The Half-life of Erythrocytes in Dogs with Pulmonary Heartworm Disease

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Anemia is commonly complicated with heartworm disease in dogs, but precise mechanism of the anemia has been unknown. In dogs with heartworm caval syndrome, intravascular hemolysis is obvious [3, 4, 7], and the hemolysis takes part in development of anemia. In dogs with pulmonary heartworm disease, acceleration in intravascular hemolysis has been suggested [6] by detection of urinary or renal hemosiderin [3, 8] and the low serum haptoglobin level [4]. Urine or renal hemosiderin might associate with the destruction of erythrocytes by adult *Dirofilaria immitis*, and low serum haptoglobin level might associate with decrease in synthesis of the protein by the liver. The half-life of erythrocytes, which is a sure indicator of hemolysis [6], has not been determined. In the present study, in order to investigate the mechanism of anemia, the apparent erythrocyte life span was determined in dogs with chronic pulmonary heartworm disease.

The animals were introduced from the regional dog pound, and bred in the Radioisotope Laboratory, Gifu University. The experimental dogs consisted of 2 heartworm-free normal dogs (control group) and 16 heartworm-infected dogs in which only the pulmonary arteries were the site of infection. The location of heartworms was confirmed by two-dimensional echocardiography (EUB-40, Hitachi Medical Corp., Tokyo). The heartworm-infected dogs were divided into 3 groups according to their clinical signs; 1) asymptomatic group (3 dogs without apparent signs), 2) mildly-affected group (8 dogs with slight to mild anemia, coughing and accentuated second heart sound) and 3) severely-affected group (5 dogs with prostration, severe anemia, intolerance to exercise, cardiac murmur and/or ascites, but without apparent hemoglobinuria).

The 16 ml of whole blood samples were collected from the dog into a syringe containing acidified citrate-dextrose solution. The blood was mixed with ⁵¹Cr-sodium chromate solution (about 150 mcci, Japan Radioisotope Association, Tokyo), and incubated at 37°C for 5 min. After addition of ascorbic acid (100 mg), labeled erythrocytes were returned intravenously in the original dogs [1, 2]. Thereafter, 3 ml of heparinized blood was sampled every day for determination of radioactivity using a well-type liquid scintillation counter (Gamma 9,000 Counting System, Beckman Instruments, Fullerton, U.S.A.). Urine hemosiderin was detected by Prussian blue reaction. At the end of the study, all dogs were euthanatized by overdosing of an anesthetic, and the heartworms harbored were counted. Hemosiderin in the renal tubules was also

Fig. 1. Half-life of erythrocytes in 4 groups.

examined histopathologically by Prussian blue reaction.

As shown in Fig. 1, the half-life of erythrocytes was 25 and 29 days in the control group, and 20, 26 and 29 days in the asymptomatic group. In the mildly-affected group, the half-life ranged from 9 to 23 days with a mean $\pm$ standard deviation of 15.3 ± 5.9 days. In 6 of 8 dogs of this group, the half-life was less than 20 days. In the severely-affected group, the half-life ranged from 5 to 18 days with a mean of 11.2 ± 5.2 days. The half-life in the mildly- and severely-affected groups was significantly ($P < 0.01$) shorter than in the control and asymptomatic groups.

Table 1 shows clinical data in the 3 heartworm-infected groups. The half-life of erythrocytes correlated significantly with RBC, reticulocyte count and number of heartworms/kg body weight (Table 2). The half-life of erythrocytes in hemosiderin-positive dogs was significantly shorter than in hemosiderin-negative dogs (Table 3).

In pulmonary heartworm disease, the half-life of erythrocytes was short in many dogs of the mildly- and severely-affected groups. Because erythroblasts and reticulocytes were found in peripheral blood, the anemia in dogs with heartworm disease was regenerative one. These indicate acceleration of hemolysis not only in dogs with caval syndrome but also in those with pulmonary heartworm disease. In dogs with pulmonary heartworm disease, adult heartworms in the pulmonary arteries produce

Table 1. Clinical data on heartworm-infected dogs

Parameter	Asymptomatic group		Mildly-affected group		Severely-affected group	
RBC ($\times 10^4/\mu\text{l}$)	3	656 $\pm$ 5	10	536 $\pm$ 119	5	492 $\pm$ 79
Reticulocyte (% of RBC)	3	2.7 $\pm$ 1.6	10	18.2 $\pm$ 12.8	5	17.1 $\pm$ 9.6
WBC ($\times 10^2/\mu\text{l}$)	3	139 $\pm$ 21	10	126 $\pm$ 56	5	181 $\pm$ 94
Total protein (g/dl)	3	5.9 $\pm$ 0.8	10	6.3 $\pm$ 0.8	5	6.7 $\pm$ 1.8
A/G ratio	3	1.45 $\pm$ 0.41	10	0.90 $\pm$ 0.29	5	0.86 $\pm$ 0.23
Albumin (g/dl)	3	3.41 $\pm$ 0.37	10	2.84 $\pm$ 0.39	5	2.99 $\pm$ 0.75
GPT (IU/l)	3	33.3 $\pm$ 3.1	10	49.7 $\pm$ 14.5	5	77.2 $\pm$ 75.7
Plasma Hb (mg/dl)	3	5.1 $\pm$ 1.3	8	6.1 $\pm$ 5.1	4	10.7 $\pm$ 3.7
Haptoglobin (mg/dl)	3	93.9 $\pm$ 32.6	10	73.1 $\pm$ 96.6	5	73.4 $\pm$ 100.5
No. of heartworms harbored	3	10.0 $\pm$ 7.5	10	18.3 $\pm$ 14.7	5	22.8 $\pm$ 10.2
No. of heartworms/kg body weight	3	1.23 $\pm$ 0.80	10	2.24 $\pm$ 1.97	5	3.90 $\pm$ 2.10
No. of microfilariae (/200 μl)	2	4230 $\pm$ 3097	9	4590 $\pm$ 4741	5	3051 $\pm$ 3741

Data are expressed as number of dogs and mean $\pm$ standard deviation.

Table 2. Correlation coefficient between the half-life of erythrocytes and other clinical data

Item	n	Correlation coefficient	P ^{a)} <
RBC	18	0.77	0.01
Reticulocyte	18	-0.60	0.01
WBC	18	-0.15	NS ^{b)}
GPT	18	-0.46	NS
Total protein	18	-0.20	NS
A/G ratio	18	0.30	NS
Plasma Hb	15	-0.19	NS
Haptoglobin	18	0.25	NS
No. of heartworms	18	-0.46	NS
No. of heartworms/kg body weight	18	-0.50	0.05
No. of microfilariae	16	-0.07	NS

a) Probability of significant coefficient.

b) Not significant.

Table 3. Half-life of erythrocytes in dogs with and without detection of hemosiderin in urine or renal tubules

Group	Half-life of erythrocytes (day)		
Hemosiderin positive	9	10.7 $\pm$ 3.8	0.01 ^{a)}
Hemosiderin negative	7	22.4 $\pm$ 4.0	

Data are expressed as number of dogs and mean $\pm$ standard deviation. a) Probability of significant difference.

severe proliferative and thromboembolic lesions in the pulmonary vasculature [6]. The presence of worms and these lesions may induce in abnormality of the bloodstream. Also in dogs with pulmonary heartworm disease, circulating erythrocytes tend to be injured easily by mechanical force following serial changes in lipid metabolism complicated with liver dysfunction as well as in dogs with caval syndrome [5]. The shearing force of abnormal bloodstream and collision with abnormal surfaces (collision between worm cuticle or injured arterial wall) may injure erythrocytes, and result in increased intravascular hemolysis. The mechanical fragility of circulating erythrocytes was greater [3], and lesions in the pulmonary arteries were more severe in dogs with advanced heartworm disease [7]. Therefore, the half-life of erythrocytes was shorter in the severely-affected group.

Injured erythrocytes are trapped by the reticuloendothelial system, and this may also accelerate extravascu-

lar hemolysis. The fact that the half-life of erythrocytes was shorter in hemosiderin-positive dogs might corroborate the acceleration of hemolysis. Significant correlations between the half-life of erythrocytes and RBC or reticulocyte counts were observed. These hemolysis-related mechanisms, acceleration of intra- and extra-vascular hemolysis might be associated with anemia in dogs with pulmonary heartworm disease.

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