JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 997-1000

Efficacy of Low Dose Diminazene Aceturate for *Babesia gibsoni* Infection

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(Received: 06 January 2015; accepted: 13 February 2015)

***Babesia gibsoni* (*B. gibsoni*) infection has been primarily reported in Asia with the region of endemicity expanding worldwide. Diminazene aceturate has a narrow clinical safety margin and can induce side effects. The purpose of the present study was to compare the efficacy of lower dose diminazene aceturate to the normal dose. Diminazene aceturate was administered to three infected dogs in acute phase, at a dosage of 5 mg/kg three times and 3 mg/kg five times every other day and at a lower dose of 1 mg/kg for 15 consecutive days. Red blood cell count of the three treated dogs increased to greater than 500×10^4/µl on day 18, and an increasing in food consumption was followed by increasing RBC count. Conversely, one of two untreated dogs died and the other untreated dog improved later than the three treated dogs, never reaching greater than 300×10^4/µl during the experimental period. These results indicate that a continuous treatment using low dose diminazene aceturate against acute phase of severe *B. gibsoni* infection can be a very beneficial method in clinical cases for four reasons: avoidance of side effects, adequate efficacy, relative low cost for large dog breeds and safety when administering to aggressive dogs.**

Key words: *Babesia gibsoni*, diminazene aceturate, dog, low dose.

Canine babesiosis caused by *Babesia gibsoni* (*B. gibsoni*) is an infection transmitted by ticks. Erythrocytes are parasitized by the organisms, inducing severe hemolytic anemia. This disease has been reported in India, Ceylon, Malaysia, Korea and Japan[1] with the region of endemicity expanding worldwide, notably in North America[2], Australia[3], South Africa[4] and Italy[5]. Infection can result in severe clinical manifestations such as anorexia, lethargy, weakness and vomiting[1]. As the disease is fulminant and fatal in some cases, treatment in the acute phase is very important. Limited medication has been applied in the management of dogs infected with *B. gibsoni*. Compounds such as quinuronium sulfate and trypan blue to which *B. canis* readily responds seem to have little therapeutic value in treating *B. gibsoni*[6,7]. It is therefore necessary to develop new medication and reconsider the use of existing treatments. Although diminazene aceturate has been used widely for the treatment, it has a narrow clinical safety margin and can induce side effects such as pain and swelling at the injection site and neurologic manifestations. The purpose of the present study was to compare the efficacy of lower dose diminazene aceturate to the normal dose for dogs infected with *B. gibsoni* in acute phase.

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MATERIALS AND METHODS

A minimal number of dogs were used for this study because the efficacy of diminazene aceturate as a treatment for *B. gibsoni* has already

been confirmed. Five experimental beagle dogs, 1 to 3 years, purchased from the Research Institute for Animal Science in Biochemistry and Toxicology were used. The dogs were housed indoors in the Research Institute of Biosciences of Azabu University and examined daily. All procedures in the study were in accordance with the guidelines approved by the Azabu University Animal Experiment Committee (No. 040304-1). The *B. gibsoni* parasites used in the study were isolated from a naturally infected dog in Oita Prefecture in Japan. The parasititzed blood was injected into a splenectomized dog to proliferate piroplasms. When the parasitized blood reached greater than 100‰, blood was taken using heparin as the anticoagulant and washed with saline 3 times to remove plasma and buffy coat. Parasitised erythrocytes of 2.5×10^{10} per one recipient dog were prepared and injected intravenously into each of the five dogs.

Diminaphen® (PHENIX PHARMA CEUTICALS N.V., Belgium) was used. Drug treatment was initiated on the day RBC count decreased to less than 300×10^4/µl. Dog No.1 was administered the drug intramuscularly at a dosage of 5 mg/kg 3 times every other day, No.2 was administered 3 mg/kg 5 times every other day and No.3 was administered 1 mg/kg for 15 consecutive days. Nos.4,5 were used as untreated controls. These two animals were not administered any drugs, and are represented by day 0 on Figure 3 when the RBC count decreased to less than 300×10^4/µl to fit with Nos.1-3.

Food consumption as clinical findings, and RBC count as hematological findings and parasitemia observations were continued until 20

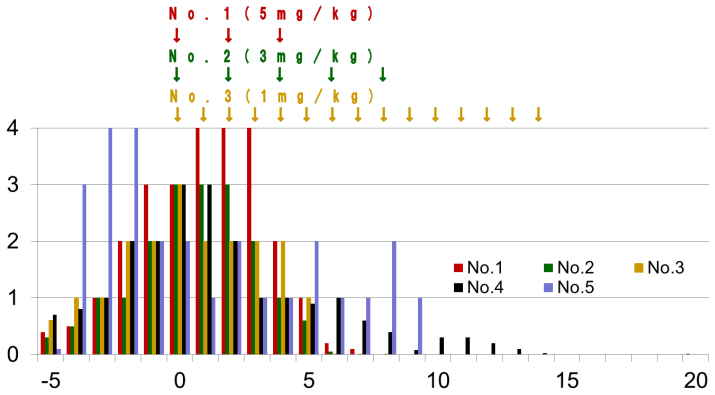

Fig. 1. Changes in parasitemia in *Babesia gibsoni* experimentally infected dogs, treated and untreated with diminazene aceturate

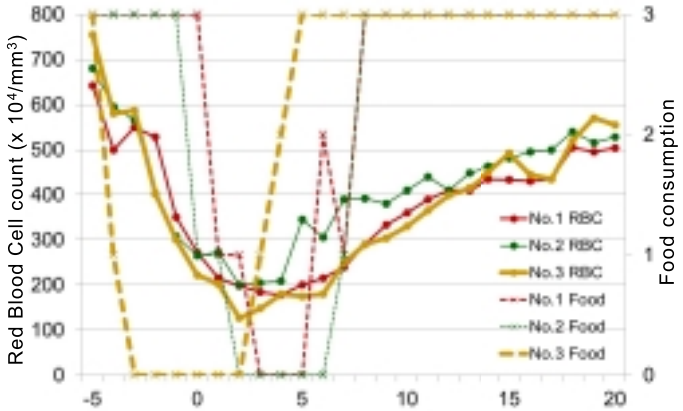

Fig. 2. Relationship in food consumption and red blood cell count in *Babesia gibsoni* experimentally infected dogs, treated with diminazene aceturate.

days after administration, a period representing the acute phase, and these findings were used as the criteria to determine drug toxicity and effectiveness. Food consumption was ranked as 1 to 3. EDTA blood samples were submitted daily for automated hematological analysis using Celltac ±® (NIHON KOHDEN, Japan). Blood smears were stained using May-Grünwald and Giemsa double stain in order to measure parasitemia. Parasitemia level was counted as the number of parasitized erythrocytes per 1,000, and ranked as 1 to 4≤.

RESULTS AND DISCUSSION

Figure 1 showed changes in parasitemia after administration in the three treated dogs. Parasitemia of Nos.1-3 was suppressed soon after administration, and was no longer detected in blood smears after days 7 to 9. In dog No.3, no side effect was observed during the period of drug therapy and until the end of the study. Conversely, parasitemia of dog No.4 was observed to decrease more slowly than for the treated dogs and the time taken for parasitemia to be undetectable on the blood smear was 15 days, 2 times that of the treated dogs. Dog No.5 died on day 10 of the investigation, although parasitemia had tended to decrease until that point.

Figure 2 shows RBC count and food consumption to establish the relationship between recovery of anemia and improvement of clinical signs. In the three treated dogs, Nos. 1-3, RBC count increased to greater than 300×10^4/µl on day 5 and 9, and to greater than 500×10^4/µl on day 18, respectively. For dog No.1, signs of recovery of appetite followed by increasing of RBC count were observed on day 6, food consumption returned to initial level on day 8. An increasing in food consumption for dog No.2 was followed by increasing RBC count, as for No.1. Furthermore, an increasing in food consumption for dog No.3 was followed by increasing RBC count, as for Nos.1 and 2, and returned to initial level on day 5. Overall findings for the three treated dogs, showed improved appetite followed by recovery of anemia, and clinical findings improved until the initial level within several days of administration. These results indicate that low dose diminazene aceturate could demonstrate suppression of parasitemia, recovery of anemia and improvement of clinical findings as effectively as the normal clinical dose.

In the case of dog No.4, anemia recovered from day 11, although the recovery was slow (Figure 3). Improvement of appetite was observed from day 16 and was entirely later than No.3. Dog No.5 died without improvement of appetite and anemia.

A study to evaluate the inhibitory activities of these drugs against *B. gibsoni* in vitro has been reported[8]. According to the report, atovaquone and diminazene aceturate are more than 1,000 times more effective than clindamycin and 300 times more effective than doxycycline. This study shows that atovaquone and diminazene aceturate have the highest activity against *B. gibsoni* among the existing drugs. However it has also been reported that atovaquone could not

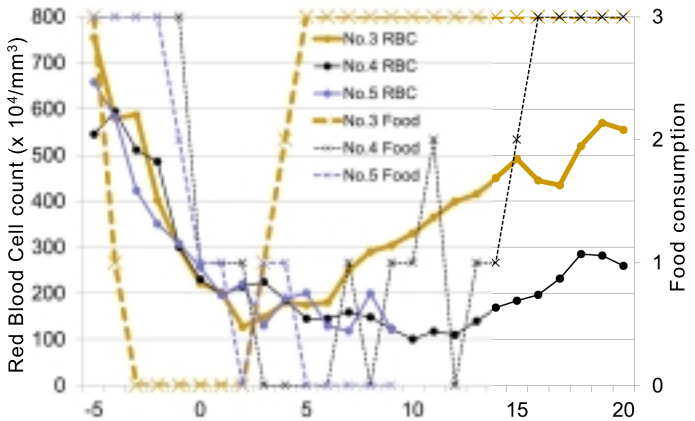

Fig. 3. Relationship in food consumption and red blood cell count in *Babesia gibsoni* experimentally infected dogs, treated and untreated with diminazene aceturate

eliminate the parasite, and drug resistance appeared to develop[9,10]. Furthermore, as the drug is orally administrated, when the patients experience vomiting from severe gastrointestinal disturbances and the dog is aggressive, administration can be difficult or impossible. Furthermore, it is expensive, especially for large dog breeds. Diminazene aceturate had been used widely as an agent for treatment of *B. gibsoni* infection of dogs. However the drug may show side effects even for the normal clinical dose, and it is therefore necessary to reexamine its use in clinical cases.

In this study, low dose diminazene aceturate showed the same efficacy as normal clinical doses in the treatment of anemia. No side effect was observed and when cases show gastrointestinal disturbances such as vomiting the drug may be used by intramuscular application.

These results indicate that a continuous treatment using low dose diminazene aceturate against severe *B. gibsoni* infection can be a very beneficial method for four reasons: avoidance of side effects, adequate efficacy, relative low cost for large dog breeds and safety when administering to aggressive dogs.

ACKNOWLEDGEMENTS

The authors would like to thank the members of Laboratory of Infectious Diseases, School of Veterinary Medicine, Azabu University for their technical assistance.

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