to be the common cause of enteritis in dogs. The transmission of the virus takes place by the fecal-oral route, after 3-10 days of virus transmission, the animal starts displaying clinical manifestation of increased body temperature, a decrease in appetite, bloody diarrhea vomiting, and marked leukopenia is also seen in this malady. On 8-10 days of post-infection, high titer can be seen in feces of infected dogs [4]. Canine Parvovirus (CPV) infection is an extremely contagious disease of dogs. CPV causes high morbidity and mortality in dogs, mainly in puppies, and is present everywhere in the world [4]. In CPV infected dogs, there were significant changes in packed cell volume, neutrophils, lymphocytes, and hemoglobin concentration was reported. Biochemical investigation revealed a significant decrease in plasma glucose, total plasma protein, A: G ratio (albumin: globulin). The level of potassium and chloride was markedly declined from the normal value. There was a marked increase in ALT (Alanine Aminotransferase) and BUN was observed [5].

Acyclovir belongs to the class of antiviral drugs. It is extremely selective and has a very low cytotoxic effect [6]. At an early age, it was primarily used against herpes simplex, chickenpox, and shingles virus infection [7]. Aciclovir monophosphate is produced from Acyclovir with the help of a viral enzyme (thymidine kinase), which is then changed to ACV-TP (Aciclovir Triphosphate) by an enzyme known as kinases to ACV-TP. This compound can block the active site of HSV-specified DNA polymerases. So the enzyme with a blocked active site not able to synthesize the DNA of the virus [8].

The main goal of our study is to investigate the antiviral effect of Acyclovir against canine parvovirus. These biologically active substances are well known because of their antiviral properties and their use against infectious diseases. We want to check its

efficacy against canine parvovirus infection so that it can be used in the development of medicine against this disease.

Materials and Methods

The study was conducted from February 2019 to August 2019 at the hospital of the University Of Veterinary and Animals Sciences Lahore, Pakistan. A total of Thirty animals (n=30) were part of the study, twenty animals (n=20) positive for CPV-2 infection were selected and were divided into two groups (A and B). While group C was kept as a control negative group having (n=10) healthy animals. The criteria for selection of dogs were irrespective of breed and sex. Ethical Committee of the University approved this study on the Use of Animals (numbers 245/2019). The animals' owners consented to participate in the study by signing an Informed Consent Form for Research.

Inclusion criteria for sampling

Fecal samples were taken from those dogs that showed similar typical clinical signs of CPV-2 infection like severe bloody diarrhea, lethargy, anorexia, fever, vomiting, and weight loss. Quacking Biotechnology rapid detection kits were used for confirmatory diagnosis of CPV-2 antigen. The procedure was used as per the protocol of manufacturer guidelines.

Hematological examination

3 ml of blood was collected on day 0 before any treatment and Day 6 after the treatment from group A,B and C animals. Cephalic or Saphenous veins were used to collect the blood and were transported into an EDTA coated vacutainer. Total Erythrocytes Count (TEC), Total Leukocytes Count (TLC), Hemoglobin (Hb), platelets count, and Pack Cell Volume (PCV) were analyzed through the hematological analyzer.

Collection of serum sample

Blood (5 ml) was collected in a disposable syringe from group A, B and C animals, through the cephalic or saphenous vein. The collected blood was poured into a gel coated vacationers, were labeled and kept in boxes containing ice, and carried to the Medicine Department, UVAS Lahore, and was placed in a refrigerator. Serum was collected by centrifugations at 3,500 rpm for 5 minutes from all the collected samples then properly labeled and placed at -20°C then Liver function test like ALP, AST and ALT were done through automatic serum chemistry analyzer.

Results

Virus recovery

At the time of admission CPV-2 antigen had recovered from group A and B. Following the completion of therapeutic trials, CPV-2 antigen had recovered from eight animals of group B after the examination of all fecal swabs, while CPV-2 antigen had recovered from only one animal of groups A (**Table 1**).

Effect of acyclovir on clinical sign of canine parvovirus

On day 0 (before treatment) it was observed that there were all clinical signs of CPV like bloody, diarrhea, lethargy, anorexia, fever, vomiting, severe weight loss, pale mucus membrane, sunken eyes and were present in the animals of group A and B while on day 6th (after treatment) when all groups were clinically examined, a significant improvement in their health status was observed and no clinical signs of CPV had found in Groups A and Group C (healthy dogs) while in Group B (only supportive treatment) we had observed a fever 40.5°C, vomiting, and foul-smelling diarrhea and vomiting. The Acyclovir was considered safe because none of the puppies assigned to the Acyclovir group developed any clinical abnormalities that were attributable to the procedure (**Figures 1a and 1b) (Table 2**).

Figure 1a CPV infected dogs showing hemorrhagic diarrhea.

Figure 1b CPV infected dogs showing dehydration and depression.

Table 1: Detail of CPV-2 Infection in different groups before and after Treatment.

Group A (10)		Group B (10)		Group C (10)	
Before R_x	After R_x	Before R_x	After R_x	Before R_x	After R_x
Positive(10/10)	Negative (9/10)	Positive (10/10)	Negative (2/10)	Positive (0/10)	Negative (10/10)

Table 2: Group A: Acyclovir and supportive treatment, Group B: Only supportive Treatment, Group C: Healthy group (control group).

Clinical sign	Group A (10)		Group B (10)		Group C (10)
	Before R_x	After R_x	Before R_x	After R_x	No R_x
Anorexia	Present (10/10)	Absent (10/10)	Present (10/10)	Absent (2/10)	Absent (10/10)
Depression	Present (10/10)	Absent (10/10)	Present (10/10)	Absent (2/10)	Absent (10/10)
Temperature	Present (6/10)	Absent (10/10)	Present (7/10)	Absent (2/10)	Absent (10/10)
Abdominal pain	Present (3/10)	Absent (10/10)	Present (5/10)	Absent (2/10)	Absent (10/10)
Diarrhea	Present (10/10)	Absent (10/10)	Present (10/10)	Absent (2/10)	Absent (10/10)
Vomiting	Present (7/10)	Absent (10/10)	Present (7/10)	Absent (2/10)	Absent (10/10)

Table 3: Group A: Acyclovir and supportive treatment, Group B**:** Only Supportive Treatment.

Parameters	Group A			Group B		
	Before treatment	After treatment	P-value	Before treatment	After treatment	P-value
WBC (10 × 9/l)	4.77 ± 0.76	9.60 ± 0.89	0	4.77 ± 0.76	5.81 ± 0.64	0.124
LYM (%)	10.00 ± 1.12	18.00 ± 1.10	0	10.00 ± 2.75	12.00 ± 2.10	0.362
NEUT (%)	30.0 ± 4.85	79.0 ± 4.60	0	34.0 ± 4.10	36.0 ± 4.177	0.301
RBC (10 × 12/l)	4.98 ± 0.43	6.36 ± 0.24	0.017	4.10 ± 0.42	4.84 ± 0.42	0.309
Hb (g/dl)	10.23 ± 0.41	12.24 ± 0.38	0.002	10.13 ± 0.43	11.32 ± 0.54	0.106
PCV%	38.00 ± 1.62	45.0 ± 0.57	0.001	38.0 ± 1.62	42.00 ± 1.66	0.168
ALT(IU/l)	204.0 ± 14.44	161.0 ± 10.68	0.024	206.0 ± 14.44	176.0 ± 14.24	0.151
AST (IU/l)	69.0 ± 1.97	61.0 ± 2.85	0.025	69.0 ± 1.44	64.0 ± 2.20	0.069
ALP (IU/l)	215.0 ± 1.20	174.0 ± 4.42	0	205.0 ± 9.59	182.0 ± 6.61	0.06

Haematological examination

The blood was collected and analyzed on day 0 and day 6 of all animals of all groups. At day 0 it was found that the mean values of WBC, Hb, neutrophil, RBCs were decreased in infected dogs as compared to healthy dogs. Following consecutive treatment with Acyclovir and supportive therapy, it was observed that WBC, Hb, neutrophil, RBCs, were significantly improved ($p<0.05$) in the animals of group A on day six. While no significant improvements ($p>0.05$) in hematological parameters was observed on day six in animals of group B following consecutive treatment with supportive therapy alone.

Biochemical examination

There was a significant elevation in ALT, AST, and ALP was observed in infected dogs (groups A and B) as compared to healthy dogs. Following six days of consecutive treatment with Acyclovir and supportive therapy, it was observed that the ALT, AST and ALP were significantly ($p<0.05$) decreased on day six. While non-significant reduction ($p>0.05$) in biochemical parameters (ALP, AST, ALT) was observed in animals of group B after treatment with only supportive therapy (**Table 3**).

Discussion

In the present study, it was observed that canine parvovirus causes a decrease in white blood cells, neutrophils, hemoglobin, and decreased packed cell volume which was correlated with the past observations [9]. There was a significant decrease in mean hemoglobin value as compared to healthy dogs which are due to loss of blood in diarrhea and decreased erythropoiesis as a result of the direct effect of parvovirus on the bone marrow, accumulation of toxic waste products during viremia and febrile phase of the disease and preexisting poor health status of the dogs. Vomiting, dehydration, hemorrhagic enteritis, anemia, dehydration, increased ALT, and ALP were the common findings in this study [10]. Leucopenia and neutropenia were attributed to the cytotoxic effect of the virus on hematopoietic and bone marrow cells during the acute phase of the disease [11]. The elevation of liver enzymes had resulted from hepatic disorders and the development of inflammatory bowel disease [12,13].

Many drugs were used in the treatment of viral infections like canine parvovirus infection. In the present study, it was observed that Acyclovir along with supportive therapy had good efficacy against CPV-2 replication and a restorative effect on haemato-biochemical recovery with $p<0.05$. The above result is also supported by the past observation made by El-Gallad [14] who reported that Acyclovir had good antiviral efficacy against canine adenovirus-1 (which is DNA, non-envelope, same symmetry, characterized by the same clinical sign as CPV. The end product of Acyclovir blocks the active site of HSV-specified DNA polymerases, which is responsible for DNA synthesis and inhibition of the further activity of this enzyme occurs [15,16].

Conclusion

In the current study, the supportive therapy alone had no effect on Hemato-biochemical recovery and it keeps hastening as the infection advances. So, no significant changes had been found in hematological and biochemical parameters recovery ($p>0.05$). Supportive treatment also prolongs the recovery period and weakens the immune system of the dog as the infection advances. Adjunct antibiotic therapy with Vitamin C and N-acetylcysteine had an additive effect in assisting early recovery with the restoration of Hemato-biochemical parameters when compared

with the groups which were administered with antibiotics alone.

So, it was concluded that Acyclovir along with supportive treatment was effective for the hastening hemato-biochemical recovery, but supportive treatment alone was not enough for the recovery of hemato-biochemical parameters and prevention of CPV-2 multiplication.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authors Contribution

All the authors contributed to the research and writing of the manuscript and approves the publication.

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