Effects of Oral Administration of “Rumen-Bypass” Vitamin D₃ on Vitamin D and Calcium Metabolism in Periparturient Cows

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ABSTRACT. Eleven late-pregnant Jersey cows were assigned to two groups; a group (PO-RBVD group) consisting of five cows treated with an oral administration of 10 million I.U. of an encapsulated form of vitamin D₃ (“rumen-bypass” VD₃; RBVD₃) and another group (IMVD group) consisting of the other six treated with an intramuscular injection of 10 million I.U. of vitamin D₃ (VD₃). The cows received the RBVD₃ or VD₃ administration at 7 days before the expected parturition. The changes in the plasma concentrations of vitamin D metabolites, ionized Ca (Ca++) and inorganic phosphorus (iP) were evaluated. Of the vitamin D metabolites, the plasma 25-hydroxyvitamin D concentrations in PO-RBVD group increased significantly after the RBVD₃ administration and remained in high levels that were significantly higher than those in IMVD group. This suggested that RBVD₃ was absorbed rapidly and excellently from the post-ruminal digestive tract without the degradation by ruminal microorganisms. The plasma Ca++ and iP concentrations in PO-RBVD group tended to be higher after the administration and around parturition than those in IMVD group. From these observations, it was suggested the oral RBVD₃ administration had more potent ability to prevent parturient paresis compared with the VD₃ injection used widely in Japan.—KEY WORDS: calcium, cattle, parturition, “rumen-bypass”, vitamin D₃.

The use of vitamin D (VD; general name of vitamin D₂ and D₃) has been popular in attempt to prevent parturient paresis in cows. These treatments include a single intramuscular injection of 10 million I.U. of vitamin D₃ (VD₃) before 1 week of the expected parturition [4, 11, 13, 17, 24] and continuous feeding of 20 to 30 million I.U. of VD for five consecutive days before calving [3, 5, 6]. Although parental administration of VD₃ has been widely used in Japan, large oral doses of VD has not been used because of instability of the prophylactic effect and inconvenience due to continuous feeding and necessity of extremely massive doses of VD. Once VD is orally administered to cows, VD metabolism actually begins in the rumen [10]. VD is converted to some unidentified metabolites having antivitamin D activities by rumen microorganisms, and as much as 80% of the VD disappears from the incubation media of the rumen fluid over 24 hrs [10]. The ruminal degradation of VD is the reason why continuous feeding of extremely massive doses of VD is needed for maximum protection against milk fever [10]. Several studies have reported that dietary proteins, amino acids and vitamin AD₃E premix encapsulated by hydrogenated oil or polymer can bypass the rumen with minimization of the ruminal degradation in cows [1, 15, 16, 23]. Therefore, we suspect that VD in a form that prevents the degradation by ruminal microorganisms will bypass the rumen and provide the prophylactic effect against parturient paresis. In the present study, cows received a single oral administration of an encapsulated form of VD₃ (“rumen-bypass” VD₃; RBVD₃) before calving. The objective of this study was to determine the effects of RBVD₃ on VD and Ca metabolism after the oral administration and around parturition. These effects were compared with a single intramuscular injection of VD₃ that was one of the popular prophylaxis in Japan.

MATERIALS AND METHODS

Animals: Eleven late-pregnant Jersey cows (aged 3 to 8 years) were assigned to two groups; a group (PO-RBVD group) consisting of five cows treated with an oral administration of RBVD₃ and another group (IMVD group) consisting of the other six treated with an intramuscular injection of commercial VD₃ preparation. The cows stayed in an outside paddock during dry period until 8 days before the expected parturition. They were housed in an individual pen until 3 days after calving. They thereafter stayed in another outside paddock during 9 a.m. to 3 p.m., and were
housed in a stanchion during 3 p.m. to 9 a.m. The cows were fed a good quality hey, corn silage, and commercial concentrate during dry period and after parturition. Commercial Ca and P supplements were added in the diet after calving. It was calculated that the cows were consuming 42 to 45 g of Ca and 27 to 31 g of phosphorus (P) per day during at least 2 weeks before the expected parturition, and 223 to 232 g of Ca and 73 to 79 g of P per day after calving.

Administration of RBVD$_3$ or VD$_3$: The cows of PO-RBVD group treated with an oral administration of 10 million I.U. of RBVD$_3$ mixed with a small amount of the concentrate at 3 p.m. of 7 days before the expected parturition. The RBVD$_3$ was in the form of VD$_3$ resin encapsulated by hydrogenated oil that was supplied by Nippon Soda Co., Ltd., Japan (Pro Vit D$_3$-1000). The cows of IMVD group treated with an intramuscular injection of 10 million I.U. of VD$_3$ dissolved in a liposoluble medium (Osvitan-1000, Denka Pharmacy Co., Ltd., Japan) at 3 p.m. of 7 days before the expected parturition. If cow did not calve within 1 week, the cows received an additional administration of RBVD$_3$ or VD$_3$ at 8 days after the previous administration.

Plasma biochemistry: Blood was taken with two heparinized syringes from the external jugular vein at 3 p.m. of every day before calving, at time soon after calving (0 days), and at 0.25, 0.5, 1, 2, 3 and 5 days (i.e., 6, 12, 24, 48, 72 and 120 hrs) after the time of calving. The blood from one syringe was immediately used to determine the plasma concentration of ionized Ca (Ca++) by 288 Blood Gas System (Ciba Corning Diagnostics Corp., U.S.A.). The blood from another syringe was immediately centrifuged to separate plasma, which was frozen at -20°C until analyzed. The plasma concentration of 25-hydroxyvitamin D (25-OHD) was measured by a radioreceptor assay described by Reinhardt et al. [11]. The plasma concentration of 1,25-dihydroxyvitamin D (1,25-(OH)$_2$D) was measured by a radioreceptor assay described by Reinhardt et al. [21]. In the present study, total concentrations of 25-OHD$_2$ and 25-OHD$_3$ (or 1,25-(OH)$_2$D$_3$ and 1,25-(OH)$_2$D$_3$) were expressed as the concentrations of 25-OHD (or 1,25-(OH)$_2$D), because 25-OHD$_3$ (or 1,25-(OH)$_2$D$_3$) cannot be separated from 25-OHD$_2$ (or 1,25-(OH)$_2$D$_2$). The plasma inorganic P (iP) concentration was determined by a molybdenum method [2].

Ca treatment for parturient paresis: If cow developed parturient paresis after calving, the cow received Ca treatment (an intravenous infusion of 500 ml of 25 % Ca borogluconate solution) [19, 25]. The criteria to start this treatment were that the cow was in recumbency and was unable to stand up itself.

Data analysis: The data in each group were expressed as means ± standard deviation. Firstly, the data during 0 to 3 days after the administration were evaluated. If cow received the repeated administration, the data after the second administration were excluded. Student’s t-test was done to compare each timed value between PO-RBVD and IMVD groups. Analysis of variance was used to observe the effects in each group. If the effects were significant, the timed values were compared with the value at 0 day by Bonferroni multiple comparison test. In the next, the data around parturition (during -2 to 5 days after parturition) were also evaluated. If cow received Ca treatment after calving, the data after the treatment were excluded. Student’s t-test was done to compare each timed value between PO-RBVD and IMVD groups. In all of the analysis, significance was set at P<0.05.

RESULTS

Duration between the administration and calving: The cows of PO-RBVD group calved at 3.2 ± 1.9 days after the oral RBVD$_3$ administration. The cows of IMVD group calved at 6.0 ± 3.1 days after the VD$_3$ injection. One cow of IMVD group received repeated VD$_3$ injections before calving.

Development of parturient paresis: Two cows of PO-RBVD group and two cows of IMVD group developed parturient paresis within 1 day (during 6 to 24 hr) after calving. They stood within 12 hr after Ca treatment.

Effects after RBVD$_3$ or VD$_3$ administration: The plasma 25-OHD concentrations in PO-RBVD group increased significantly from 55.4 ± 27.5 ng/ml at 0 day to about 125 to 145 ng/ml during 1 to 3 days after the RBVD$_3$ administration (P<0.05 or 0.01) (Fig. 1). The 25-OHD levels during 1 to 3 days were significantly higher in PO-RBVD than in IMVD group (P<0.05 or 0.01). The plasma 25-OHD concentrations in IMVD group did not change significantly (around 54 to 75 ng/ml). There were no significant changes in the plasma 1,25-(OH)$_2$D concentrations (around 30 and 62 pg/ml) in both PO-RBVD and IMVD groups (Fig. 1). The plasma Ca++ and iP concentrations in PO-RBVD group (about 1.31 to 1.38 mmol/l and 6.0 to 7.6 mg/dl, respectively) tended to be higher than those in IMVD group (about 1.22 to 1.27 mmol/l and 5.0 to 6.1 mg/dl, respectively) during 1 to 3 days, although there were no significant differences between the groups (Fig. 2).

Effects around parturition: Although the plasma 25-OHD concentrations did not show marked changes in each group (Fig. 3), the levels in PO-RBVD group (about 134 to 185 ng/ml) during 70 to 90 ng/ml during -2 to 2 days after parturition (P<0.05 or 0.01). The cows of PO-RBVD group showed the small increase in the plasma 1,25-(OH)$_2$D levels after parturition (80 to 94 pg/ml), whereas the cows of IMVD group revealed the marked rise after parturition (more than 100 pg/ml). There were no significant differences in the each timed values between PO-RBVD and IMVD groups. The plasma Ca++ concentrations in PO-RBVD group seemed be higher than those in IMVD group around parturition except for 0 to 1 day (Fig. 4). At -2 and 3 days after parturition, the plasma Ca++ levels in PO-RBVD group (1.43 ± 0.08 and 1.21 ± 0.04 mmol/l, respectively) were
significantly higher than those in IMVD group (1.28 ± 0.06 and 1.11 ± 0.04 mmol/l, respectively; P<0.05). The plasma Ca++ concentrations decreased to about 0.9 mmol/l in PO-RBVD group and to about 0.87 mmol/l in IMVD groups during 0 to 1 day after parturition. The plasma iP concentrations in PO-RBVD group seemed to be higher than those in IMVD group around parturition. At 3 days after parturition, the plasma iP level in PO-RBVD group (4.1 ± 0.7 mg/dl) was significantly higher than that in IMVD group (2.9 ± 0.9 mg/dl; P<0.05). The cows of PO-RBVD group revealed the decrease in the plasma iP levels (about 3.5 mg/dl) at 0 and 1 days after parturition, and those of IMVD group showed the low iP concentrations (about 2.6 to 3.2 mg/dl) during 0 to 3 days.

**DISCUSSION**

Once VD enters the blood, it accumulates to the liver [8–10, 20]. The increase in the plasma 25-OHD after the VD₃ administration is thought to be caused by the acceleration of the production of 25-OHD in the liver, because VD is converted into 25-OHD by the hepatic microsomal and mitochondrial 25-hydroxylase enzymes [8, 9, 10, 20]. The mitochondrial 25-hydroxylase enzyme, especially, can hydroxylate VD to 25-OHD under excess concentration of VD over the physiological range, because this enzyme is immense in quantity [12]. The 25-OHD is present in the plasma at concentrations of 20 to 50 ng/ml in the normal cows and at 200 to 300 ng/ml in VD toxicosis [9]. In the present study, the plasma 25-OHD concentrations in PO-RBVD group after the RBVD₃ administration increased significantly to approximately 2 to 3 times of the values in the normal cows. The plasma 25-OHD levels around
parturition were significantly higher in PO-RBVD group than in IMVD group, although the levels in IMVD group were higher than the values in the normal cows. These results suggest that the most of RBVD$_3$ administered orally bypassed the rumen to be absorbed from the post-ruminal digestive tract. In addition, the results suggest that the absorption of 10 million I.U. of RBVD$_3$ from the digestive tract was more rapid and excellent compared with that of the same dose of VD$_3$ from an injection site of the muscles.

Hollis et al. [7] reported that the plasma 25-OHD concentrations did not change until 7 days after an intramuscular injection of 15 million I.U. of VD$_3$ to pregnant cows and that the values increased significantly at 28 days. Their results [7] seemed to be similar to the data of the present study, because the plasma 25-OHD concentrations increased immediately after an intramuscular injection of 10 million I.U. of VD$_3$ to lactating cows and pregnant cows. This discrepancy may be due to different solvents of VD$_3$. The VD$_3$ used by Hollis et al. [7] and in the present study was dissolved in an liposoluble medium, whereas Naito et al. [14] and Takaki et al. [22] used commercial VD$_3$ that had been reported as a form of VD$_3$ dissolved in an aqueous medium [4]. We supposed that the absorption of VD$_3$ in the liposoluble medium from an injection site of the muscle might be slower than that of VD$_3$ in the aqueous medium, and we suspect that the VD$_3$ in liposoluble medium may distribute easily to the fat tissues before the accumulation into the liver.

Once 25-OHD is formed, it can be converted directly into at least four metabolites. Of these metabolites, 1,25-(OH)$_2$D$_3$, the most active form of VD, is converted from 25-OHD by 1α-hydroxylase in the kidney [20]. The control of 1α-hydroxylation process is tightly regulated by directly
parathyroid hormone (PTH) and P and indirectly by Ca [9, 10, 20]. For example, the decline in the plasma Ca concentration stimulates to secrete PTH from the parathyroid gland, and PTH stimulates the activation of 25-OHD by up-regulating 1α-hydroxylase enzyme to form 1,25-(OH)2D [9]. When the plasma Ca is high, PTH secretion is depressed, and 1,25-(OH)2D synthesis is depressed [9]. In the present study, there was no significant elevation of the plasma 1,25-(OH)2D concentrations in PO-RBVD group, although the plasma 25-OHD concentrations increased significantly. We suggested that the cows described here maintained the tight control of kidney 1α-hydroxylase enzyme during the high plasma 25-OHD concentrations after the oral RBVD3 administration.

The present study showed the cows of IMVD group had the decrease in the plasma Ca++ levels during 0 to 1 day after parturition and the decline in the plasma iP concentrations during 0 to 3 days. The plasma 1,25-(OH)2D concentrations in this group increased markedly after parturition. This increase in the 1,25-(OH)2D levels seemed to be a response to the decline in the plasma Ca++ and iP concentrations. The cows of PO-RBVD group showed only a tendency of mild increase in the plasma 1,25-(OH)2D concentrations after parturition (Fig. 3), although the cows had the decrease in the plasma Ca++ and iP concentrations during 0 to 1 day after calving. These different degree of the rise in the plasma 1,25-(OH)2D levels was thought to be due to the different degree of the elevation in the plasma Ca++ and iP concentrations after the oral RBVD3 administration and VD3 injection. The plasma Ca++ and iP concentrations in PO-RBVD group tended to be higher after the oral RBVD3 administration and were significantly higher at -2 and/or 3 days after parturition than those in IMVD group.

Conrad et al. [3] reported that a massive oral doses (20 to 30 million I.U.) of VD increased Ca and P absorption from the digestive tract and induced an increase in the blood Ca and iP levels. Hollis et al. [7] reported that bone resorption was activated immediately by an increase in the plasma 25-OHD concentrations after the injection of 15 million I.U. of VD3. We suggested that the higher values of the plasma Ca++ and iP concentrations in PO-RBVD group resulted from increased intestinal absorption and bone resorption of Ca and P due to higher concentrations of the plasma 25-OHD.

In the present study, the single oral administration of 10 million I.U. of RBVD3 at 7 days before the expected calving showed the evidence of rapid and excellent absorption of RBVD3 from the digestive tract and the tendency to maintain the higher plasma levels of Ca++ and iP. These results suggested that the RBVD3 administration provided more potent ability to prevent parturient parapares compared with the VD3 injection used widely in Japan. However, the occurrence of parturient parapares in the several cows of both PO-RBVD and IMVD groups suggested that the prophylaxis with VD3 was not always complete, as shown in the previous investigations [4, 11].

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REFERENCES