Suppression of Mitogenic Response of Bovine Peripheral Blood Lymphocytes by Ketone Bodies

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ABSTRACT. Mitogenic response of peripheral blood lymphocytes from ketotic cows and effect of ketone bodies on lymphocyte blastogenesis were investigated. Glucose consumption index (GCI) values for phytohaemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM) in the ketotic cows were significantly lower than those in the healthy dry cows. A significant negative correlation was observed between the GCI values and serum concentrations of total ketone and β-hydroxybutyric acid. When lymphocytes from healthy dry cows were preincubated in the medium with acetoacetic acid or β-hydroxybutyric acid, the GCI values for PHA and PWM were significantly lower than those in the lymphocytes without ketone bodies. These findings indicate that increased serum concentrations of acetoacetic acid and β-hydroxybutyric acid in ketotic cows suppress lymphocyte blastogenesis.—KEY WORDS: cattle, ketone body, lymphocyte blastogenesis.

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In vitro studies [3, 16] indicated that toxic and subtoxic concentrations of β-hydroxybutyrate and a toxic concentration of acetoacetate inhibited bovine lymphocyte proliferation. Mitogenic responses of lymphocytes from calves with experimentally induced ketonemia by feeding a diet containing 1, 3 butanediol were lower than those of normal calves [18]. Suppression of lymphocyte blastogenesis has been also recognized in cows with periparturient period [5, 7, 8] and in mastitic cows [6, 12], however, impaired lymphocyte blastogenesis has not been reported in the clinical ketotic cows. In the present study, mitogenic responses of peripheral blood lymphocytes from ketotic cows and effects of ketone bodies on lymphocyte blastogenesis were investigated to determine the immunosuppressive condition in clinical ketosis.

Blood samples were obtained by jugular venepuncture from 15 Holstein cows with naturally occurring clinical ketosis. They were within 30 days after parturition, and diagnosed clinically due to several symptoms such as a decrease in appetite and milk production, and positive urine ketone bodies. The cows did not have any clinical features such as retained placenta, pyrexia or mastitis. Blood samples were also obtained from 27 healthy Holstein cows in the dry period, and from 10 healthy cows early in lactation. Mean serum concentrations of total ketone, acetoacetic acid and β-hydroxybutyric acid determined with a commercial kit (Ketone test, Sanwa Chemical, Nagoya) in the ketotic cows were significantly higher than those in the healthy dry and lactating cows (Table 1).

Peripheral blood leukocytes counted with a hemocytometer (Celltac, Nihon Koden, Tokyo) in the ketotic, healthy dry and healthy lactating cows were 5,750±2,110 (mean ± standard deviation)/μl, 7,370±1,310/μl and 6,430±1,560/μl, respectively. Lymphocyte counts determined with May-Gimsa-stained blood smears in the ketotic, healthy dry and healthy lactating cows were 3,430±1,170/μl, 5,000±1,530/μl and 4,210±1,070/μl, respectively. Leukocyte and lymphocyte counts in the ketotic cows were significantly ($p<0.05$ and $p<0.01$, respectively) lower than those in the healthy dry cows, but no significant difference in leukocyte and lymphocyte counts was observed between the ketotic cows and the healthy lactating cows.

Table 1. Serum concentrations of total ketone, acetoacetic acid, and β-hydroxybutyric acid in the ketotic cows, healthy dry and lactating cows

Measurement	Serum concentrations (mM/l)		
	Ketotic cows (n=15)	Dry cows (n=15)	Lactating cows (n=10)
Total ketone	2.17±0.80	0.62±0.18[a)]	1.17±0.55[a)b)]
Acetoacetic acid	0.15±0.15	0.02±0.01[a)]	0.04±0.03[a)]
β-hydroxybutyric acid	2.02±0.71	0.60±0.18[a)]	1.13±0.53[a)b)]

Data are expressed as the mean±standard deviation. Statistical analyses are carried out using the Student's and Welch's *t*-test.

a) Significant difference ($p<0.01$) was observed among the ketotic cows, the healthy dry and lactating cows.

b) Significant difference ($p<0.05$) was observed between the healthy dry and lactating cows.

Blastogenic responses of lymphocytes to mitogens were performed as described previously [15]. The mean glucose consumption index (GCI) value for PHA in the ketotic cows was much lower than that in the healthy dry cows (Fig. 1). The GCI values for Con A and PWM in the ketotic cows were lower than those in the healthy lactating cows, and were much lower than those in the healthy dry cows. Significant ($p<0.01$) negative correlations were observed in the ketotic and healthy cows between the GCI values and the concentrations of total ketone and β-hydroxybutyric acid. Coefficients of correlations between total ketone and the GCI values for PHA, Con A, and PWM were −0.407, −0.493 and −0.449, respectively. Furthermore, coefficients of correlations between β-hydroxybutyric acid and the GCI values for PHA, Con A, and PWM were −0.412, −0.524 and −0.476, respectively. In calves with experimentally induced ketonemia, blood β-hydroxybutyrate was high and mitogenic responses of lymphocytes to PHA and Con A were low [18]. The findings obtained in the present study indicate that lymphocyte blastogenesis is suppressed in naturally occurring ketosis.

On the other hand, the effects of incubation and preincubation of lymphocytes with ketone bodies on the

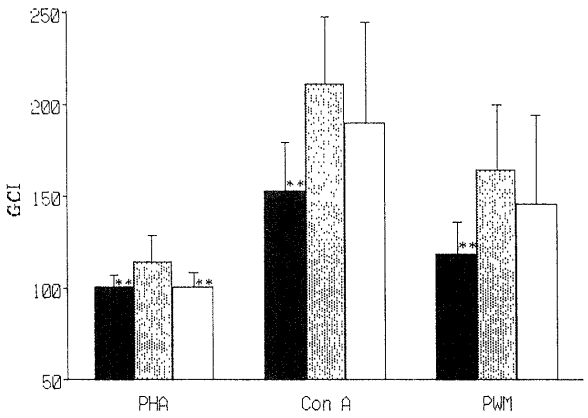

Fig. 1. Glucose consumption index (GCI) values in the ketotic cows (■; n=15), healthy dry (□; n=15) and lactating cows (□; n=10). Peripheral blood lymphocytes were stimulated by phytohaemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM). Data are expressed as the mean±standard deviation of GCI values from triplicate results in the indicated cow. Significant difference compared with the healthy dry cows is indicated by double asterisks (**: $p<0.01$).

GCI values were investigated to estimate the suppressive effect of ketone bodies on lymphocyte blastogenesis. Lymphocytes from healthy dry cows (n=8) were incubated in the medium with mitogens and various concentrations of acetone (Wako Pure Chemical, Osaka), acetoacetic acid (Sigma Chemical Co., MO) or β-hydroxybutyric acid (Sigma). The final concentrations of the substance in a well ranged from 0.1 to 5.0 mM. The control lymphocytes were incubated without ketone bodies. A slight reduction of mitogenic response was observed when lymphocytes were incubated in the medium with β-hydroxybutyric acid at concentrations from 0.1 to 5.0 mM. The reduction was not statistically significant, but lymphocytes incubated in the medium with acetone or acetoacetic acid at concentrations from 0.1 to 5.0 mM did not reduce mitogenic response.

In some experiments, lymphocytes from healthy dry cows (n=4) were preincubated in the medium containing acetoacetic acid or β-hydroxybutyric acid, and then washed 3 times and incubated in the medium without ketone bodies. The final concentration of the substance in suspension was 5.0 mM. The control lymphocytes were preincubated without ketone bodies. The viabilities of lymphocytes preincubated in the medium with acetoacetic acid or β-hydroxybutyric acid were more than 95% by trypan blue exclusion. No significant difference was observed in the viabilities of lymphocytes preincubated with ketone bodies and the control. Preincubation of lymphocytes with acetoacetic acid or β-hydroxybutyric acid suppressed the mitogenic response (Fig. 2). When lymphocytes were preincubated in the medium with acetoacetic acid or β-hydroxybutyric acid from 0.5 to 4 hr, the GCI values were lower than those in the lymphocytes without ketone bodies. The GCI values for PHA and

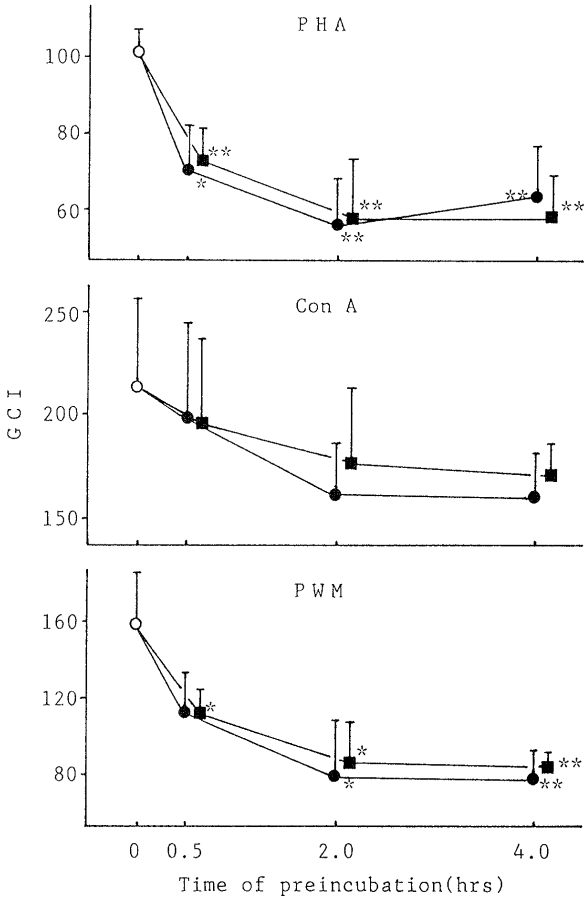

Fig. 2. Effect of preincubation of lymphocytes with acetoacetic acid (●) and β-hydroxybutyric acid (■) on glucose consumption index (GCI) values in mitogen-stimulated lymphocyte culture. Lymphocytes from 4 healthy dry cows were preincubated in the medium containing ketone bodies (5.0 mM), and then washed and incubated in the medium with mitogen (phytohaemagglutinin; PHA, concanavalin A; Con A, and pokeweed mitogen; PWM). Data are expressed as the mean ± standard deviation of GCI values from triplicate results in the indicated cows. Significant difference compared with the control lymphocytes preincubated without ketone bodies (○) is indicated by single (*: $p<0.05$) and double asterisks (**: $p<0.01$).

PWM were significantly lower than those in the control culture.

Toxic and subtoxic concentrations of β-hydroxybutyrate (0.08, 0.8, and 4.0 mM) or toxic concentration of acetoacetate (0.8 and 4.0 mM) significantly suppressed the PHA-stimulated response of bovine lymphocytes, but acetone did not affect the mitogenic response [16]. Only supraphysiological levels of β-hydroxybutyrate (6.25 mM) inhibited proliferation of bovine lymphocytes stimulated with PHA and PWM [3], and also inhibited immunoglobulin M secretion by bovine blood lymphocytes [13]. Reduction of mitogenic response to PHA has been also shown when lymphocytes were only preincubated for 2 hr or longer with β-hydroxybutyrate or acetoacetate (0.8 mM) [16]. Furthermore, increasing concentrations of

β-hydroxybutyrate and acetoacetate ranging from 0.1 to 4.8 mM, in contrast to acetone, impaired mitogenic responses of bovine T-lymphocytes isolated from milk and peripheral blood [9, 10]. The findings obtained in the present study indicate that acetoacetic acid and β-hydroxybutyric acid suppress mitogenic responses of lymphocytes.

Recent epidemiologic studies [1, 2] indicated that ketosis was interrelated with several infectious diseases in dairy cows. The cows with ketosis had an increased incidence of metritis and mastitis. Furthermore, a high blood concentration of β-hydroxybutyrate has been associated with a higher frequency of infections such as mastitis and metritis than in cows with a normal concentration of β-hydroxybutyrate [4]. Increased blood concentrations of acetoacetic acid and β-hydroxybutyric acid in the ketotic cows might suppress lymphocyte blastogenesis. This may be one of the reason for a higher frequency of infectious diseases such as mastitis and metritis observed in cows during subclinical and clinical ketosis, which were indicated previously [1, 2]. Further study is needed to clarify the relationships between suppression of lymphocyte blastogenesis and blood concentrations of ketone bodies, including cortisol and ammonia which are known to have a suppressive effect on the immune system [11, 14, 17].

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