NOTE *Internal Medicine*

Changes of Serum Alkaline Phosphatase Activity in Dry and Lactational Cows

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(Received 9 July 2004/Accepted 8 April 2005)

ABSTRACT. Serum alkaline phosphatase (ALP) activities were measured during dry and lactational periods to investigate the influence of lactation on serum ALP activity in cows. Higher levels of serum ALP activity were seen in lactational periods than in dry periods. The serum activities of bone-specific ALP (BALP), liver ALP (LALP), tartrate resistant acid phosphatase (TRAP) and aspartate aminotransferase also increased in lactational periods. ALP activities in the bone extract and in whey were decreased at similar rates by the addition of lectin. Moreover, since the ALP band in whey was observed to have the same migration in polyacrylamide gel (PAG) disk electrophoresis as that of the bone extract, analysis of ALP isoenzymes by lectin affinity or PAG disk electrophoresis could not distinguish ALP originating from the mammary gland from that of bone. In this study, it was clear that the increased level of serum ALP activity was due to increases of BALP and LALP in lactational periods. However, the extent of the influence of ALP originating from the mammary glands on serum ALP activity was unknown. Judging from changes of BALP and TRAP activities in the serum and the correlation between the both, it was guessed that ALP originating from the mammary glands influenced serum ALP activity.
KEY WORDS: cattle, lactation, serum ALP activity.

J. Vet. Med. Sci. 67(8): 813–815, 2005

Alkaline phosphatase (ALP) in serum has been shown to consist of isoenzymes originating from the liver and bone, which have a long half-life, and these isoenzymes are mainly utilized as indices of liver function or bone metabolism in cattle [2]. Serum ALP activity increases in cases of hepatitis, biliary disorders, or during growth due to active bone metabolism [2].

A lot of ALP originating from the mammary glands is found in the whey [1, 3]. Since the activity of ALP originating from the mammary glands in milk is completely inhibited by heating, measuring the ALP activity in milk is utilized as one of the methods for certifying sufficient heat treatment of milk in pasteurization [1, 3]. However, the influence of lactation on serum ALP activity has remained unknown.

ALP isoenzymes originating from the bone and liver have different features of inhibition by heating or lectin affinity, and these isoenzymes have been measured individually using those features for clinical examination in humans [10] and dogs [6]. However, in cattle, the features of the ALP isoenzyme from the mammary gland have not been examined. There are reports in the literature on serum ALP isoenzymes in horses [11], cattle [9], dogs [6] and cats [5]. However, the relationship between lactation and variations of serum ALP activity have not been examined in these animals. In this study, we examined the variation of bovine serum ALP activity and its isoenzymes during dry and lactational periods to investigate the influence of lactation on serum ALP activity and the features of the ALP isoenzymes from bone, liver, and the mammary gland.

Blood samples were obtained from 52 healthy Holstein Friesian cows (3–9 years old) kept on two farms. The cows were divided into five groups by number of days after calving; dry period (dry off to calving, n=7), early lactation period (8 to 50 days, n=8), peak lactation period (51 to 110 days, n = 11), mid lactation period (111 to 220 days, n=12), and late lactation period (221 days to dry off, n=14).

The blood was incubated at 37°C for 1 hr, centrifuged to separate the serum, and frozen at –20°C until analysis. Serum ALP activities were determined using an automatic analyzer (Automatic Analyzer 7060, Hitachi, Ltd., Japan) and a commercially available ALP kit (Liquitech ALP, Roche Diagnostics Co., Ltd., Japan). The fractions of ALP isoenzymes in the serum were measured by Rosalki's method using lectin [10]. Furthermore, the serum activities of osteoclast-derived tartrate resistant acid phosphatase (TRAP) and aspartate aminotransferase (AST) were measured as indices of bone metabolic activity and hepatic function, respectively. Serum TRAP activity was measured by an enzymatic method [12]. Serum AST activity was measured using the automatic analyzer described above and a commercially available AST kit (Liquitech GOT IFCC, Roche Diagnostics Co., Ltd., Japan).

Parenchymatous tissue of the bone and the liver were obtained from five Holstein Friesian cows (5–8 years old) after euthanasia. Each tissue was suspended in physiological saline, then homogenized using an ultrasound homogenizer, and centrifuged to separate the supernatant. Milk obtained from five healthy Holstein Friesian cows (3–6 years old) was mixed with 1N HCl to lower the pH to 4.6, and centrifuged at 4°C to separate the whey. All extracts were frozen at –20°C until analysis.

These extracts were divided into the following three groups: heating, supernatant after addition of lectin, and

levamisole addition. The 1st group of samples was incubated at 56°C for 30 min. The 2nd group of samples was mixed with an equal volume of 0.5% wheat germ lectin solution (lectin from *Triticum Vulgaris*, Sigma-Aldrich Co., U.S.A.), incubated at 37°C for 30 min and centrifuged to separate the supernatant fluid. Levamisole was added to the third group of samples with a final concentration of 4.2 mM. Following these treatments, ALP activities in these extracts were measured. Inactivation rates were calculated as follows:

Inactivation rate (%) = {(Intact ALP activity – ALP activity after treatment) / Intact ALP activity} × 100.

Serum obtained from the cows in dry and lactational periods, the extracts of bone and liver, and the whey were examined for ALP isoenzymes by polyacrylamide gel (PAG) disk electrophoresis. PAG disk electrophoresis of ALP isoenzymes was performed using a commercially available kit (Alkphor System, Jokoh Co., Ltd., Japan). The extracts were treated with Triton X-100 (final concentration, 0.2%, Polyscience Inc., U.S.A.) and phosphatidylinositol specific phospholipase C (final concentration, 0.02 U/*l*, Funakoshi Co., Ltd., Japan) before the electrophoresis.

All results were presented as mean ± SD. Significant differences in ALP, BALP, LALP, TRAP, and AST between each period were analyzed by one-way ANOVA, followed by Tukey-Kramer's test. Correlations between ALP and BALP, ALP and LALP, BALP and TRAP, and LALP and AST were analyzed by Pearson's correlation coefficient. Values of $p<0.05$ were regarded as significant.

Serum activities of ALP, BALP, LALP, TRAP, and AST were slightly higher in lactational periods than in the dry period, respectively (Fig. 1). However, significant differences were not found in all. Significant correlations between ALP and BALP and ALP and LALP were found, respectively ($p<0.01$), but not in others. The percent inactivation of ALP activity in extracts of bone and liver and in whey after various treatments is shown in Table 1. The ALP activity in whey was completely inactivated by heating. The percent inactivation of ALP activity in extracts of bone and liver was 70.5 ± 8.0% and 58.4 ± 9.4%, respectively. Lectin addition caused a similar percent inactivation in the extract of bone and in whey. The ALP activity in all samples was almost completely inactivated by levamisole addition.

In PAG disk electrophoresis, a single major band was observed at the same migration position for the bone extract and the whey (Fig. 2). In the case of the liver extract, double bands were observed, and a cathodal band disappeared in the supernatant after lectin addition (Fig. 2). In serum obtained from cows in dry and lactational periods, double bands were observed at the same migration position (Fig. 2). They corresponded to the bands observed in the bone extract, whey, and liver, respectively.

In this study, serum ALP activity was found to be higher in lactational periods in comparison with the dry period, and it was clear that the increase in the serum activity was attributed to increases of BALP and LALP in the former period. Judging from the variation of LALP and AST, it appeared that liver function in the lactational period was not the same as in the dry period. Serum activities of BALP and TRAP have been used as indices for the bone metabolic state in cat-

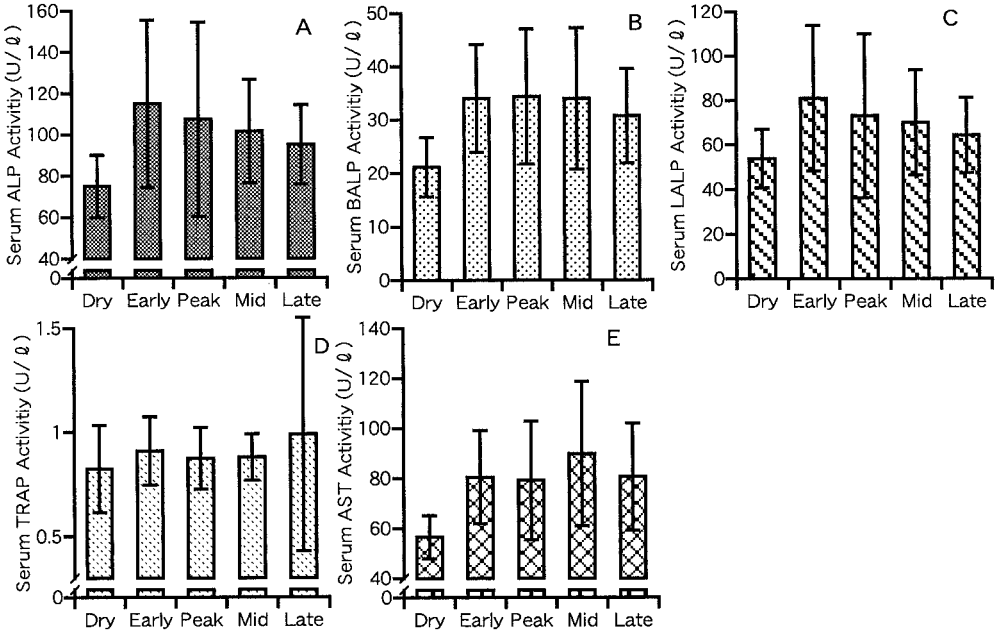

Fig. 1. Serum activity of ALP (A), BALP (B), LALP (C), TRAP (D), and AST (E) in the dry and 4 lactational periods.

Table 1. Effect of various treatments on ALP activity in extracts of bone and liver and in whey

	Heat	Lectin	Levamisole
Bone	70.5 ± 8.0	91.5 ± 7.9	100 ± 0
Liver	58.4 ± 9.4	61.7 ± 12.1	98.9 ± 0.7
Whey	100 ± 0	95.0 ± 1.53	100 ± 0

Values are expressed as percent inactivation of the original activity (means ± SD).

tle [12]. A previous report has shown that bone metabolism is more active in lactational periods than in the dry period [13]. In this study, Serum activities of BALP and TRAP showed high levels in lactational periods, suggesting that bone metabolism is more active in these periods, as in the previous report [13]. Bone resorption and formation changed by the same levels as did TRAP and BALP in non-lactational women [4]. In this study, BALP activity did not correlate with TRAP, with BALP showing an increase of about 40% in lactational periods in comparison to the dry period, and TRAP, however, showing an increase of only a 20%. The measurement of BALP isoenzyme by Rosalki's method used in this study is performed by calculating the difference between the total ALP fraction and the non-lectin-conjugated ALP fraction, since BALP is specifically bound to lectin [10]. Thus, part of the ALP originating from the mammary glands may have been concluded inaccurately to be BALP in lactational periods.

One of the purposes of this study was to observe the effect of lactation on serum ALP activity. The ALP isoenzyme originating from the mammary glands could not be distinguished here from BALP, because the two isoenzymes have the same lectin affinity and migration pattern in PAG disk electrophoresis. Consequently, we were unable to clarify the influence of lactation on serum ALP activity. Judging from changes of BALP and TRAP activities in the serum and the correlation between both, it was guessed that ALP originating from the mammary glands influenced serum ALP activity to some extent in this study.

In cattle, serum ALP activity is increased in cases of hepatic disease and in young animals [2]. However, it is of little value in hepatic disease because of the broad range of reference values with which the patient's values must be compared [8]. The reference range of serum ALP activity in cattle is very broad and has been shown to be 0–488 U/*l* [7]. This value was determined without regard to lactation [7]. It is probable that the broad range of reference values for cattle is due to the high level of serum ALP activity in lactating cows.

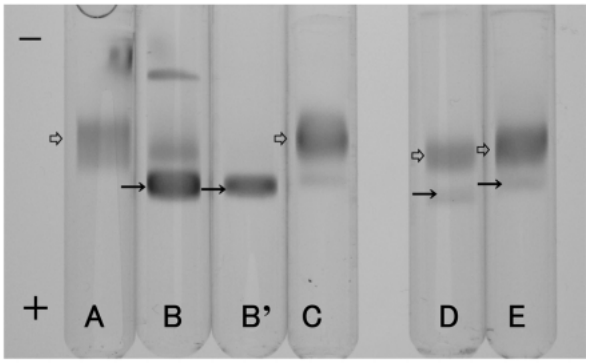

Fig. 2. PAG disk electrophoresis of ALP isoenzymes in extracts of bone (A) and liver (B), and in whey (C). A single band is observed in the extract of bone (⇨ , A) and in whey (⇨, C). Double bands are observed in the extract of liver (→, B). The cathodal band disappears in the supernatant of the liver extract after lectin addition (B'). PAG disk electrophoresis of serum ALP obtained from cows in the dry (D) and lactational (E) periods. Double bands are observed in both.

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