Experimental Hypocalcemia Induced by Hemodialysis in Goats

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ABSTRACT. To evaluate whether hemodialysis with a dialysate containing no calcium (Ca-free HD) can induce hypocalcemia and restore the clinical signs and blood biochemical changes in naturally occurred hypocalcemic disorder in ruminants, the clinical signs and the changes in plasma electrolytes and minerals concentrations were observed in goats during 6-hr hemodialysis. The four goats received hemodialysis with the dialysate containing calcium (Ca HD), and 10 days later they had Ca-free HD. The plasma ionized Ca (Ca^{++}) and total Ca (TCa) concentrations were not affected by Ca HD, whereas the levels significantly decreased during whole period of Ca-free HD. The Ca^{++} and TCa concentrations were 0.69 ± 0.06 mmol/l and 5.9 ± 0.3 mg/dl at 6 hr of Ca-free HD, respectively. The clinical signs observed during Ca-free HD seemed to resemble to those in naturally occurred hypocalcemic cases that were reported previously. Therefore, Ca-free HD was suggested to be one of the possible methods to induce experimental hypocalcemia in ruminants.—**KEY WORDS:** experimental hypocalcemia, goat, hemodialysis, ruminant.

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There are numerous reports dealing with an experimentally induced hypocalcemia in ruminants as a model for studies of the pathophysiology and prophylaxis of milk fever (parturient paresis, parturient hypocalcemia) [3, 8, 9, 12, 13, 18, 19, 22]. Most of the reports have described an intravenous infusion of disodium ethylenediaminetetraacetic acid (Na_2EDTA) solution to induce experimental hypocalcemia. Some of the authors have reported that accidental death sometimes occurs in the experimental animals as a result of the Na_2EDTA infusion [8, 22]. The cause of death remains unclear, but they have assumed that death occurs due to the side effects of the Na_2EDTA infusion [8, 22]. Nephrotoxicity and hepatotoxicity are known to be side effects of the Na_2EDTA infusion [6, 7, 10].

Hemodialysis is one of safe therapeutic methods for human patients with severe renal failure. It has been reported that hemodialysis with a dialysate containing no calcium (Ca-free HD) can lower the plasma calcium concentration in dogs [4, 5, 23]. But less is known as to whether Ca-free HD can restore the clinical signs and blood biochemical changes in naturally occurred hypocalcemic disorder in ruminants. We therefore examined the clinical signs and the changes in plasma electrolytes and minerals concentrations during 6-hr Ca-free HD in goats.

MATERIALS AND METHODS

Animals: Four clinically healthy crossbred goats (female, aged 2 to 4 years, and weighing 34 to 46 kg) were penned

and accustomed to handling for at least 1 month. They were fed a commercial grain mixture and timothy hay *ad libitum*. Each goat had two catheters (Anthron Catheter II-1470, Toray Medical Co., Ltd., Tokyo) inserted into the left carotid artery and jugular vein for hemodialysis and another one into the right jugular vein for collecting blood samples. The goats received 6-hr hemodialysis with a dialysate containing calcium (Ca HD) 10 days after the catheterization. They received 6-hr Ca-free HD 10 days after Ca HD.

Dialysate: One hundred l of dialysate was used in each hemodialysis. The contents of the electrolyte (in mmol/l) in the dialysate for CaHD were NaCl (105), KCl (4), $\text{MgCl} \cdot 6\text{H}_2\text{O}$ (1), $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ (12), NaHCO_3 (25), $\text{C}_6\text{H}_{12}\text{O}_6$ (3.5) and CaCl_2 (1.25). The dialysate for Ca-free HD contained the same electrolytic contents except for CaCl_2 . The pH of the dialysate was adjusted to 7.3 with 10 N HCl and the osmolality was about 280 mOsm/kg.

Hemodialysis: A dialyzer (Mera Crystal S-SP 0.8, Senko Medical Instrument Mfg. Co., Ltd., Tokyo), filled with heparinized sterile saline, was connected to a dialysate inflow-outflow equalizer (Dialife S-11, Senko Medical Instrument Mfg., Co., Ltd., Tokyo) and a blood pump (Dialife S-7, Senko Medical Instrument Mfg. Co., Ltd., Tokyo). Before 10 min of hemodialysis, the goats received an intravenous administration of heparin sodium (300 IU/kg). The flow of dialysate was maintained at 500 ml/min at 38°C. The blood flow was regulated to 3 ml/kg/min with no pressure gradient across the membrane. Each of the goats stood in a small wooden crate during the hemodialysis.

Clinical observation and blood biochemistry: Clinical observation and blood sampling were done immediately before (0 hr) and at 1 hr intervals during hemodialysis (1 to 6 hr). The rectal temperature, heart rate and respiration rate were recorded. The clinical signs observed during hemodialysis were mobility, demeanor, shivering, ruminal motility and heart sound quality by auscultation. Blood

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Fig. 1. Changes in rectal temperature, heart rate and respiration rate during Ca HD and Ca-free HD.

was taken in a 2.5-ml heparinized syringe. The plasma concentrations of ionized calcium (Ca^{++}), sodium (Na^+), potassium (K^+) and bicarbonate (HCO_3^-) were determined by means of the 288 Blood Gas System (Ciba Corning Diagnostics Corp., CA, U.S.A.), and the plasma ionized chloride (Cl^-) by C-80A (Jookoo Co., Ltd., Tokyo). The plasma total calcium (TCa) and magnesium (Mg) concentrations were determined by atomic absorption spectrophotometry [20], and inorganic phosphorus (P) by colorimetry [2].

Statistical analysis: The rectal temperature, heart rate, respiration rate and blood biochemical data were expressed as means $\pm$ standard error. One-way analysis of variance was used to observe the effect of each Ca-free HD and Ca HD. If the effects were significant, the values at 1 to 6 hr during each hemodialysis were compared with those at 0 hr by Turkey multiple comparison test. The significance was taken at $P < 0.05$.

RESULTS

Clinical findings: The rectal temperature and heart rate tended to reach the maximum at 1 hr of hemodialysis, but these changes had no statistical significance in Ca HD and Ca-free HD (Fig. 1). The respiration rate was not affected by either Ca HD or Ca-free HD. In Ca HD, one of the goats temporally staggered and shivered at 1 hr of hemodialysis. The other goats with Ca HD did not show any abnormal signs. During the entire period of Ca-free HD, three goats became ataxic and there was repeated reeling and falling associated with muscle tremor. All of the goats showed signs of dullness, shivering, ruminal stasis and decreased heart sound intensity as detected by auscultation during Ca-free HD.

Blood biochemical results: There were statistically

Fig. 2. Changes in plasma Ca^{++} and TCa concentrations during Ca HD and Ca-free HD. Significant difference from value at 0 hr in Ca-free HD, b: $P < 0.01$.

significant changes in the plasma Ca^{++} , TCa, P, Mg and HCO_3^- concentrations during Ca HD and/or Ca-free HD (Figs. 2 and 3). Ca HD had no effect on changes in the plasma Ca^{++} and TCa concentrations, but Ca-free HD induced a significant reduction at 1 to 6 hr. The plasma Ca^{++} (1.13 ± 0.03 mmol/l) and TCa levels (9.2 ± 0.4 mg/dl) at 0 hr decreased to 0.69 ± 0.06 mmol/l and 5.9 ± 0.3 mg/dl at 6 hr of Ca-free HD, respectively ($p < 0.01$). The plasma P

Fig. 3. Changes in plasma P, Mg, HCO_3^- , Na^+ , K^+ and Cl^- concentrations during Ca HD and Ca-free HD. Significant difference from value at 0 hr in Ca HD or Ca-free HD, a: $P < 0.05$, b: $P < 0.01$.

concentrations were not affected by Ca HD, but the levels (5.6 ± 1.1 mg/dl at 0 hr) were low at 4 hr (2.7 ± 0.5 mg/dl) during Ca-free HD ($P < 0.01$). The plasma Mg concentrations at 0 hr were 1.9 ± 0.2 mg/dl in Ca HD and 2.2 ± 0.3 mg/dl in Ca-free HD. These values were significantly high during 2 to 6 hr of Ca HD (2.6 ± 0.1 mg/dl at 6 hr, $p < 0.01$) and during 4 to 6 hr of Ca-free HD (3.1 ± 0.2 mg/dl at 6 hr, $p < 0.01$). The plasma HCO_3^- levels during Ca-free HD were higher at 5 hr (29.2 ± 1.3 mmol/l; $p < 0.05$) than those at 0 hr (25.9 ± 2.3 mmol/l). The plasma Na^+ , K^+ , and Cl^- concentrations were not affected by Ca-free HD or Ca HD.

DISCUSSION

Clinical signs of hypocalcemia in goats generally resemble those in cows [21]. The affected goat shows signs of ataxia, shivering and muscle tremor, followed by recumbency. Experimental studies on goats made hypocalcemic by intravenous infusion of 4% Na_2EDTA solution [12, 13] described how staggering and restlessness occurred in a mild hypocalcemic state (more than a 6 mg/dl Ca concentration in blood). The goats had depressed anal and palpebral reflex and repeated reeling and falling with moderate hypocalcemia (4 to 5 mg/dl), and exhibited complete recumbency in a severe hypocalcemic state (less than 2 mg/dl) [12, 13]. In the present study, the goats showed signs of marked hypocalcemia during Ca-free HD. The plasma Ca^{++} and TCa concentrations were around 0.69 mmol/l and 5.9 mg/dl during Ca-free HD, which were similar levels to a mild to moderate hypocalcemic state in

previous experimental studies on hypocalcemic goats [12, 13]. The clinical signs found in the goats on Ca-free HD were ataxia (i. e., staggering and repeated reeling and falling, associated with muscle tremor), dullness, shivering, ruminal stasis and decreased heart sound intensity. These clinical signs seemed to resemble those in milk fever cows and experimental hypocalcemic ruminants previously reported [8, 12, 13, 17, 18, 21, 22].

Although Ca HD did not affect the plasma Ca^{++} and TCa concentrations, only one goat temporally staggered and shivered during the early period of Ca HD. The rectal temperature and heart rate showed a tendency to increase during both Ca HD and Ca-free HD. In human medicine, it has been noted that headache, chest and back pain, mental confusion, muscle twitching, dyspnea and diaphoresis often occur during hemodialysis [14, 25]. It has been reported that these symptoms occur in human patients during hemodialysis as a result of complications that may arise following the interaction between blood and the dialyzer [25]. It has been considered that possible factors contributing to this interaction are the excessive removing of body water, the rapid shift in the acid-base status, and the participation of cytokines and endotoxins [11, 16]. In the present study, it was supposed that the temporal clinical signs during Ca HD and the tendency to a high rectal temperature and heart rate during both Ca HD and Ca-free HD occurred as complications similar to those in human patients on hemodialysis.

It is well known that hypophosphatemia occurs in cows with milk fever [17, 21]. This may be caused by phosphorus

loss in colostrum, inadequate phosphorus intake around parturition, inadequate phosphorus mobilization from bone, and/or phosphorus diuresis caused by the high blood concentrations of parathyroid hormone (PTH) due to hypocalcemia [17]. In the present study, the plasma P concentrations significantly decreased followed by a reduction in the Ca concentrations during Ca-free HD, which was the same result as in previous studies on experimental hypocalcemia induced by Na₂EDTA infusion in cows and sheep [3, 9, 18, 19, 22]. In experimental hypocalcemia, it is suggested that the hypophosphatemia is due to PTH action. The secretion of PTH is stimulated by falling plasma Ca⁺⁺ levels. The high blood concentrations of PTH lower the plasma P concentrations by increasing renal phosphate excretion and by increasing the salivary phosphate secretion [3, 15].

In the present study, the plasma Mg concentrations increased during both of Ca HD and Ca-free HD. There was concern that this Mg elevation resulted from a relatively high Mg concentration in the dialysate; so it was necessary to reduce the Mg concentration in the dialysate in this experiment.

The present study describes how the plasma HCO₃⁻ concentrations increased temporally at 5 hr of Ca-free HD. Recently, Barzanji and Daniel [1] and Setia and his associates [24] have reported that a rise occurs in the plasma HCO₃⁻ concentrations as well as in arterial and venous blood pH, carbon dioxide pressure and base excess in experimental hypocalcemic cows and sheep induced by the Na₂EDTA infusion. It is suggested that these changes were due to an impairment of oxygen uptake by the pulmonary blood flow and an impairment of peripheral tissue uptake of oxygen during hypocalcemia [1, 24].

In conclusion, the present study suggested that Ca-free HD was one of the possible experimental methods to induce hypocalcemia and to restore the clinical signs and blood biochemical changes in naturally occurring hypocalcemic disorder in ruminants. But some unfavorable clinical signs sometimes occurred in the animals probably due to the complications that may arise following the interaction between blood and the dialyzer during hemodialysis.

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