

Influence of Immunosuppressants against *Dirofilaria immitis* Infection in Dogs

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(Received 13 January 1989/Accepted 6 July 1989)

ABSTRACT. Two immunosuppressive drugs, azathiopurine (AZP) and prednisolone (PDS), were examined on dogs experimentally infected with *Dirofilaria immitis* in order to estimate the involvement of immunological assail in rejecting the parasite by the host. AZP was orally administered to 3 dogs daily at a dosage of 1–10 mg/kg for a period from 3 days before infection until the end of the experiment. The dose was then varied and transiently ceased according to the severity of the side effects. PDS was subcutaneously administered daily to 2 dogs. They were administered 10 mg/kg of PDS from 3 days before infection to day 15 and 5.0–8.5 mg/kg from day 60 after infection to day 70. The serum *D. immitis*-specific antibody level assessed by an indirect hemagglutination test was steadily decreased in the AZP-medicated dogs. However, in the PDS-medicated dogs, the antibody titer was decreased until day 32 and, thereafter, was recovered. When all dogs were sacrificed between days 145–148, an average recovery rate of worms in both the AZP- and PDS-medicated dogs was 52.5% and 49.6%, respectively, while the controls showed 42.5%. The statistical analysis revealed no significant difference among the groups, indicating that the administration of AZP and PDS was not effective in protecting the larvae from the host's immune attack.—**KEY WORDS:** azathiopurine, *Dirofilaria immitis*, dog, immunosuppressant, prednisolone.

Jpn. J. Vet. Sci. 51(5): 955–960, 1989

In our previous studies, about 40% of the inoculated larvae of *Dirofilaria immitis* were confirmed to reach the heart in dogs in experimental infection [10–12]. In these dogs inoculated, an antibody specific to *D. immitis* was produced against the excretory and secretory products released during the fourth molting stages of the larvae [10, 11]. Thus, it is conceivable that the remaining inoculated larvae (about 60%) might be failed to grow by some protective mechanism of the host including immune-mediated one. Therefore, the present study was carried out to estimate the role of the canine immune system in the rejection of *D. immitis* larvae in dogs administered immunosuppressive drugs.

MATERIALS AND METHODS

Dogs: Seven mongrel dogs, aged 4–8

months old and free from *D. immitis* infection, were used in this study.

Infective larvae: Infective larvae (L₃) of *D. immitis* were obtained from *Aedes togoi* experimentally infected with *D. immitis* and were subcutaneously inoculated according to a previous report [11].

Immunosuppressive drugs and schedule of administration and experimental infection: Azathiopurine (AZP) and prednisolone (PDS) were used in this study. AZP is commercially marketed as ImuranTM (Tanabe Seiyaku Co., Osaka). One tablet of ImuranTM contains 50 mg of pure AZP as a derivative of 6-mercaptopurine.

Three dogs (group 1) were orally administered 1 mg/kg of AZP daily from 3 days before the inoculation until day 7 after the inoculation. The oral dose was then gradually increased on day 8 and reached 10 mg/kg, as a maximum dose, on day 45 of the

infection. The medication was transiently ceased or the dose was reduced when an adverse reaction appeared. When the adverse reactions were abated or disappeared, the drug was administered again. On day 0, the 3 dogs were experimentally infected with 252, 209 or 216 of L_3 , respectively. PDS (Takeda Yakuhin Co., Osaka) were subcutaneously injected into 2 dogs (group 2) in two phases. The first phase involved the administration of 10 mg/kg daily from 3 days before infection until day 15 of the infection. The second phase involved the administration of 8.5 mg/kg to one dog (No. 4) and 5 mg/kg to another dog (No. 5) on day 60 through 70 of the infection. Each injection of PDS was alternately administered into the sides of the body in attempts to avoid any dermatological side effects. They were experimentally infected with 191 or 194 of L_3 on day 0, respectively.

Two dogs (group 3) were inoculated with 161 or 159 of L_3 as the control group.

All 7 dogs were housed in a mosquito-proof room and fed a commercial dog food and normal tap water *ad lib.* during the study.

White blood cell (WBC) counts: Adverse reaction of AZP was monitored by means of WBC counts.

Serology: The antibody production specific to *D. immitis* was monitored at various postinfection intervals by means of the indirect hemagglutination (IHA) test, according to the method of Hayasaki [9].

Worm recovery: All dogs were sacrificed on the final day (between day 145–148) of the experiment and the numbers of the parasitic adult worms found in the right ventricle and pulmonary arteries were counted. The effects of 2 drugs against *D. immitis* infection were assessed by calculating the means of the rate of recovery from worm infection.

Statistical analysis: The experimental data

were statistically analyzed based on Student's *t* test.

RESULTS

Administration of drugs and clinical findings of dogs: The dogs of group 1 showed several side effects throughout the observation period. Time course changes of the WBC count are shown in Fig. 1. These data indicate the AZP induced a strong adverse reaction in the dogs.

Dog No. 1 indicated severe anorexia and dullness on day 34 when the administrations of 4 mg/kg of AZP were continuously administered. Therefore, administration of the drug was ceased. Nevertheless, the dog succumbed to the complications on day 35. Dog No. 2 showed a marked leucopenia of $3,500/\text{mm}^3$ on day 39 of the infection, after the oral dose reached 4 mg/kg of AZP. Therefore, the medication was stopped until the number of the leucocytes increased (day 48). This dog indicated moderate but wide-

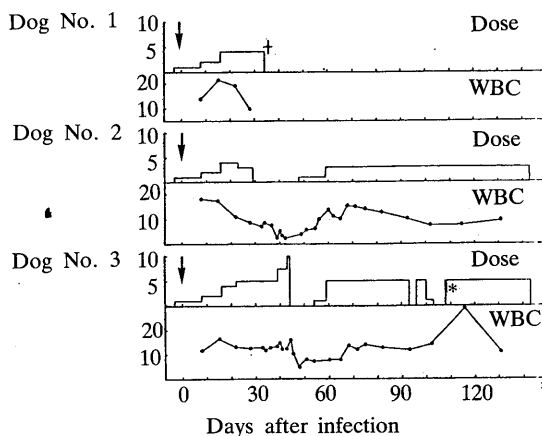


Fig. 1. Diagrammatic relation in dose (mg/kg) of azathiopurine administration and WBC counts ($\times 1,000$) of the three dogs in group 1. Arrows show the inoculation of *D. immitis* L_3 .

Remarks: †: Dog No. 1 died on day 35 as a result of a severe adverse reaction to the drug. *: Administration of 10 mg/kg of prednisolone was taken the place of azathiopurine administration at the onset of the adverse reaction.

spread dermatitis throughout the course of the drug administration. Dog No. 3 was initially given a low dose of AZP which was gradually increased until it reached 10 mg/kg (day 43). However, administration of the drug was halted on day 44 when a fungal diarrhea was observed. Antibiotics were administered for treatment (Trichomycin, Fujisawa Yakuhin Co., Osaka). Once the clinical findings stabilized, AZP was readministered at a dose of 5 mg/kg (day 54). In this dog, the drug course was halted a further 2 times as a result of anorexia, dullness, hemorrhaging and severe, widespread dermatitis. Due to these complications, administration of the drug was changed to PDS (day 108) until the end of the experiment period (Fig. 1).

Each dog in group 2 showed acute dermatitis and marked alopecia around the injection sites. After the first phase of the administration, these side effects disappeared. During the second phase of drug administration, severe dermatitis with hemorrhagic and purulent inflammation of the skin was noted. Each dog received one injection of an antibiotic (Tylocin, Takeda Yakuhin Co., Osaka) which sufficiently cleared up the symptoms.

Production of *D. immitis*-specific antibody: Time course changes of the antibody production of the experimental dogs during the observation period are shown in Fig. 2.

The AZP-medicated dogs (group 1) showed the suppression of the antibody production throughout the observation period. The PDS-medicated dogs (group 2) consistently showed a reduction of the antibody titer after the first phase of administration until day 32. These data indicate that a steady suppression of the antibody production was induced in groups 1 and 2. In group 2, a substantial amount of antibody production was observed from day 50, suggesting that the second phase of administration failed to suppress the antibody pro-

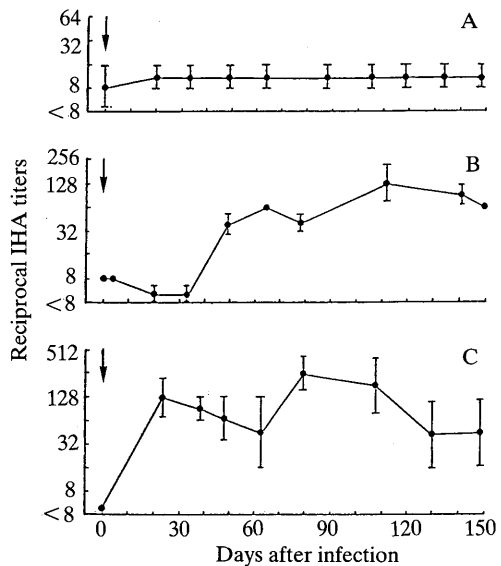


Fig. 2. Indirect hemagglutinating antibody productions of dogs administered with azathiopurine 1–10 mg/kg/day (A) and prednisolone 5–10 mg/kg/day (B), and controls (C). Arrows show the inoculation of *D. immitis* L₃.

duction. The dogs in the control group consistently produced the specific antibody during the experiment.

Worm recovery: The recovery rate was determined by dividing the number of adult worms obtained from the dog's heart by the number of L₃ inoculated (Table 1). The AZP- and PDS-medicated dogs showed an average recovery rate of 52.5% and 49.6%, respectively, whereas the control dogs showed an average recovery rate of 42.5%. The statistical analysis indicate that there is no significant difference among the three groups.

DISCUSSION

The dose range of AZP for the treatment of immune diseases is from 2.0 to 2.2 mg/kg in dog and cat cases [8], while that of PDS is from 1.0 to 4.0 mg/kg in dog and cat cases [8]. Comparing to these dosage, 1 to 10 mg/kg (average 4 to 5 mg/kg) of AZP and 5 to 10 mg/kg of PDS used in this study were

Table 1. Worm recovery in dogs infected with *Dirofilaria immitis* after administration of immunosuppressants

Group	Dog No.	No. of L ₃	Period(Days) at necropsy after infection	Worm recovery		
				Total No. of worms(Male/Female)	Worm recovery rate (%)	
					Individual	Mean(SD)
Azathiopurine	1 ♀	252	—	—	—	52.5(4.8)
	2 ♀	209	146	120(44/76)	57.4	
	3 ♀	216	145	103(42/61)	47.7	
Prednisolone	4 ♀	191	148	90(40/50)	47.1	49.6(2.5)
	5 ♀	194	148	101(47/54)	52.1	
Control	6	161	146	75(35/40)	46.6	42.5(4.1)
	7	159	146	61(25/36)	38.4	

Dog No. 1 died on day 35, as a result of a severe adverse reaction to the drug. There is no significant difference among the groups.

considerably high doses. In group 1, the *D. immitis*-specific antibody production was prevented during the experiment, indicating that the host's immune response was suppressed by the drug. The suppression of host's immune response was also supposed by the decrease of WBC counts and the occurrence of acute prurient dermatitis. In group 2, the antibody production was suppressed by the first phase of administration until about one month after the inoculation. However, subsequently, the antibody titer was increased with no effects of the second phase of the administration on antibody production. These phenomenon may lead to the hypothesis that the B-lymphocytes of the dogs were already sensitized by L₃, as an antigen, during the interval between the first and second phase of PDS administration, since the B-lymphocytes not yet sensitized by an antigen are susceptible to the steroid hormone, while the B-lymphocytes already sensitized are less susceptible [1-3, 5, 13, 17-19]. To the contrary, in group 3, the antibody adequately produced after the inoculation of L₃.

The worm recovery rate in group 1 and in group 2 was 10% and 7% higher than that in the control group, respectively, although there is no significant difference among the three groups. However, the results obtained

in this study did not provide sufficient evidence on the acceleration of larval development as a result of the immunosuppressive drug. These findings may indicate that, in dogs as a definitive host, the immunological attack against the larvae of *D. immitis* may weakly disturb the development of the larvae, although some immunological problems still remain to be studied. The mechanism which is unsusceptible to the immunosuppressive drug may be involved in the immunoprotectivity of dogs to *D. immitis* infection and the immunosuppressive drugs may be ineffectual for recipient dogs in suppressed immune response to *D. immitis* infection, or the design of drug administration may not be suitable. As in other parasitic infections [4, 7, 21, 22, 24, 25], dogs with *D. immitis* infection usually show an depressed cell-mediated immune response [6, 10, 23], although a specific antibody with parasitocidal effects was predominantly produced.

In contrast, the immunoprotectivity against *D. immitis* infection may be a principal mechanism to refuse the parasitic infection in underdefinitive hosts [14-16], because rodents steadily rejected the infection of *D. immitis* L₃. Wong [26] also suggested a role of immunoprevention of monkeys. According to their report, the macaques

monkeys were medicated with 10 mg/day of PDS, which was the equivalent to approximately 3 mg/kg/day and then experimentally infected with 90 to 100 L₃ of *D. immitis*. The resulting worm recovery count showed positive infection with 1.1 to 3% of recovery rate, while monkeys of the control group never permitted the infection. Additionally, the cats permitted very slight percentage (0.4%) of *D. immitis* L₃ infection [20]. These data suggest that the immunoprotection mechanism of the undefinitive host may play an important role in prevention of parasitic infection.

The difference of infectivity between definitive and undefinitive hosts was also revealed in *Angiostrongylus cantonensis* infection to rat and to the guinea pig [27-30].

Further studies, therefore, are necessary to determine a role of immunoprotectivity in a definitive host against *D. immitis* infection in dogs.

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要 約

犬糸状虫感染に対する免疫抑制剤の影響：早崎峯夫・大石 勇(東京農工大学農学部家畜内科学教室)——宿主(犬)の免疫防御能による犬糸状虫感染防御効果を検討するために2種類の免疫抑制剤を用いて、犬糸状虫感染実験を行った。アザチオプリン(AZP)投与群の3頭は、家験感染前3日より感染後7日までAZP 1 mg/kgを、8日以後は投与量を増加(最大10 mg/kg)、減少あるいは休薬しながら連続経口投与した。プレドニゾロン(PDS)投与群の2頭は実験感染前3日より感染後15日の17日間に10 mg/kgを、60日—70日までの11日間は8.3 mg または5 mg/kgを連日皮下注射した。犬糸状虫特異抗体産生の推移は間接赤血球凝集反応により観察した。AZP投与群では実験期間を通じて確実に抑制され、PDS投与群では32日まで確実に抑制されたが以後抗体産生がみられた。実験犬は感染後145-148日に剖検し虫体回収率を比較したが、両投与群とも対照群2頭との間に有意差は認められなかった。