INHIBITORY EFFECT OF NUCLEOTIDES ON THE MULTIPLICATION OF BABESIA GIBSONI IN CANINE ERYTHROCYTES

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ABSTRACT

The inhibitory effects of nucleotides on the multiplication of Babesia gibsoni was studied on in vitro culture of canine erythrocytes. B. gibsoni was cultured at 37°C for 3 days under a humidified atmosphere containing 5% CO₂, 5% O₂ and 90% N₂ in culture media with or without nucleotides (5',CAMP), cyclic 5'-adenosine monophosphate (5',AMP), cyclic 5'-guanosine monophosphate (5',GMP), cyclic 5'-adenosine monophosphate (5',AMP), cyclic 5'-guanosine monophosphate (5',GMP), cyclic 5'-adenosine monophosphate (5',AMP) and cyclic 5'-guanosine monophosphate (5',GMP) at a final concentration of nucleotides was 0.1 mM. The adding of 5',CAMP (5',AMP) and 5',GMP in nucleic acid significantly (p<0.01) inhibited the multiplication of B. gibsoni in canine erythrocytes. Further more, the 5',GMP and 5',AMP showed the dose-dependently significant inhibitory effect on the multiplication of B. gibsoni. It may be concluded from these results that the accumulation of specific nucleotides might have inhibited the multiplication of B. gibsoni in canine erythrocytes.

Key words: Babesia gibsoni, multiplication inhibition, canine erythrocytes, nucleotides

INTRODUCTION

Babesiosis is a widespread tick-borne blood protozoan disease of domestic animals. Babesia gibsoni is well-known causative pathogen of canine babesiosis and causes chronic disease associated with hemolytic anemia in infected dogs (Borror et al., 1973; Pawlewicz et al., 1972). Although severe anemia often occurs in dogs infected with this protozoan, most of the studies show a percentage of parasitized erythrocytes in the peripheral blood (Carson and Phillips, 1981). Kawamura et al. (1987). It was proved that the nucleotides such as cyclic 5'-adenosine monophosphate (5',CAMP) and cyclic 5'-guanosine monophosphate (5',GMP) might be accumulated in the young erythrocytes and/or in clumps in dogs infected with B. gibsoni as a result of decreased activity of pyrimidine 5'-nucleotidase (5'NPN) and purine 5'-nucleotidase, reducing the debased maturation of reticulocytes and parasites in the peripheral circulation of infected dogs (Hosoi et al., 2003). Deficiency of the erythrocyte enzyme pyrimidine 5'-nucleotidase (5'-nucleotidase) may result in a hematologic syndrome in which the affected cells accumulate high levels of cytidine and uridine nucleotides, affected individuals retain 10-15% of MCV even after normal turnover, and are mildly anemic. The purpose of the present study to investigate the effects of nucleotides on multiplication of B. gibsoni and discuss the relationship between accumulation of nucleotides and low parasitemia in canine babesiosis in vivo.

MATERIALS AND METHODS

Babesia gibsoni was cultivated according to the method reported by Yamazaki et al. (2000) with some modifications. Venous blood was collected from clinically healthy dogs using ethylenediaminetetraacetic acid as an anticoagulant. The buffy coat was removed after centrifugation and the erythrocytes were washed twice with 10-fold phosphate-buffered saline (PBS, pH 7.4) and then washed three times with 0.5-Molar solution of Eagle medium (o-MEM, Life Technologies, Grand Island, NY, U.S.A.) containing with sodium-pyruvate (5.61 mg/ml), glutamine (0.3 mg/ml), and sodium bicarbonate (2 mg/ml). Cultivation was performed in plastic microtiter plate and incubated using a incubator (APW-36, Anest, Fukushima, Japan) at 37°C for 7 days under a humidified atmosphere containing 5% CO₂, 5% O₂ and 90% N₂ in culture media with or without nucleotides (5',CAMP). The cultured parasite was visualized in each well of a 96-well flat-bottom microplate and incubated using a incubator (APW-36, Anest, Fukushima, Japan) at 37°C for 3 days under a humidified atmosphere containing 5% CO₂, 5% O₂ and 90% N₂ in culture media with nucleotides (5',CAMP).

Control open column Cyclic 5'-adenosine monophosphate (5',CAMP), cyclic 5'-guanosine monophosphate (5',GMP), cyclic 5'-adenosine monophosphate (5',AMP) and cyclic 5'-guanosine monophosphate (5',GMP), in which a final concentration of nucleotides was 0.1 mM. This dose-dependently effect of nucleotides on the in vitro multiplication of B. gibsoni was studied at 37°C for 6 days under a humidified atmosphere containing 5% CO₂, 5% O₂ and 90% N₂ in culture media with nucleotides (5',CAMP) and 5',GMP, 5',AMP (0.1-10 mM) in an incubator (APW-36, Anest, Fukushima, Japan). Every 24 h, 50% of culture supernatant was removed without disrupting the sedimented erythrocytes and replaced with an equal volume of the fresh culture medium. Percentage of parasitemia was calculated by counting the number of parasitized cells per 2000 cells on a Giemsa stained blood smear. Statistical analysis was performed using Student's t-test. These analyses were carried out on a computer using a statistical software package, PSSTAT (2006, IBM, IL, U.S.A.).

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Inhibitory effect of nucleotides on multiplication of B. gilvus

RESULTS AND DISCUSSION

Bacteria gilvus was cultivated with canine erythrocytes in culture media with or without 5 mM of pyrimidine and purine nucleotides (Fig. 1). The level of parasitaemia was significantly (p < 0.01) enhanced when 5-CMP, 5-UMP, 5-IMP, 5-SAMP and 5-SIMP were added to the culture medium, but 5-IMP and 5-SUMP showed no significance (p > 0.05) effect on the multiplication of B. gilvus. Furthermore, the dose-dependent effect on the multiplication of B. gilvus was examined using 5-CMP, 5-IMP and 5-SIMP (Fig. 2). 5-CMP and 5-SIMP inhibited the multiplication of B. gilvus dose-dependently while 5-IMP showed no effect at the concentrations of up to 10 mM. The inhibitory effect of 5-S-CMP seemed to be stronger than that of 5-S-IMP.

Fig. 1. The in vitro effect of nucleotides on the multiplication of Babesia gilvus. Values are Means ± SD (N = 4). *p < 0.05.

Fig. 2. The dose-dependent effect of nucleotides on the multiplication of Babesia gilvus. Values are Means ± SD (N = 4). *p < 0.05, **p < 0.01, ***p < 0.001.

As described elsewhere, canine erythrocytes have two isozymes equivalent to human pyrimidine 5'-nucleotidase (PN5-N, PN5-E) and purine 5'-nucleotidase (Anits et al., 1991; Meyers, 1985; Hulthen et al., 2000). The PN5-N is mainly involved in the degradation of pyrimidine 5'-monophosphate, whereas PN5-E preferentially catalyzes the breakdown of pyridine 5'-monophosphate. 5-CMP and 5-SAMP are the most effective and specific substrates of PN5-N.
PSN-I-like characterized by its high Michaelis constant and maximum velocity of Y-TMP and Y-UMP. In addition, a third cytosine 5'-nucleotidase, a partial 5'-nucleotidase, is also present in human erythrocytes, and it preferentially hydrolizes p[NH4] creatine and their deoxy counterparts (Brenchley et al., 1988).

We previously also reported that the serum from dogs infected with B. gibsoni and the Jaranui strain inhibits PSN-I-like activity and purine-specific 5'-nucleotidase activity in dogs, but no PSN-I-like activity (Hassan et al., 2003). Furthermore, Y-TMP and Y-UMP, which are specific substrates for the two enzymes inhibited by the multiplication of B. gibsoni in vivo culture, are monophosphates specific for PSN-I-like and had no effect on human erythrocytes. However, we also demonstrated that a third cytosine 5'-nucleotidase present in human erythrocytes is characterized by marked haptoglobin staining in Wright-stained blood films and an accumulation of pyrimidine nucleotides (Weaver et al., 1995). A further reduction of PSN-I-like results in the inhibition of pyrimidine nucleotide and pyrimidine salvage pathways, which are characteristic in the inhibition of B. gibsoni-induced haptoglobin staining in Wright-stained blood films and an accumulation of pyrimidine nucleotides (Weaver et al., 1995).

REFERENCES