Calcium Metabolism in Hypocalcemic Cows with Myocardial Lesion

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ABSTRACT. This paper deals with blood levels of calcium (Ca), inorganic phosphorus, parathyroid hormone and 1,25-dihydroxyvitamin D in 6 cows treated for milk fever. Four of the cows stood within 1 day after Ca therapy, whereas 2 other cases showed an unsatisfactory response to Ca therapy and did not rise. The necropsy revealed microscopic necrotic myocardial lesions scattered in the heart of these 2 unrecovered cows. The degree of hypocalcemia and hypophosphatemia were similar in the 6 cows. However, the recovery from hypophosphatemia was markedly delayed in the cows with an unsatisfactory response. — KEY WORDS: calcium metabolism, hypocalcemia, myocardial lesion.

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We recently reported a hypocalcemic disease that occurs in 13 cows around parturition associated with recumbency, moaning, writhing, tachycardia and dyspnea, and leading to death within a few days [13]. They showed a complete lack of effective clinical response to calcium (Ca) therapy. Necropsy revealed that multifocal myocardial necrosis was invariably found in the heart. The lesion was often accompanied by cellular infiltrates and interstitial fibrosis. Blood biochemical examination revealed extremely severe hypocalcemia (3.6 ± 1.3 mg/d*l*) at the time of the clinical onset (i.e., the first visit of the referring veterinarians).

In the present study, we examined blood levels of Ca, inorganic phosphorus (iP), parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] in 6 cows (cases 1–6) which were treated for milk fever (parturient paresis, parturient hypocalcemia) by referring veterinarians on 2 farms between June 1994 and May 1995. These cases were tested for the hypothesis that the hypocalcemia might be more severe in cows being in the process of dying associated with the myocardial lesion than that in cows recovered after Ca therapy.

The clinical onset was within 4 days after parturition (Table 1). Only 1 cow (case 6) received an intramuscular injection of 10 million I.U. of vitamin D_3 (Duphafral D_3-1000, Duphar Co., Amsterdam, Netherlands) before 1 day of calving. Cases 1 and 3 were in lateral recumbency associated with flaccid paresis at the onset. Cases 2 and 4 were nervous (hypersensitive to sound and touch) in sternal recumbency associated with muscle twitch and stiffening of the hind limbs. These 4 cases stood within 1 day after Ca therapy (an intravenous injection of 400–500 m*l* of a 25% Ca borogluconate solution). Case 5 was lethargic in sternal recumbency and exhibited the head extension, dyspnea (breathing through the mouth), complete anorexia and a decreased intensity of the heart sounds. This case was slaughtered at the 5th day of the clinical course because of a complete lack of effective clinical response to twice Ca therapy during the first 2 days. Case 6 was in lateral recumbency with head extension, writhing in agony with mild pyrexia (39.2°C) and hyperpnea just before the calving. After Ca therapy, she sat up and delivered a calf. Next day she was lethargic in lateral recumbency with complete anorexia in spite of the 2nd Ca therapy. On the 3rd day, she developed acute mastitis and died the next morning.

At necropsy, case 5 revealed that the myocardium was flabby. The liver was enlarged and pale yellowish-brown. Histologically, small foci of necrotic myocytes were scattered throughout the myocardium in the left ventricular free wall and intraventricular septum (Fig. 1A). The necrotic myocytes showed nuclear pyknosis and eosinophilic cytoplasm with hematoxylin and eosin (HE) stain. The lesions were often associated with mild mononuclear cell infiltrations in the myocardial interstitium. Necropsy in case 6 revealed hemorrhagic necrotizing mastitis and congestive lung. The spleen was enlarged and mushy in consistency. The liver was enlarged and yellowish-brown. Retained fetal membrane was observed. The right ventricular chamber of the heart was markedly dilated. Microscopically, focal myocardial necrosis was observed in both atria associated with mononuclear cell infiltration in the myocardial interstitium (Fig. 1B).

Heparinized blood samples were obtained from the external jugular vein at 2 weeks before expected day of calving (prepartum) and during clinical course. The blood samples were taken at the onset (the 1st day of the clinical course) in case 1, at the 1st and 2nd days of the clinical course in cases 2–4, at the 1st, 2nd, 3rd and 5th days in case 5, and at the 1st, 2nd and 3rd days in case 6. The samples were obtained immediately before Ca therapy, when the cows were treated. The blood was centrifuged to separate plasma frozen at −20°C until analyzed. The plasma Ca concentration was determined by atomic absorption spectrophotometry [11], and iP by a molybdenum method [1]. The plasma PTH concentration was determined by two-site immunoradiometric assay (Parathyroid hormone IRMA kit, Incstar Co., MN, U.S.A.) using human intact PTH standards [4, 5]. The plasma 1,25-$(OH)_2$D concentration was determined by radioreceptorassay [12].

The plasma Ca concentrations at prepartum were 8.1–10.8 mg/d*l* (Fig. 2A). Plasma Ca reduction occurred at the onset in all cases, mildly in case 1 (8.2 mg/d*l*). The plasma

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Table 1. Clinical information of six hypocalcemic cows

Case No.	Age (years)	Parity	Clinical onset (postpartum days)	Clinical course (days)	Day(s) of Ca therapy*	Outcome
1	3.9	2	4	1	Onset	Stand immediately after Ca therapy
2	7.7	6	1	1	Onset	Stand immediately after Ca therapy
3	8.1	6	1	1	Onset	Stand immediately after Ca therapy
4	5.3	4	1	1	Onset	Stand within 1 day after Ca therapy
5	5.7	4	1	5	Onset and next day	Slaughter after 4 days of onset
6	7.2	5	0	3	Onset and next day	Death until 3 days after onset

* Ca therapy: an intravenous injection of 400–500 m*l* of a 25% Ca borogulconate solution.

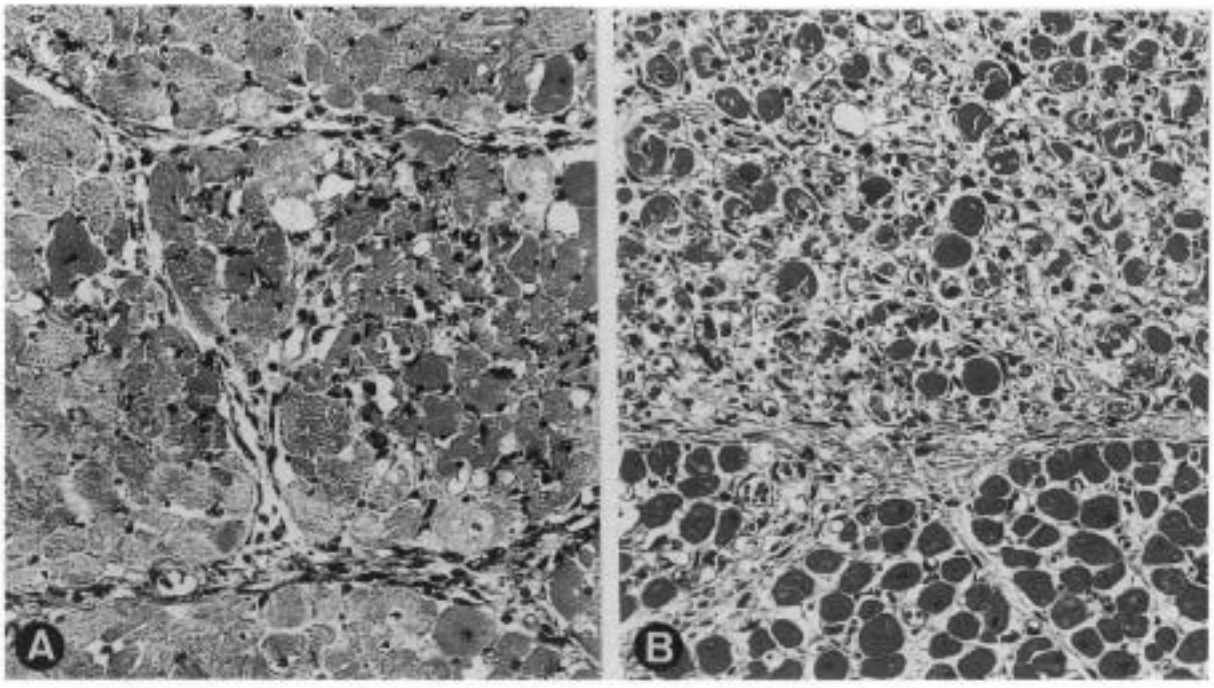

Fig. 1. Microscopic photographs of the myocardium in two hypocalcemic cows lacking a satisfactory response to Ca therapy. A: Necrotic myocytes with eosinophilic cytoplasm accompanied by mild mononuclear cell infiltration in the left ventricular free wall in case 5. HE stain, × 236. B: Interstitial inflammation including mononuclear cell infiltration associated with necrotic myocytes in the left atrium in case 6. HE stain, × 236.

Ca levels in cases 2–4 were 4.7–5.8 mg/d*l* at the onset and increased to 6.9–8.5 mg/d*l* at the 2nd day of the clinical course. The plasma Ca concentrations at the onset in cases 5 and 6 were 5.2 and 5.8 mg/d*l*, respectively. In case 5, the plasma Ca was 5.9 mg/d*l* at the 2nd day of the clinical course and increased to > 8.4 mg/d*l* after the 3rd day. In case 6, the plasma Ca concentration increased to 6.9 mg/d*l* on the 2nd day and 10.8 mg /d*l* on the 3rd day of the clinical course. The plasma iP concentrations in all cases were 4.4–7.7 mg/d*l* at prepartum and decreased to 1.2–2.9 mg/d*l* on the day of clinical onset (Fig. 2B). The plasma iP levels in cases 2–4 returned to > 4.3 mg/d*l* at the 2nd day of the clinical course. In cases 5 and 6, the plasma iP levels were low (2.0 and 1.4 mg/d*l*) on the 2nd day of the clinical course and then returned to > 5.6 mg/d*l* after the 3rd day. The plasma PTH concentrations at prepartum were < 5.0

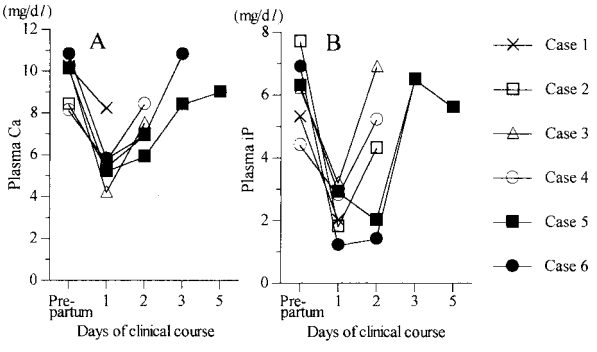

Fig. 2. Change of plasma Ca (A) and iP (B) concentrations in six hypocalcemic cows with and without a satisfactory response to Ca therapy.

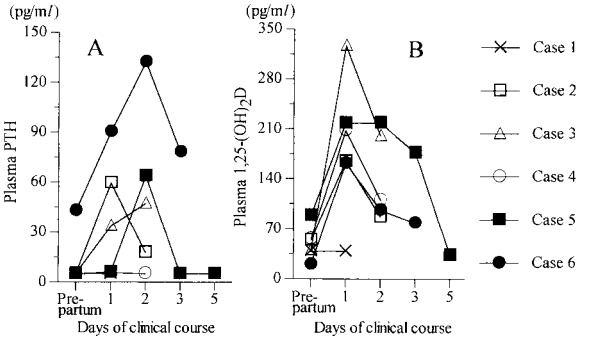

Fig. 3. Change of plasma PTH (A) and 1,25-$(OH)_2$D (B) concentrations in six hypocalcemic cows with and without a satisfactory response to Ca therapy.

pg/m*l* in cases 1–5, and 42.8 pg/m*l* in case 6 (Fig. 3A). After the onset, the plasma PTH values elevated in cases 2, 3, 5 and 6. In case 2 , the plasma PTH level showed a temporary peak (59.5 pg/m*l*) at the onset. The plasma PTH concentration in case 3 revealed a continual rise until the 2nd day of the clinical course (47.1 pg/m*l*). In case 5, it showed a transient rise (63.7 pg/m*l*) on the 2nd day. The plasma PTH value in case 6 was at a maximum (132.2 pg/m*l*) on the 2nd day. In cases 1 and 4, the plasma PTH levels did not increase. The plasma 1,25-$(OH)_2$D concentrations were 20.0–88.4 pg/m*l* at prepartum in all cases (Fig. 3B). In cases 2–6, the plasma 1,25-$(OH)_2$D concentrations were higher during the clinical course than the prepartum levels and showed a peak (161.9–326.1 pg/m*l*) at the onset. In case 1, plasma 1,25-$(OH)_2$D was not elevated at the onset.

The cows in the present study showed a similar degree of hypocalcemia and hypophosphatemia at the onset (except that the fall in plasma Ca was mild in case 1). The plasma Ca recovery from hypocalcemia in case 6 was little slower than that in cases 2–5. Previous studies have revealed that the plasma Ca concentration decreases to the pre-therapeutic level immediately after Ca therapy in milk fever cases and slowly elevates to normal levels over several days showing a satisfactory response to single Ca therapy [7–9]. Another study has suggested that a lack of 1,25-$(OH)_2$D production is an important factor predisposing the cows to a relapse of hypocalcemia and milk fever [5]. The results of the present study revealed that case 6 as well as cases 2–5 showed a marked rise in plasma 1,25-$(OH)_2$D after the onset, which suggested that 1,25-$(OH)_2$D production was maintained.

Hypophosphatemia in cases 5 and 6 remained during the first 2 days of the clinical course, whereas the plasma iP concentrations in cases 2–4 returned to normal levels by the 2nd day of the clinical course. Some previous studies [2, 8] have suggested that hypophosphatemia relates to prolonged recumbency in cows treated for milk fever, because phosphate is one of important components to induce contraction of the muscles. Although these studies have not fully explained a reason why hypophosphatemia remained in the cows with prolonged recumbency, one hypothesis has arisen as to a relation of PTH action to such the prolonged hypophosphatemia. PTH lowers the plasma iP concentration either by increasing renal excretion and salivary secretion of phosphate [6, 9]. The secretion of PTH is immediately stimulated by falling plasma Ca levels. In the present study, case 5 showed a transient rise in plasma PTH on the next day of beginning of hypophosphatemia, and the plasma PTH levels in case 6 varied markedly higher than those in the others. But, the behavior of the plasma PTH in case 3 did not seem to support this hypothesis because the plasma PTH showed a continuous rise until the day when the plasma iP level returned to normal value. A further study is needed to clarify the relation of PTH action to the prolonged hypophosphatemia and to answer whether the behavior of the plasma PTH was unusual in cases 5 and 6.

In the present study, the myocardial lesion in case 5 and 6 was mainly composed of necrosis. The lesion of the present cases seemed to be similar to that of the previous cases [13], although case 6 had mastitis and retained fatal membrane that were known as complications occurring in the recumbent cows. It is difficult to answer whether this myocardial lesion resulted from hypocalcemia and/or prolonged hypophosphatemia, because the cows with a satisfactory response to Ca therapy were not subjected to necropsy. But, several studies on milk fever have failed to detect any lesions in the myocardium at the necropsy in the cows without any response to Ca therapy [3, 8].

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