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ORIGINAL ARTICLE

Effectiveness of Danazol as an adjunctive therapy in dogs with immune-mediated hemolytic anemia

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Immune-mediated hemolytic anemia (IMHA) is autoimmune disease which is anemia caused by own immune system destroying the red blood cells (RBC). It can be diagnosed with spherocytosis, positive auto-agglutination of RBCs and direct antiglobulin test (DAT, Coomb's test). The treatment for IMHA are blood transfusion, immunosuppressive agents including glucocorticoids and other supportive therapies. Danazol is synthetic androgen that has effect of interfering the autoimmune reaction to RBCs. It can be used as an adjunctive agent in addition to glucocorticoids. To investigate its effectiveness, the medical records of 10 IMHA-diagnosed dogs were evaluated. All subjects were treated with blood transfusion, prednisolone, mycophenolate mofetil, and intravenous human immunoglobulin G. Additionally, 6 subjects were administered with danazol and 4 subjects were not. The results of initial blood examination and responses to the treatment for IMHA were compared between the groups. There were significant differences in the number of blood transfusions; once in group with danazol, twice in group without danazol, duration of recovery to normal hematocrit; 7.67±3.08 days in group with danazol, 22.00±5.66 days in group without danazol, and hospitalization; 5.17±0.75 days in group with danazol, 12.75±2.22 days in group without danazol. Therefore, danazol has potential effective on treating IMHA for rapid improvement.

Key Words: Immune-mediated hemolytic anemia, Danazol, Dog

INTRODUCTION

Immune-mediated hemolytic anemia (IMHA) is one of autoimmune disease that is associated with high mortality in dogs (Garden et al, 2019). It is characterized by destruction of red blood cells (RBC) with own antibodies resulting in severe anemia (McCullough, 2003; Swann and Skelly, 2013). Clinical signs of IMHA are lethargy, inappetence, pale mucous membrane, and hemoglobinuria (McCullough, 2003). IMHA is diagnosed with complete blood cell counts (CBC) and serum biochemistry reflecting the evidence of anemia (McCullough, 2003; Piek et al, 2008; Swann and Skelly, 2013; Garden et al, 2019). CBCs of patients with IMHA show decreased hematocrit (Hct) and may exhibit increased white blood cells (WBC) (McCullough, 2003). There is no diagnostic gold standard for IMHA, but spherocytosis, positive auto-agglutination of RBCs and direct antiglobulin test (DAT, Coomb's test) support the confirmation of IMHA (Balch and Mackin, 2007; Piek et al, 2008; Garden et al, 2019). The therapeutic intervention for IMHA starts with transfusion of packed red blood cells to increase tissue oxygen delivery followed by administration of immunosuppressive agents and other supportive therapies such as intravenous human immunoglobulin (HIG) (McCullough, 2003; Balch and Mackin, 2007; Swann et al, 2019). Immunosuppressive agents used for the treatment include glucocorticoids, mycophenolate, azathioprine, cyclosporine, and danazol (McCullough, 2003).

Danazol is a synthetic androgen that inhibits IgG pro-

duction and binding of antibody to RBCs (McCullough, 2003). It is reported that danazol had beneficial effects in human patients with IMHA such as reduction of RBC osmotic fragility and increase of antithrombin Ⅲ concentrations (Grundy and Barton, 2001). Previous studies about use of danazol in veterinary IMHA patients showed that there was no significant difference in probability of survival to discharge in dogs who received danazol (Reimer et al, 1999; Grundy and Barton, 2001), but there was no study about the recovery time of IMHA patients who were administered danazol comparing to those who were not.

The objective of this study was to investigate the effectiveness of danazol administration on improvements and the time of recovery of IMHA patients promoting the efficient treatment.

MATERIALS AND METHODS

Animals

Medical records of the client-owned patients with anemia at the Sungsim Animal Medical Center from January 2021 to December 2021 were reviewed retrospectively. The dogs with clinical signs of anemia were examined with CBC, serum biochemistry, and microscopic evaluation of blood film. Inclusion criteria were hematocrit <32%, presence of spherocytes, auto-agglutination or positive Coomb's test. The dogs who had evidence of secondary anemia from underlying causes such as ehrlichiosis and babesiosis were excluded. The subjects were consisted of 10 dogs who were diagnosed with IMHA. The specific data of the subjects are presented in Table 1.

Blood transfusions with combination of IV fluids and human IgG were given to all subjects. The number of blood transfusions given to the subject was noted. Protocol of immunosuppressive agents was initiated. Prednisolone with dosage of 2 mg/kg BID and mycophenolate mofetil 15 mg/kg SID were administered orally. Additionally, 6 subjects had prescribed danazol with dosage of 5 mg/kg TID. After the first blood transfusion, the second transfusion was initiated when judged necessary.

The subjects were divided into two groups; the ones who received additional danazol, and others who did not. The responses to the blood transfusion and immunosuppressive agents were recorded. Variables for the response included number of blood transfusions, hematocrit after transfusion, duration of recovery time to normal hematocrit, and hospitalization period. The results of initial blood examination and response to the treatments were compared between groups.

Statistical analysis

Statistical analysis was performed using a commercial software program (IBM SPSS 26.0, SPSS Inc., Chicago, IL, USA). Data of blood examination on visiting day (WBCs, hematocrit, platelets, creatinine, BUN, D-dimer), he-

Table 1. Descriptive data of subjects

Group	Breed	N	Sex	N	Age (year)
With danazol	Shih tzu	2	Spayed female	3	5.50±3.39
	Maltese	1	Female	2	
	Poodle	1	Male	1	
	Miniature pinscher	1			
	Mongrel	1			
Total	6				
Without danazol	Shih tzu	2	Spayed female	3	9.00±4.40
	Schnauzer	1	Female	1	
	Cocker spaniel	1			
Total	4				

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Table 2. Comparison of variables between groups

Variables	Group		*P* value
	With danazol	Without danazol	
WBC (1×10^9/L)	27.51±15.73	29.44±3.63	0.783
RBC (1×10^{12}/L)	1.92 (1.13~3.57)	1.71 (1.09~2.15)	0.610
Platelet (1×10^9/L)	283.17±226.96	195.25±105.92	0.496
Hematocrit (%)	13.35±6.75	9.88±2.20	0.357
Creatinine (mg/dL)	0.60±0.30	0.45±0.13	0.375
BUN (mg/dL)	19.50±11.31	32.50±21.70	0.244
Auto-agglutination	2.00 (2.00~3.00)	2.50 (2.00~3.00)	0.762
Spherocytosis	2.00 (1.00~2.00)	2.00 (2.00~3.00)	0.257
D-dimer (mg/L)	0.70±0.49	1.55±0.72	0.055
Number of transfusions	1.00 (0.00~1.00)	2.00 (2.00~2.00)	0.016*
Hematocrit after transfusion (%)	24.13±3.13	24.50±1.96	0.846
Duration of recovery time to Normal Hct (days)	7.67±3.08	22.00±5.66	0.001**
Hospitalization period (days)	5.17±0.75	12.75±2.22	0.004**

*P<0.05, **P<0.01. Normally distributed data are presented as the mean±SD.
Data that were not normally distributed are presented as the median (range).

matocrit after the first transfusion, duration of recovery time to normal hematocrit, and hospitalization period were normally distributed and represented as means with standard deviations; data for the variables above were compared using paired t-test. Data of blood examination on admission (RBCs, degree of spherocytosis, degree of auto-agglutination) and number of transfusions were not normally distributed and represented as median; data of those were compared using Mann-Whitney U tests. A P value <0.05 was considered to be statistically significant.

RESULTS

The study subjects consisted of 10 dogs divided into two groups depending on danazol prescription. Differences in group with danazol prescription and the group without danazol were analyzed (Table 2). Results showed significant differences on duration of recovery time to normal hematocrit, hospitalization period, and number of transfusions on admission. The mean of duration of recovery time to normal hematocrit was significantly lower in group with danazol prescription (7.67±3.08) compared to that of group without danazol (22.00±5.66) (P<0.01). Hospitalization period was significantly different which were 5.17±0.75 days in danazol-prescribed group and 12.75±2.22 days in the other group (P<0.01). There was significant difference in the median number of transfusions which were 1.00 in group with danazol and 2.00 in group without danazol (P<0.05). Other variables showed no significant differences.

DISCUSSION

IMHA is associated with loss of self-tolerance, immune dysregulation, and production of auto-antibodies (Goggs, 2020). It is usually treated with immunosuppressive drugs such as glucocorticoids and mycophenolate mofetil (Goggs, 2020).

Danazol is another agent that can be used as immunosuppressive drug. It has properties of inhibiting TNF-alpha and IL-1 (Chai et al, 2019). It had shown its effectiveness in humans treating aplastic anemia, myelodysplasia, myelofibrosis, pure red cell aplasia, and bone marrow failure syndrome (Chai et al, 2019). Danazol has effects of decreasing macrophage Fc receptor expression, reducing the binding of antibodies to RBCs, stabilizing the RBC membranes, and alteration of T-cell regulation (Whitley and Day, 2011). It is used to treat immune-mediated diseases and corticosteroid-resistant

immune-mediated diseases in dogs (Bloom et al, 1989; Holloway and Meyer, 1990; Miller, 1997).

If IMHA patients are unresponsive to continuous treatment with immunosuppressive agents or refractory, administration of intravenous HIG or splenectomy are followed (Swann et al, 2019; Goggs, 2020). But these therapeutic interventions have risks. We investigated the outcome using danazol as adjunctive agent to previous treatment, since there were not sufficient veterinary researches about danazol's effectiveness on treating IMHA. In this study, we compared differences in results of initial blood examination and response to the treatments between groups.

The results of this study showed that the subject group with danazol administration had a significant lower number of blood transfusion compared to the group without danazol. After treating with blood transfusions, the recovery time of normal hematocrit in danazol-administered group was significantly lower than the group of no danazol administration. Also, the hospitalization period of the danazol-administered group was significantly lower than the group without danazol. Previous studies showed that there is no significant difference on mortality whether danazol was used (Bloom et al, 1989; Holloway and Meyer, 1990; Miller, 1997; Grundy and Barton, 2001). In this study, we evaluated the responses to the treatment between the groups and danazol showed its effectiveness on treating IMHA. These results may suggest that danazol can be used for more rapid improvement of IMHA.

There are two limitations in this study. First, a small number of subjects are included. Second, the adverse effect of long-term usage of danazol are not evaluated. Further studies with larger number of subjects and about effects in longer period are needed.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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