Investigation of the antiviral effect of acyclovir on canine parvovirus infection

Abstract
The aim of the present study was to evaluate the Acyclovir as antiviral agent in prophylactic treatment of puppies experimentally infected by canine parvovirus 2. Fifteen apparently healthy native puppies less than 9 wks. old were grouped into 3 groups, each containing 5 puppies. These puppies were found to be free from CPV antibodies. The 1st group was prophylactically treated with Acyclovir; the 2nd group was kept infected without treatment, while the 3rd group was kept without infection and without treatment as control. Blood samples and fecal samples were collected at 0 day and daily up to 5th day post infection from all groups. Our results indicated that The Acyclovir regime was succeeded in prevention of CPV2 replication in puppies through absences of viral particles in fecal swabs.

Keywords: acyclovir, antiviral agents, canine parvovirus 2, puppies

Abbreviations: CPV2, canine parvovirus enteritis; TCID_{50}, tissue culture infected dose 50; AST, serum aspartate aminotransferase; ALT, serum alanine aminotransferase; SEM, standard error of the mean

Introduction
One of the most devastating canine diseases is canine Parvovirus enteritis which is a highly contagious disease that occurs worldwide, but is still evolving. Since the initial parvovirus enteritis pandemic following the emergence of canine parvovirus type 2 (CPV-2). Three variants, namely CPV-2a, 2b and 2c completely replaced the original type 2 viruses. Canine parvovirus (CPV) is an important cause of morbidity and mortality in puppies younger than 6months. CPV-2 induced disease is observed mainly in 6-12weeks-old pups; whereas, younger dogs are generally protected from CPV-2 infection by maternally-derived immunity. CPV-2 spreads from infected to susceptible dogs by the fecal-oral route and reaches high titers in the feces of infected dogs. Survival rate depends on how quickly CPV is diagnosed, the age of the animal and how aggressive the treatment is. Treatment usually involves extensive hospitalization, due to the severe dehydration and damage to the intestines and bone marrow. A CPV test should be given as early as possible. If CPV is suspected in order to begin early treatment and increase survival rate if the disease is found. It is an interesting subject to know something about antiviral drugs which developed progressively over the last few years. The list of antiviral agents approved for use by the US Food and Administration, includes acyclovir and ribavirin. Generally, antiviral agents inhibit steps in virus-specific replication. Acyclovir is guanine analogue commonly used antiviral drug of low Cytotoxicity and primarily used for treatment of herpes simplex virus infection. The objectives of this study were to record the therapeutically evaluation of an Acyclovir as a prophylactic antiviral agent for canine parvovirus enteritis.

Materials and methods

Puppies
Fifteen apparently healthy native puppies less than 9weeks old were grouped in to 3 groups each contain 5 puppies. These puppies were found to be free from CPV antibodies as screened by serum neutralization test. The 1st and 2nd groups were experimentally infected with the virulent CPV2 through the intranasal route using dose of 5log_{10} TCID_{50}/animal. The 1st group was treated with Acyclovir using dose of 20mg/kg every 8hours injected intravenously for 5days according to the manufacturer direction. While the 2nd group was kept infected without treatment. The 3rd group was kept without infection and without treatment.

Virus
Virulent and live attenuated canine parvovirus was kindly supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. The virulent virus was used in experimentally infection of puppies. While the live attenuated virus was used for detection of CPV antibodies using serum neutralization test.

Acyclovir
Acyclovir (90\{2-hydroxyet-hoxy\} methyl\} guanine) was obtained from Sigma Chemical Company (St. louis, Mo.). The commercial acyclovir 5% injection was obtained from a local pharmacy and used for treatment of experimentally infected animals.

Clinical scoring system
Regarding experimentally infected puppies; a published clinical scoring system14 was used to evaluate 5 clinical attributes of each patient: attitude, appetite, vomiting, temperature and feces. A score of 0 represented a clinically normal parameter, with increasing severity of signs as the score increased up to a maximum of 3 for each variable (Table 1).
Samples

Blood samples were collected without anticoagulant from all puppy groups for serum separation to follow up liver functions. The separated serum samples were kept at -20°C till used. In addition blood samples were collected on anticoagulant from all puppies for hematological examination. In addition, seventy five fecal swabs one from each one daily up to 5 days were collected from infected treated puppies and infected non-treated puppies for trails of viral recovery.

Estimation of serum proteins

Serum total protein was estimated in the sera of tested dogs according to Weichselbauz, while serum albumin was estimated according to Ness. Serum globulin was determined by subtraction of serum albumin from the total serum protein.

Evaluation of liver functions

Serum asparate aminotransferase (AST) and serum alanine aminotransferase (ALT) were evaluated in the sera of all dogs groups using the specific kits according to Reitman and Frankel.

Hematological examination

Total leukocytic count was carried out using hemocytometer and turkey’s solution as diluents; while the differential leucocytic count was carried out using a stained blood film with Giema’s stain according to Coles.

Virus recovery

Trials for recovery of CPV from experimentally infected puppies were carried out on Vero cell culture and through the detection of viral antigen using Antigen Rapid CPV/CCV Ag test kit was supplied by BIONOTE, 2-9, Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea (445-170) in collected fecal samples according to the manufacturer directions.

Statistical analysis

Data were expressed as mean±standard error of the mean (SEM). All data were tested for normal distribution by using Kolmogrov-Smirnov test for normality. Then, subjected to Analysis of Variance (one way-ANOVA) followed by post hoc LSD test for multiple comparisons. This was carried out by using Statistical Package for Social Sciences (SPSS Inc, version 17 Chicago, USA).

Results and discussion

The development of antiviral drugs is still in its infancy with rapid changes and progressive milestones encountered almost daily. The last two decades have been the most dynamic in the history of viral infections and their management. Unfortunately, antiviral drugs have been effective for only a few groups of viruses up until now. Most antiviral drugs do not produce a cure, but rather allow control of the infection. However, the limitations of antiviral therapy, including the high costs of drugs, make the need for prevention even more urgent. Clinical examination to the three groups showed that there was no clinical signs observed in 1st and 3rd groups (treatment and control ones), but in 2nd group (untreated one) showed fever 39.5°C, mild vomiting and soft feces at 2nd day and then increase in 3rd day, which become moderate watery diarrhea, fever 40°C and vomiting (4-10 times) daily and in fourth and fifth days observed sever signs fever 41°C watery bloody diarrhea and sever vomiting more than 12 times daily (Table 1) (Table 2) and this result were similar to result of, who revealed that the experimental infection by CPV2 showed high fever and bloody diarrhea and vomiting at 4th day post infection. Virological examination for all fecal swabs revealed CPV2 recovered from 2nd group, while the 1st group failed in virus recovery and control group still sterile for CPV2 and this result were similar to result of Spibey et al., who revealed that the virus was detected in swabs taken from untreated group from 1st day to 5th day post infections.

Clinical pathological examination as showed in Table 3; the white blood cell values that were evaluated were compared between groups. There were differences between groups. It was noted that 1st group which treated by acyclovir, showed decrease in the main of WBC and lymphocyte count at 3rd and 4th days, while in 2nd group, showed that significant decrease in total leukocyte and lymphocytes after infection and no any changes were noticed in 3rd group. Concerning to the liver function and total serum protein (Table 4), decrease in total protein (hypoproteinaemia) and increase liver function (ALT and AST) in 2nd group, while the 1st and 3rd groups were within normal value, these results with agree with Otto et al., who mentioned that leukopenia is considered a characteristic and often diagnostic quality of CPV infection, in addition to our results are going in harmony with those obtained with they found that the white cell counts demonstrated that virus causes a leukopenia in the unvaccinated controlled animals, whereas the vaccinated group remained normal. Hypoproteinaemia, in particular hypoalbuminemia, is another common clinical pathological abnormality associated with CPV enteritis. This is a result of a combination of factors, including intestinal loss, decreased synthesis as a negative acute phase protein and decreased nutritional intake. Other laboratory abnormalities vary and can include increased liver enzymes.

Concerning to the effect of Acyclovir on treatment of CPV2 in experimentally infected puppies. It was successes in preventing of CPV2 replication in puppies as showed in Tables 2–4 and virus recovering, which revealed absences of viral particles in fecal swabs, leukopenia, lymphopenia and hypoproteinaemia in compared to 2nd group and this supported by Piriet et al., who mentioned that the Acyclovir differs from previous nucleoside analogues in containing only a partial nucleoside structure: the sugar ring is replaced with an open-chain structure. It is selectively converted into acyclo-guanosine monophosphate (acyclo-GMP) by viral thymidine kinase, which is far more effective (3000 times) in phosphorylation than cellular thymidine kinase. Subsequently, the monophosphate form is further phosphorylated into the active triphosphate form, acyclo-guanosine triphosphate (acyclo-GTP), by cellular kinases. Acyclo-GTP has approximately 100 times greater affinity for viral than cellular polymerase. As a substrate, acyclo-GTP is incorporated into viral DNA, resulting in chain termination. It has also been shown that viral enzymes cannot remove acyclo-GTP from the chain, which results in inhibition of further activity of DNA polymerase. Acyclo-GTP is fairly rapidly metabolised within the cell, possibly by cellular phosphatases. Similar results were obtained by Gertrudeb who tested the antiviral effect of Acyclovir against herpes virus type-1 (which is a DNA virus as CPV) and these results come in complete agreement with obtained using Acyclovir against HV-1 in skin samples from experimentally infected mice by Piriet et al. Detectable antiviral effect against canine hepatitis virus and it was valuable to reduce the severity of experimental infection of puppies with the virulent virus as CPV were recorded.

Table 1 Clinical scoring system

<table>
<thead>
<tr>
<th>Score</th>
<th>Attitude</th>
<th>Appetite</th>
<th>Vomiting</th>
<th>Feces</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Normal</td>
<td>Absent</td>
<td>Well-formed or absent</td>
<td>Normal (38)</td>
</tr>
<tr>
<td>1</td>
<td>Mild to moderate depression</td>
<td>Voluntarily eats small amounts</td>
<td>Mild; once per 12hours</td>
<td>Soft or pasty Feces</td>
<td>Mild increase (39.5)</td>
</tr>
<tr>
<td>2</td>
<td>Severe Depression</td>
<td>No interest In Food</td>
<td>Moderate; 2-5times per 12hours</td>
<td>Watery diarrhea, non-bloody</td>
<td>Increase (40)</td>
</tr>
<tr>
<td>3</td>
<td>Collapsed or Moribund</td>
<td>Not offered</td>
<td>Severe;&gt; 6times per 12hours</td>
<td>Watery, bloody diarrhea</td>
<td>Sever increase (41)</td>
</tr>
</tbody>
</table>

*Scores for each category were assigned to each dog twice daily to encompass the previous 12hour period

Table 2 Clinical scoring system. Scores for each category were assigned to each dog twice daily to encompass the previous 12hrs

<table>
<thead>
<tr>
<th>Tested groups</th>
<th>Clinical score</th>
<th>1st Day</th>
<th>2nd Day</th>
<th>3rd Day</th>
<th>4th Day</th>
<th>5th Day</th>
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</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Group 1, Treatment by acyclovir
Group 2, Challenge by virulent CPV without treatment
Group 3, Normal without treatment or infected

Table 3 Differential leukocyte count in treated group with acyclovir and in non-treated group

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group 1</td>
<td>13980</td>
<td>2487.6</td>
<td>12026</td>
<td>1896</td>
<td>10026</td>
<td>1159.8</td>
<td>10500</td>
<td>1732</td>
<td>11348</td>
<td>1357</td>
</tr>
<tr>
<td>2</td>
<td>Group 1</td>
<td>11980</td>
<td>1897.4</td>
<td>10560</td>
<td>1335.5</td>
<td>9114</td>
<td>908.7</td>
<td>7950</td>
<td>691.9</td>
<td>7278</td>
<td>607.1</td>
</tr>
<tr>
<td>3</td>
<td>Group 1</td>
<td>15000</td>
<td>4500</td>
<td>14950</td>
<td>4250</td>
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<td>4500</td>
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<tr>
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<td>12026</td>
<td>1896</td>
<td>10026</td>
<td>1159.8</td>
<td>10500</td>
<td>1732</td>
<td>11348</td>
<td>1357</td>
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<tr>
<td>Group 3</td>
<td>11980</td>
<td>1897.4</td>
<td>10560</td>
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<td>9114</td>
<td>908.7</td>
<td>7950</td>
<td>691.9</td>
<td>7278</td>
<td>607.1</td>
<td></td>
</tr>
</tbody>
</table>

TLC, total leukocytic count; Ly, lymphocytes
Group 1, treatment by acyclovir
Group 2, challenge by virulent CPV without treatment
Group 3, normal without treatment or infected

Table 4 Estimation of ALT, AST and total protein in acyclovir group with untreated group

<table>
<thead>
<tr>
<th>Tested groups</th>
<th>ALT</th>
<th>AST</th>
<th>Total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>30</td>
<td>25</td>
<td>7.3</td>
</tr>
<tr>
<td>Group 2</td>
<td>103</td>
<td>70</td>
<td>3.96</td>
</tr>
<tr>
<td>Group 3</td>
<td>25</td>
<td>20</td>
<td>7.6</td>
</tr>
</tbody>
</table>

ALT, 10-94IU/L
AST, 10-62IU/L
Total protein, 5.3-7.6g/dl

Conclusion

The results of this study highlight the successes of Acyclovir regime in treatment of CPV infection in puppies.

Acknowledgements

None.

Conflict of interest

Author declares that there is no conflict of interest.

References