EFFECTS OF GS-441524 ON CLINICAL AND HEMATOCHEMICAL PARAMETERS OF CATS WITH EFFUSIVE FIP OVER 60 DAYS FOLLOW-UP

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ABSTRACT

Feline infectious peritonitis (FIP) is an important disease characterized by granulomatous changes in various organs including the ocular and central nervous system. FIP infection is referred as the best candidate for antiviral drug development studies due to the ineffectiveness of vaccines and being a cause of high mortality. The most important antiviral drugs developed against RNA viruses are nucleoside analogues such as GS-441524. In this study, GS-441524 was used in the treatment of three naturally infected cats with ocular lesions diagnosed with effusive FIP. The treatment of cats was planned as GS-441524 (5 mg / kg, SC, daily) administration for 12 weeks along with fluid therapy on the admission day. The treatment process included follow-ups with clinical and laboratory examinations on the admission 5th, 15th, 30th, 60th, 90th and 120th days. On the 5th day, clinical signs including ocular abnormalities and on the 30th day, laboratory findings were significantly improved in all 3 cats. While the cats were expected for monthly follow-ups, it was learned that the cats died on the 58th, 60th and 62nd days. It was concluded that GS-441524 (5 mg/kg/day, SC) has been proven to be beneficial for improving clinical, laboratory findings and animal welfare. However, it was evaluated that clinical studies involving more cases are required in order to determine the clinical efficacy of nucleoside analogues and investigate the causes of deaths occurred on the 58th, 60th and 62nd days of the treatment period with no symptoms that would grab the attention of the owners.

Keywords: Coronavirus; feline infectious peritonitis; hematology; nucleoside analogues; treatment

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INTRODUCTION

Coronaviruses can be seen in many mammal species, including humans and birds, causing acute or chronic infections due to cell tropism (Enjuanes *et al*., 2006). Feline coronaviruses (FCoV) are pleomorphic, enveloped, single-stranded and non-segmented RNA viruses (Pedersen, 2009). There are 2 pathotypes of feline coronaviruses: feline enteric coronavirus (FECV), which is the enteric biotype and feline infectious peritonitis virus (FIPV), which is the virulent biotype (Kipar and Meli, 2014). Feline infectious peritonitis (FIP) is generally seen in cats younger than 2-3 years and older than 10 years. Although the morbidity rate is low (10%), the mortality rate is quite high (90%). The course of the disease varies depending on the immune status of the host (Hartmann, 2005). FIP has 3 major clinical forms: effusive (exudative), dry (non-effusive, non-exudative, granulomatous, parenchymatous) and mixed. Non-specific symptoms such as anorexia, weight loss, depression and fever develop in FIP infection (Kipar and Meli, 2014). The clinical appearance may vary according to the involvement of kidney, liver, eye, pancreas and central nervous system (CNS). Clinical and pathological laboratory findings are related to vasculitis and related organ failure. The effusive form is characterized by fibrinous peritonitis, pleuritis or pericarditis with effusion in the abdomen, thorax and/or pericardium. The non-effusive form is characterized by granulomatous changes in the organs (Hartmann, 2005; Pedersen, 2009; Kipar and Meli, 2014).

Treatment of RNA and DNA virus infections in humans has focused on drugs that inhibit virus replication (De Clercq and Li, 2016). FIP infection is referred as the best candidate for antiviral drug development studies due to the ineffectiveness of vaccines, difficulty of protection and being cause of high rate of mortality (Dickinson, 2020). The most important antiviral drugs developed against RNA viruses are GS-5734 (Remdesivir, Gilead Sciences®) and GS-441524 (Gilead Sciences Inc.®, Foster City, California), which have been used against FIP infection in cats (Pedersen *et al*., 2019). In a study conducted on 31 cats naturally infected with FIP (Pedersen *et al*., 2019), the use of a nucleoside analogue GS-441524 at a dose of 5-10 mg/kg (SC, daily) it has been reported that regardless of the clinical form of the infection, it provides rapid clinical improvement in mentation, appetite and general activity in a period of 24-36 hours (Dickinson *et al*., 2020).

Although GS-441524 was reported to provide clinical improvement, to the best of the authors' knowledge, no study has been found that focused on the hematological and biochemical changes in cats with effusive FIP. Therefore, in this study, clinical and laboratory examination outcomes of GS-441524 (5 mg/kg, SC, daily) treatment over a period of approximately 2 months in 3 cats naturally infected with effusive FIP are evaluated.

MATERIALS AND METHODS

The animal material of the present study was 3 owned cats, which were admitted to Selcuk University Animal Hospital of Faculty of Veterinary Medicine for diagnosis and treatment purposes. Clinical examinations of all the cats were performed in the clinics of Department of

Internal Medicine of the Animal Hospital and the laboratory analyzes of the samples of the cats were performed in the Central Laboratory of the Animal Hospital between February–April 2021. The diagnosis of effusive FIP was made on the basis of CBC, serum biochemistry analysis including albumin, globulin and total protein concentrations (Kipar and Meli, 2014) and albumin:globulin ratio (A:G <0.8) (Worthing *et al*., 2012) measurements, presence of clinical findings such as anorexia, lethargy, weight loss, jaundice, effusion, ocular lesions, upper respiratory signs and positive FCoV Ag test (Pedersen *et al*., 2019) along with imagining techniques (Figure 1). In order to rule out the diseases that may cause similar clinical findings to FIP, FIV Ab (Asan Easy Test® FIV Ab / FeLV Ag, ASANPharm, Korea), FeLV Ag (Asan Easy Test® FIV Ab / FeLV Ag, ASANPharm, Korea) and FPV Ag (Asan Easy Test® FPV, ASANPharm, Korea) tests were performed. Also, diseases causing clinical symptoms similar to FIP infection such as lymphoma, cardiomyopathy, septic peritonitis, pyothorax, and chylothorax (Barlough, 1984; Addie and Jarrett 1998; Andrew, 2000) were considered in the differential diagnosis list. Permission was obtained from the owners of the three cats included in the study to use the images and data of the cats.

Animals
Cat 1. Domestic, Bombay, one-year-old, 3.6 kg, non-neutered male cat was brought with complaints of weakness, loss of appetite, stagnation and increasing abdominal distention.

Cat 2. Eleven-month-old, 3.4 kg, non-neutered male Scottish cat was brought with complaints of weakness, loss of appetite and constant sleepiness.

Cat 3. Bombay, nine-month-old, 2.9 kg, non-neutered male cat was brought with complaints of stagnation, loss of appetite and decreased defecation.

Sample Collection
Blood samples of all the cats, with minimal restraint in order to not cause stress, were taken by vena cephalica venepunction (3-5 mL) by the same personnel. Effusion samples of all the cats were taken under ultrasound guidance (Mindray DC-6, USA).

Laboratory Analyzes
To perform a complete blood count (CBC), an autoanalyzer (MS4e®, Melet Schloesing Laboratoires, France) and to perform serum biochemistry, a chemistry analyzer (BT 3000® plus analyser, Biotecnica Instruments SpA, Rome, Italy) was used. All measurements were performed within the 15-30 minutes after sampling.

Statistical Analysis
SPSS 21.00 (IBM®, USA) software was used for the statistical analysis of the variation of hematological and biochemical parameters. Kolmogorov-Smirnov test was performed to determine whether the data were parametric or non-parametric. All parametric data were presented as mean ± standard deviation (SD). The ANOVA procedure was used to compare all the data based on the follow-up intervals. Statistical significance was considered as $P < 0.05$. Statistical analysis results of time-dependent hematological and biochemical parameters were presented in Table 1 and 2, respectively.

RESULTS

Clinical Findings

Cat 1. During physical examination, abdominal ballotman, normothermia (38.8 °C), tachycardia (220 beats/min), dyspnea, tachypnea (60 breaths/min) and icterus were determined. Abdominal effusion was confirmed by direct radiography and ultrasonography and it was determined to be exudate based on the physical and dipstick examinations (3+ leukocytes, 3+ total protein, 1.030 SpG) (Figure 1). Also, the Rivolta test was positive (Figure 2). Pigmented keratic precipitates and corneal edema were detected in ocular fundus examination (Figure 3).

Cat 2. Physical examination revealed hypothermia (37.8 °C) and bradycardia (132 beats/min), abdominal ballotman as well as pain on kidney palpation. Abdominal effusion was confirmed to be exudate based on positive Rivolta rest, physical and dipstick examinations (2+ leukocytes, 3+ total protein, 1.040 SpG) results (Figure 1 and 2). Pigmented keratic precipitates, aqueous flare and corneal edema were detected in ocular fundus examination (Figure 3).

Cat 3. Physical examination revealed marked loss of skin turgor, hyperthermia (39.4 °C), tachycardia (200 beats/min), tachypnea (64 breaths/min), prolonged capillary filling time (>4 sec) and pallor of mucous membranes. Similar to the results of the other cats, the effusion was exudate in character (1+ leukocytes, 3+ total protein, 1.035 SpG) (Figure 1 and 2). Severe aqueous flare and corneal edema were detected in ocular fundus examination (Figure 3).

As a result of daily use of 5 mg / kg SC of GS-441524 along with fluid treatment with crystalloids, significant improvement in clinical findings including ocular lesions in such a short period of 5 days were determined in the present report (Figure 3).

Hematological Findings

As a result of statistical analysis of hematological parameters based on the follow-up intervals, fluctuant courses were observed in MCH and MCHC levels ($p < 0.05$). Hematological findings were presented in Table 1.

Biochemical Findings

As a result of statistical analysis of biochemical parameters based on the follow-up intervals, fluctuant courses were observed in amylase, magnesium, triglyceride and CPK levels ($p < 0.05$). Biochemical findings were presented in Table 2.

While the treatment was continuing, it was learned from the owners that all the cats died (Cat 1 on 60th day; Cat 2 on 58th day; Cat 3 on 62nd day) and the causes of death could not be investigated due to the owners' refusal of necropsy.

Table 1. Hematological findings

Parameters*‡	0th day	5th day	15th day	30th day	P value
WBC (x10^9/L)	10.27±2.09	10.48±5.29	12.19±7.06	10.80±2	0.955
Lym (x10^9/L)	3.79±0.47	4.46±2.91	3.85±1.59	3.30±1.82	0.902
Mon (x10^9/L)	1.63±0.26	1.47±0.75	2.83±3.37	0.63±0.41	0.526
Gra (x10^9/L)	4.84±2.16	4.53±2.46	5.51±2.66	8.62±3.26	0.292
RBC (x10^12/L)	7.29±3.13	9.38±3.57	9.02±2.43	7.96±1.44	0.782
MCV (fl)	41.83±3.36	46.23±9.28	50.13±5.98	59.23±12.58	0.159
Hct (L/L)	30.43±13.49	41.26±8.80	44.60±9.39	46.63±7.56	0.277
MCH (pg)	13.83±1.09 ^ab	9.30±0.78^b	14.10±1.99^ab	16.80±3.31^a	0.013
MCHC (g/dL)	33.36±4.66^a	20.96±6.26^b	28.43±1.93 ^ab	28.70±4.68 ^ab	0.050
Hb (g/dL)	8.70±3.16	8.93±4	12.10±2.52	13.16±0.46	0.214
RDW (%)	11.93±2.40	11.80±1.96	10.63±0.35	10.36±1.02	0.576

WBC: leukocyte, Lym: lymphocyte, Mon: monocyte, Gra: granulocyte, RBC: red blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, Hct: hemotocrit, Hb: haemoglobin, The letters (a, b and c) show the statistically significant differences between the follow-up days.*‡Units are based on Klaassen, 1999 and Moritz *et al.,* 2004.

Table 2. Biochemical findings.

Parameters*‡	0th day	5th day	15th day	30th day	P value
BUN (mmol/L)	83.96±45.38	49.63±48.80	14.50±0.62	11.36±4.54	0.091
Creatinine (mmol/L)	7.56±6.71	3.63±3.23	1±0.30	1.13±0.50	0.189
AST (U/L)	66.73±58	84.87±67	19.33±3.21	22.33±7.02	0.628
ALP (U/L)	31.33±22.03	643.26±405.33	216.81±160	41.66±23.75	0.535
ALT (U/L)	34±10.14	295.91±207.33	60±47.28	22.33±3.51	0.449
Amylase (U/L)	2174.33±687.82^a	1940.33±207.05 ^ab	733.33±381.58^c	918.66±297.04^bc	0.008
Glucose (mmol/L)	146.33±48.26	106.66±23.11	105.66±6.42	95±14.10	0.197
LDH (U/L)	433.66±324.09	153±52.45	110.66±75.95	252.66±211.59	0.272
Total bil. (umol/L)	0.83±0.55	1.10±0.78	0.6±0.6	0.33±0.32	0.471
Calcium (mmol/L)	9.40±3.10	9.13±1.51	9.33±1.85	8.50±2.40	0.960
Direct bil. (umol/L)	0.43±0.15	0.43±0.35	0.13±0.05	0.16±0.11	0.196
Phosphorous (mmol/L)	11.63±4.36	9.26±5.23	3.83±0.15	5.80±2.17	0.105
Cholesterol (mmol/L)	282.33±201.79	221.33±127.41	198.33±74.09	153±25.23	0.662
Magnesium (mmol/L)	3.43±0.90^a	2.63±0.90 ^ab	1.46±0.47^b	1.60±0.17^b	0.025
Triglycerides (mmol/L)	144.66±73.24^a	92.66±32.12 ^ab	44±2 ^ab	37±17.77^b	0.040
GGT (U/L)	34.07±22.66	19.07±14	4.66±0.57	4.66±2.88	0.642
CPK (U/L)	337±146.11^a	203.33±81.24 ^ab	61±3.60^b	145.66±76.42^ab	0.034
Total protein (g/L)	7.73±0.55	7.63±1.20	6.80±0.26	7.20±2.02	0.775
Albumin (g/L)	3±0.20	2.73±0.83	2.93±0.49	2.66±0.5	0.821
Globulin (g/L)	4.73±0.35	4.90±1.49	3.86±0.50	4.53±2.07	0.787
A:G †	0.6±0	0.60±0.36	0.76±0.20	0.66±0.25	0.815

BUN: Blood urea nitrogen, AST: aspartate aminotransferase, ALT: alanine transaminase, ALP: alkaline phosphatase, LDH: lactate Dehydrogenase, GGT: gamma-glutamyl transferase, CPK: creatine phosphokinase. The letters (a, b and c) show the statistically significant differences between the follow-up days.
*‡Units are based on Klaassen, 1999 and Moritz *et al*., 2004 †Inclusion and exclusion criterias (>0.8 probably not FIP, <0.6 probably FIP) are based on Hartmann *et al*., 2003.
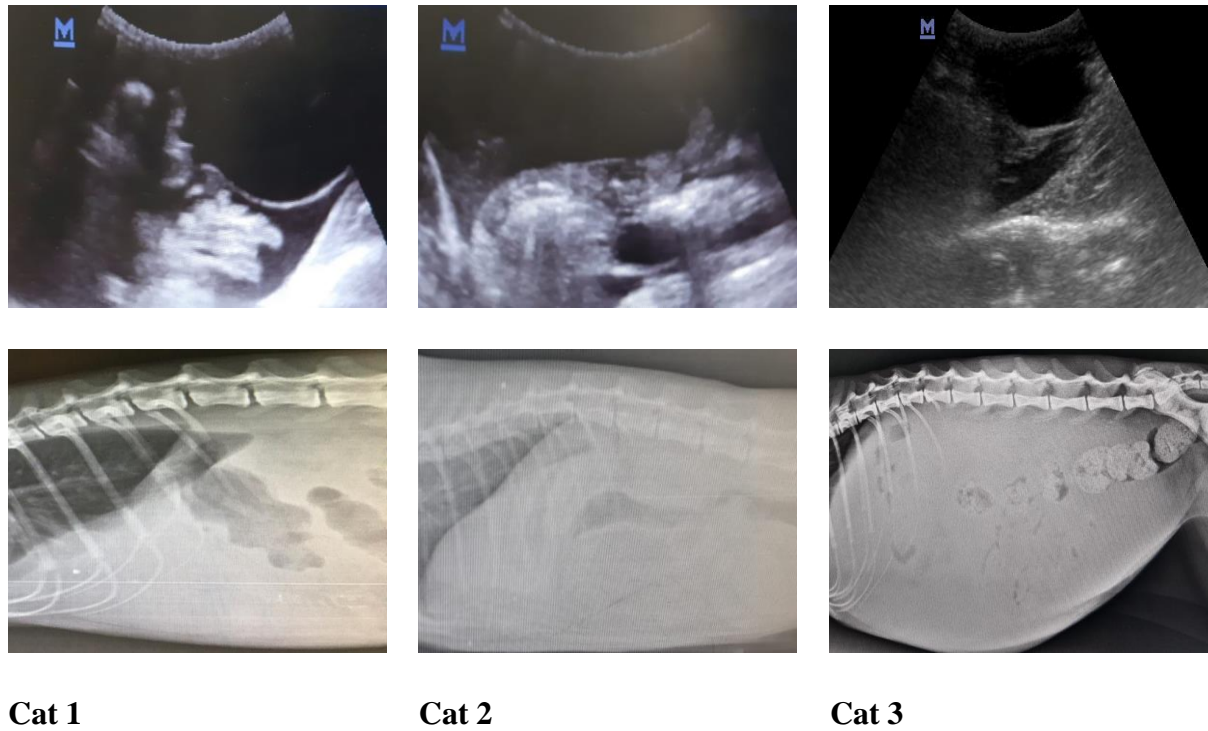
Cat 1   **Cat 2**   **Cat 3**

Figure 1. Abdominal ultrasonography (top row) and radiography (bottom row) findings of all the cats and the presence of effusion

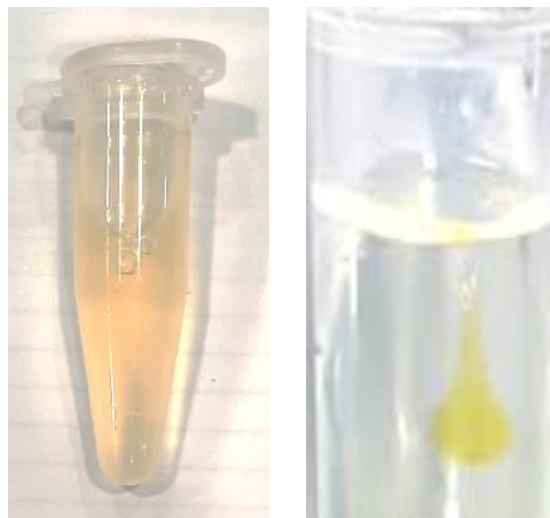**Figure 2.** Physical appearance of the fluid sample taken by abdominocentesis and positive Rivolta test

Cat 1   **Cat 3**   **Cat 3**
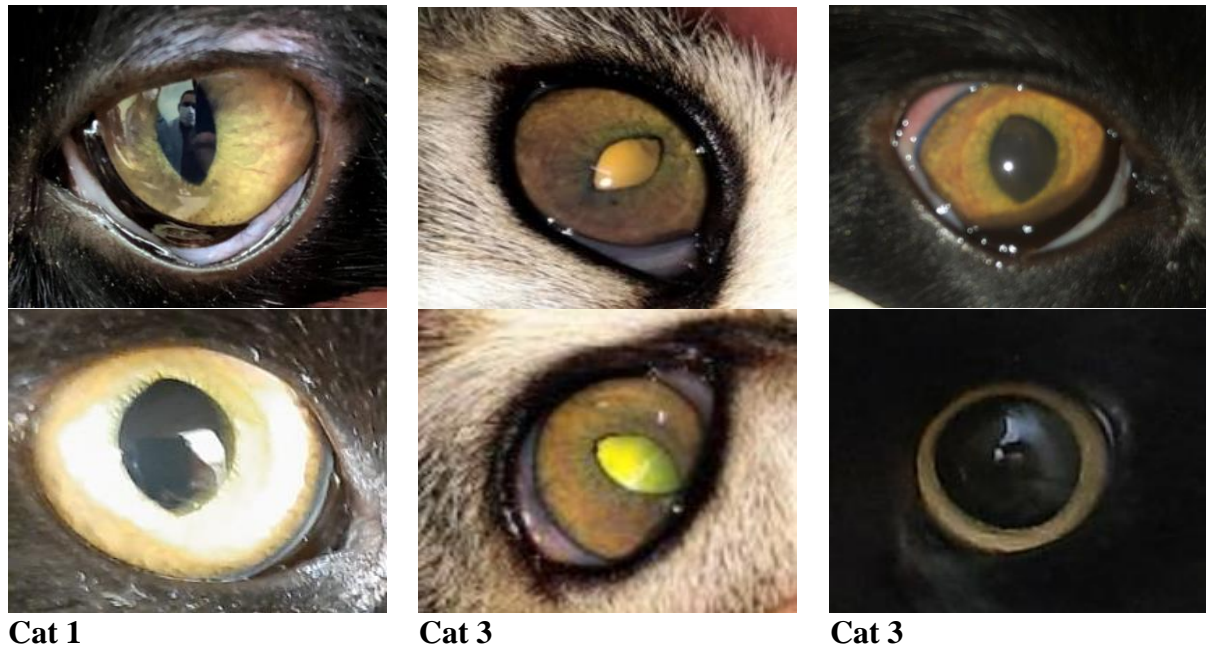
Figure 3. Presence of keratic precipitates and edema in all of the cats at first admission (top row) and clinical improvement after the treatment (bottom row)

DISCUSSION

Feline infectious peritonitis is an important cause of mortality in cats, and many experimental treatment studies have failed (Kim *et al*., 2016; Pedersen *et al*., 2019). In FIP infection, fatal immune-mediated vasculitis develops regardless of the clinical form (Andrew, 2000). Common clinical signs reported in the effusive form are anorexia, weight loss, lethargy, and fever (39-39.5 °C) (Andrew, 2000). Ocular changes such as aqueous flare, ocular pain, corneal edema, keratic precipitate, fibrin, hyphema, perivascular cuffing, optic neuritis, retinal detachment and uveitis; neurological changes such as posture disorders and mental changes were also reported (Riemer *et al*., 2016). Although ocular lesions are more common in non-effusive form, they have also been reported in effusive FIP cases (Sparkes *et al*., 1994). In this study, the presence of ocular lesions such as pigmented keratic precipitate and corneal edema (Cat 1),

pigmented keratic precipitate, aqueous flare and corneal edema (Cat 2), severe aqueous flare and corneal edema (Cat 3) were detected by ocular fundus examination. It was determined that eye lesions of all cats improved from the 5th day of GS-441524 treatment. However, it could be interpreted that the death of all three cats around 60 days may be associated with vasculitis and related organ damage (Addie and Jarrett, 1998), and there is a need for a treatment strategy for these complications with antiviral therapy.

Recently, interest in non-invasive tests to support or exclude antemortem diagnosis of FIP has increased. Of these, is serum A:G ratio. As cut off values, A:G <0.6 or <0.8 (0.80 sensitivity, 0.82 specificity) (Hartmann *et al*., 2003) were reported and A:G <0.6 ratio was reported to have high diagnostic value in inflammatory processes such as FIP (Hirschberger *et al*., 1995). Also, in a study conducted in a population with a high prevalence of

FIP, it was determined that A:G <0.8 ratio has a 92% positive predictive value (Hartmann *et al*., 2003). The fluctuant A:G course which was determined in the present study was interpreted as a result of the inflammatory process caused by FIP and the effect of the GS-441524 administration.

In the diagnosis and prognosis of FIP, evaluating A:G ratio along with hematological and biochemical analyzes are reported to be beneficial (Felten and Hartmann, 2019). In effusive FIP cases, hematological abnormalities such as monocytosis with regenerative or non-regenerative anemia or microcytosis are reported (Sparkes *et al*., 1994; Paltrinieri *et al*., 2002). In addition, lymphopenia and band neutrophilia have been reported in 50% of cats diagnosed with effusive form of FIP (Felten and Hartmann, 2019). In the present study, in terms of hematological analysis, low MCH levels in the 5th day and low MCHC levels in the 5th, 15th and 30th days were determined (Table 1). The observed changes as a result of hematological analysis were interpreted as being related to the hydration status of the cats (Kipar and Meli, 2014). Moreover, these hematological abnormalities were related to the chronic course of FIP and as a result of disruption of the hematopoietic system (Weiss and Goodnough, 2005; Pedersen, 2009; Kipar and Meli, 2014; Pedersen *et al*., 2018).

Biochemical analysis, along with routine hematological measurements, should be performed in every cat suspected of FIP (Felten and Hartmann, 2019). Also, in treatment protocols, it is recommended to follow biochemical parameters including serum total protein, globulin, albumin, BUN, creatinine and liver enzymes such as ALT, ALP and GGT along with

hemogram parameters (Paltrinieri *et al*., 2002; Pedersen *et al*., 2018). Hyperproteinemia, hypoalbuminemia and hyperglobulinemia with/without total serum protein increase and azotemia, high liver enzyme levels due to the organs affected by vasculitis are more common in cats with effusion and these have been reported in 89% of cases (Steinberg *et al*., 2008; Riemer *et al*., 2016; Felten and Hartmann, 2019). In the present study, initially high amylase and magnesium levels were determined. Similarly, increased liver enzymes and triglyceride levels indicate liver and high CPK levels indicate muscle interaction (Pedersen, 2009; Kipar and Meli, 2014; Takano *et al.,* 2019). These findings were related to the vasculitis and involved inadequate organ and tissue perfusion which may result in failure (Hartmann, 2005; Pedersen, 2009; Kipar and Meli, 2014). The parameters that were initially high decreased on the 15th and 30th days (Table 1 and 2) These changes were interpreted as a positive outcome of the treatment protocol implemented in the present study (Dickinson *et al*., 2020). Magnesium is the most abundant cationic mineral in the body which has many roles in immune system mechanisms and it has been reported that magnesium deficiency plays a role in the development of some severe and chronic viral infections. The high magnesium level determined at the beginning of our study was interpreted as the overstimulation of the immune system (Addie and Jarrett, 1998; Dominguez *et al.,* 2021).

The mechanism of action of 1'-cyano substituted adenine C-nucleoside ribose analogues such as GS-441524, which targets RNA viral replication, is based on the inhibition of viral RNA synthesis (Murphy *et al*., 2018). The parent

molecule that turns into GS-441524 in the body is Remdesivir, which is a competitive viral RNA-dependent RNA polymerase inhibitor (Frediansyah et al., 2021). Remdesivir, which is applied as a pro-drug, turns into its active form GS-441524 in the body, passes to the intercellular area and shows its antiviral effect. Remdesivir in active form is used by cell viral RNA bound to RNA polymerase instead of adenosine during viral genetic material replication. The combination of viral genetic material does not inhibit the replication process and cannot repair the viral genetic material (Ram et al., 2020). Remdesivir has hepatic (increased aminotransferase enzyme concentrations), gastrointestinal (anorexia, nausea, vomiting), respiratory (acute respiratory distress) and renal (renal tubular damage, urinary cast formation) adverse effects (Wang et al., 2020). It has been reported in naturally infected and non-neurological FIP cases in which there are recurrences or prolonged treatment period (>12 weeks) in cats that received 2-4 mg/kg SC of GS-441524 and GS-441524 once a day. In a study, it was reported that administration of 4 mg/kg SC once a day could be beneficial and essential for further studies (Pedersen et al., 2019; Dickinson, 2020). In this study, it was determined that GS-441524 treatment provided significant improvement in clinical and laboratory parameters of the 3 cats. The death of the cats over a period of about 60 days indicates that this therapy should be improved in order to prevent early deaths.

It has been reported that early deaths should be evaluated in the use of nucleoside analogues such as GS-441524 or its parent GC376 (Pedersen et al., 2019). In this context, it is emphasized that determining the virus load at the time of death is important and the early deaths may be related to the excessive progression of the disease, although the drug is effective (Dickinson, 2020). Pedersen et al. (2019) stated that the viral RNA load of a cat which was treated with GS-441524 and died on the 26th day did not decreased and the clinical findings were worsened, and interpreted the cause of death of the cat as failure of the inhibition of the virus replication. GS-5734, which is the pro-drug of GS-441524, has also been reported to develop resistance to Remdesivir, which is associated with amino acid mutations in RNA polymerase and proofreading exonuclease in coronaviruses reproduced by tissue culture (Agostini et al., 2018). It is indicated that in early deaths, viral RNA load, disease progression and resistance development should be considered. Inadequate or no response to treatment in cats treated with nucleoside analogues has been shown as an issue to be considered in future studies (Pedersen et al., 2019). The change in blood parameters determined in our study and early deaths before the completion of the recommended treatment period (>12 weeks) may be related to the persistence of virus replication and the progression of the disease or the development of resistance to GS-441524 (Agostini et al., 2018; Dickinson, 2020). Nevertheless, the effect of hepatic, gastrointestinal and renal adverse effects of Remdesivir (Wang et al., 2020) on the hematological and biochemical parameters of the present study which were determined to have fluctuant courses (Tables 1 and 2) should be considered. Also, failure to perform antemortem laboratory analyzes and postmortem necropsy / histopathological examinations in these cases and the fact that viral RNA load

were not measured significantly limited the search for causes of death in cats.

CONCLUSION

In this study, the effects of GS-441524 administration and fluid therapy on clinical, hematological and biochemical parameters in 3 cats which were diagnosed with effusive FIP on the basis of clinical, laboratory and rapid Ag test kit result were evaluated during the follow-ups of 0th, 5th, 15th and 30th days. It was determined that the treatment protocol provided significant improvement in clinical findings on the 5th day and in the laboratory findings on the 30th day in 3 cats. It was learned that the cats whose were recommended to continue the treatment and monthly follow-up, died on the 58th, 60th and 62nd days and the causes of deaths could not be investigated due to lack of necroscopic examination. As a result, the use of GS-441524 has been found to be beneficial for improving clinical, laboratory findings and animal welfare in a short time. It was concluded that clinical studies involving more cases are needed to investigate the early deaths observed during the treatment process and to determine the clinical efficacy of GS-441524 in FIP treatment.

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