Effects of 1α-Hydroxyvitamin D₃ Injection on Plasma 1α, 25-Dihydroxyvitamin D Concentration in Lactating Cows

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ABSTRACT. The present study was carried out to compare the effects of the injection of 1α-hydroxyvitamin D₃ (1αOHD₃) on the plasma 1α, 25-dihydroxyvitamin D(1,25(OH)₂D) and calcium (Ca) concentrations in lactating cows, and to determine the efficacy of 1αOHD₃ as prophylactic of parturient hypocalcemia. The cows were divided into two groups, a control group consisting of 6 cows and treatment(1αOHD₃) group consisting of 5 cows. Each cow in the treatment group once received intramuscularly 500 µg of 1αOHD₃ resolved in 3 ml ethanol. The plasma 1,25(OH)₂D concentration in the 1αOHD₃ group was significantly higher than that in the control group from 6 hr to 5 days after injection, and became significantly lower than that in the control group at 10 days after injection. The plasma total Ca concentration was significantly higher in the 1αOHD₃ group than that in the control group during 2 to 7 days after injection, while the plasma ionic Ca concentration in the former was significantly higher than that in the latter at 1, 2, 3, and 7 days after injection but lower than that at 14 days. Therefore, it is suggested to be appropriate for the prevention of the parturient hypocalcemia to inject 500 µg of 1αOHD₃ intramuscularly within 2 to 5 days before parturition. However, if 1αOHD₃ was injected earlier than 10 days before parturition, this might produce a contrary result because the injection of 1αOHD₃ caused the decrease of plasma 1,25(OH)₂D and ionic Ca concentration at 10 and 14 days, respectively, after injection.—KEY WORDS: lactating cow, parturient paresis, 1αOHD₃

Although the injection of large amounts of vitamin D₃ (VD₃) has extensively been adopted as one of the effective methods to prevent the parturient hypocalcemia (milk fever) [13], the effects of such injection on plasma VD₃ metabolites have not yet been elucidated in detail. In the previous study, therefore, we injected large amounts of VD₃ to lactating cows as a preliminary step to examine its preventive effects on the parturient hypocalcemia and observed the continuous high level of plasma 25-hydroxyvitamin D (25OHD) and the transitory rise of plasma 1α,25-dihydroxyvitamin D(1,25 (OH)₂D) concentration after VD₃ injection [14].

On the other hand, derivatives of VD₃ such as 1α-hydroxyvitamin D₃(1αOHD₃) and 1α, 25-dihydroxyvitamin D₃ (1,25 (OH)₂D₃) have newly been synthesized, expected to have preventive effects on the parturient hypocalcemia, and studied by many workers [2, 5, 6, 8, 12, 16, 19]. Among them, Hove et al. [8] reported that the injection of 500 µg of 1αOHD₃ to nonlactating and nonpregnant cows kept the high level of plasma 1,25(OH)₂D longer than did that of equivalent amount of 1,25(OH)₂D₃.

Therefore, the present study has been carried out to compare the effects of the injection of 1αOHD₃ on the plasma 1,25(OH)₂D and Ca concentrations in lactating cows with the results obtained in the previous study after VD₃ injection [14], and to determine the efficacy and safety of injection of 1αOHD₃ as prophylactic of parturient hypocalcemia.
MATERIALS AND METHODS

Eleven lactating Holstein-Friesian cows aged 4 to 7 years were used in the present study. These cows had calved 3 to 6 times and were at 45 to 102 days after the latest parturition at the beginning of the experiment. They were divided into two groups, a control group consisting of 6 cows and a treatment (1αOHD₃) group consisting of 5 cows, and were fed 23 kg corn silage, 9.4 kg concentrated diet, 2.7 kg beet pulp and 1 kg soy bean per day throughout experiment. Each cow in the treatment group once received intramuscularly 500 µg of 1αOHD₃ (provided by Chugai Pharmaceutical, Co., Ltd.) resolved in 3 ml ethanol, while each cow in the control group received only 3 ml of ethanol. In both groups, blood samples were taken with heparinized syringes from the external jugular vein at 0, 0.25, 0.5, 1, 2, 3, 5, 7, 10, and 14 days after injection, and immediately centrifuged to separate and freeze (-20°C) the plasma until assay.

The plasma concentration of 1,25(OH)₂D was measured by high pressure liquid chromatography using 0.4×30 cm μ-porasil columns (Waters Associates, Inc., Milford, Mass.) according to a modified method of Lambert et al. [11]. Because the concentration of 1,25(OH)₂D₃ cannot be measured apart from that of 1α, 25-dihydroxyvitamin D₂(1,25(OH)₂D₂) by usual chromatography [18], values obtained as the total concentration of 1,25(OH)₂D and 1,25(OH)₂D₃ were by usage described as the concentration of 1,25(OH)₂D in the present study. The plasma concentration of total Ca was determined with an atomic absorption spectrophotometer [20] (Model 170–10, Hitachi, Japan), while that of ionic Ca was determined with an ionic calcium analyzer (NOVA 2, AHS/Japan Corporation). The plasma concentration of inorganic phosphorus (Pi) was measured by the method of Chen et al. [4]. Data from the present investigation were subjected statistical analysis using the t-test.

RESULTS

Figs. 1~4 show the changes in the concentration of the plasma 1,25(OH)₂D, total and ionic Ca, and Pi.

The plasma 1,25(OH)₂D concentration in the 1αOHD₃ group was significantly higher than that in the control group at 0.25, 0.5, 1, 2, 3, and 5 days after injection (P<0.05). It reached the peak of 307.1 pg/ml at 2 days after injection, recovered to the normal level of 44.9 pg/ml at 7 days, and became significantly lower than that in the control group at 10 days after injection (P<0.05). It decreased to the level of 24.2 pg/ml at 10 days after injection. In contrast, the plasma 1,25(OH)₂D concentration in the control group did not change significantly and remained in the range from 44.0 to 71.9 pg/ml (58.6±21.6 pg/ml) during the experiment (Fig. 1).

The plasma total Ca concentration in the 1αOHD₃ group was significantly higher than that in the control group at 2, 3, 5, and 7 days after injection (P<0.05), reached the peak of 10.86 mg/dl at 3 days and recovered
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Fig. 2. Changes in the plasma total Ca concentrations in the 1\alpha\text{OHD}_3 (●) and control (○) groups.

Fig. 3. Changes in the plasma ionic Ca concentrations in the 1\alpha\text{OHD}_3 (●) and control (○) groups.

Fig. 4. Changes in the plasma Pi concentrations in the 1\alpha\text{OHD}_3 (●) and control (○) groups.

2.3% measured in 110 samples from both 1\alpha\text{OHD}_3 and control groups.

The plasma Pi concentration in the 1\alpha\text{OHD}_3 group became significantly higher than that in the control group at 2 days after injection, increased to reach the peak of 7.43 mg/dl at 7 days, but rapidly decreased thereafter and finally reached at 14 days to the value of 3.27 mg/dl, which was significantly lower than that in the control at the same period (Fig. 4).

DISCUSSION

The VD_3 is converted in the liver into 25OH\text{D}_3, which is then hydroxylated in the kidney to 1,25(OH)_2\text{D}_3. The hydroxylation in the kidney is strictly regulated by the Ca or Pi concentration in the plasma and activities of 1\alpha-hydroxylase and parathyroid hormone (PTH). On the other hand, because 1\alpha\text{OHD}_3 is independent of kidney hydroxylation and converted into 1,25(OH)_2\text{D}_3 by only the hydroxylation in the liver, the former is linearly hydroxylated to the latter by a large quantity of 25-hydroxylase enzyme of the liver [7]. Hove et al. [8] gave 500 \mu g of 1\alpha\text{OHD}_3 intramuscularly to nonlactating and non-pregnant Jersey cows to examine the change of 1,25(OH)_2\text{D} concentration in the plasma,
and reported that the plasma 1,25(OH)₂D concentration rapidly increased after injection, reached the peak of 500 pg/ml at 2 days and then gradually decreased. Carstairs et al. [3] administered 400μg of 1αOH₃D intramuscularly to lactating cows and observed that the plasma 1,25(OH)₂D reached the peak of 140 pg/ml during 18 to 72 hr after injection.

In the present study, the plasma 1,25(OH)₂D concentration in the 1αOH₃D group showed the rapid increase to reach the peak of about 300 pg/ml at 2 days, decreased gradually thereafter and became significantly lower than that in the control group at 10 days after injection. The pattern of increase of 1,25(OH)₂D after 1αOH₃D injection was almost similar to that reported by Hove et al. [8], but the value of its peak in our experiment was slightly lower than that in the study of Hove et al. [8]. This was thought to be caused by the difference in the mean body weight between their cows and ours. However, the peak value of the plasma 1,25(OH)₂D concentration in the present study was fairly higher than that in the study of Carstairs et al. [3], even after consideration of the difference in the dose of administration between their studies and ours. On the other hand, the decrease of plasma 1,25(OH)₂D at 10 days after injection of 1αOH₃D in the present study was thought to be due to the short duration of biological half life of 1,25(OH)₂D₃ [9]. Because its half life is reported to be 1.5 days, 1,25(OH)₂D₃ converted from 1αOH₃D in the liver might become depleted at about 7 days after 1αOH₃D injection [9]. Additionally, the decrease of plasma 1,25(OH)₂D in the present study might be correlated with the decrease in the production of 1,25(OH)₂D₃ in the kidney, which was directly suppressed by the increase of plasma 1,25(OH)₂D₃ concentration, and indirectly by the increase of plasma Ca concentration.

In our previous study [14], 10 million I.U. of VD₃ was injected to lactating cows to examine the change of plasma 1,25(OH)₂D concentration, which reached the peak of 121 pg/ml at 1 day after injection. From the biochemical point of view, the quantity of 1,25(OH)₂D₃ converted from 10 million I.U. of VD₃ is explained to be almost equal to that of 1,25(OH)₂D₃ converted from 500 μg of 1αOH₃D [15]. In the present study, however, the plasma 1,25(OH)₂D concentration reached higher and decreased more gradually after the injection of 500 μg 1αOH₃D than after that of 10 million I.U. VD₃. This might be resulted from the differences in the synthesizing process of 1,25(OH)₂D₃ from VD₃ and 1αOH₃D; because the former was strictly regulated by 1α-hydroxylase in the kidney, and because the latter depended only on the amount of 25-hydroxylase in the liver, 1αOH₃D increased the plasma 1,25(OH)₂D₃ concentration to a higher level than did VD₃ [15].

The injection of 1αOH₃D is explained to increase the plasma Ca concentration higher than that after injection of VD₃ or 25OH₃D [12, 15]. In a report of Hove et al. [8], 500 μg of 1αOH₃D was injected intramuscularly to nonlactating and nonpregnant cows to examine the change of plasma Ca concentration, which increased at 2 days after injection and continued to be high until 11 days after injection. On the other hand, according to the report of Bar et al. [2], when 250 μg or 350 μg of 1αOH₃D was injected to nonlactating cows to examine the change of plasma Ca concentration, it increased at 1 day after injection and continued to be high until 7 days. Bar et al. [1] further administered 700 μg of 1αOH₃D to dry pregnant cows to examine its changes, and reported that it increased at 1 day after injection and continued to be high for more than 15 days after injection. In the present study, the plasma Ca concentration in the 1αOH₃D group increased significantly at 2
days after injection and continued to be high until 7 days. The increasing pattern of plasma Ca concentration in this study was similar to that reported by Bar and his colleagues [1, 2]. However, the duration of high plasma Ca concentration was shorter in this study than that in the study of Hove et al. [8]. This discrepancy between our result and that of Hove et al. [8] may be due to the large demand of Ca of the cows used in the present study, because they were lactating during the experimental period.

Ionic Ca is a physiologically active fraction of Ca [17] and thus the ideal Ca fraction to be measured for assessment of disorders in Ca metabolism [10]. In the present study, the plasma ionic Ca concentration in the 1αOHD₃ group was significantly higher than that in the control group for the first 7 days, and became significantly lower than that in the control group at 14 days after injection. This increasing pattern of ionic Ca after 1αOHD₃ injection was almost similar to that of total Ca. On the other hand, the significant decrease of plasma concentration was observed in the ionic Ca at 14 days after 1αOHD₃ injection, although it did not occur in the total Ca. This decrease in the ionic Ca is thought to be caused by the decrease of Ca absorption in the intestine, which is induced by the increase of plasma 1,25(OH)₂D concentration at 10 days after injection. The injection of 10 million I.U. VD₃ in the previous study increased total Ca concentration in plasma higher than did that of 500 μg 1αOHD₃ in the present study; the plasma Ca concentration continued to be high until 15 days after injection of VD₃ [14]. This difference might be resulted from the continuous high level of plasma 25OHD concentration which was caused by the injection of VD₃ in the previous study [14].

Hove et al. [8] reported that the plasma Pi in nonpregnant and nonlactating cows given 500 μg 1αOHD₃ became significantly higher at 3 days after injection as compared with that before injection and tended to remain so throughout the sampling period. Similarly, Bar et al. [1] gave 700 μg 1αOHD₃ to dry pregnant cows to examine the change of plasma Pi concentration. According to their results, the plasma Pi concentration began to increase at 1 day, peaked at 8 days, and remained higher as compared with that before injection until 15 days after injection. The increasing pattern of plasma Pi concentration in the present study was similar to that reported by Hove et al. [8] and Bar et al. [2], and was thought to be mainly caused by the increase of intestinal Pi absorption as suggested by Bar et al. [2]. However, although the plasma Pi concentration became significantly lower at 14 days after injection in our experiment, this decrease was observed neither by Hove et al. [8] nor by Bar et al. [2]. This discrepancy was thought to be caused by the difference in the decreasing pattern of plasma 1,25(OH)₂D concentration between their [2, 8] and our studies; because 1,25(OH)₂D₃ was suggested to stimulate Pi absorption dose-dependently from the intestine [2, 8], the significantly low concentration of 1,25(OH)₂D induced the low plasma Pi concentration in the present study.

In conclusion, the intramuscular injection of 500 μg of 1αOHD₃ to lactating cows kept high level of plasma concentration of 1,25(OH)₂D until 5 days after injection, which caused the increase of plasma Ca and Pi concentrations.

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REFERENCES

1. Bar, A., Perlman, R., and Sachs, M. 1985. Observation on the use of 1α-hydroxyvitamin D₃ in the prevention of bovine parturient paresis; the effect of a single injection on plasma 1α-hydroxy-
vitamin D₃, 1,25-dihydroxyvitamin D₃, calcium,
Observation on the use of 1α-hydroxycholecalciferol in
106: 529–532.
3. Carstairs, J.A., Treutelaar, M.K., Jorgensen,
combination of 1,25-dihydroxyvitamin D₃
(1,25(OH)₂D₃) and 1α-hydroxyvitamin D₃(1α-
OH D₃) as a milk fever preventive. J. Dairy Sci.
64 (Suppl. 1): 156.
28:1756–1758.
5. Davies, D.C., Allen, W.M., Hoare, M.N., Pott,
J.M., Riley, C.J., Sansom, B.F., Stenton, J.R.,
and Vagg, M.J. 1987. A field trial of 1α-hydroxy-
cholecalciferol (1α-OH D₃) in the prevention of
6. Gast, D.R., Marquardt, J.P., Jorgensen, N.A.,
and DeLuca, H.F. 1977. Efficacy and safety of
1α-hydroxyvitamin D₃ for prevention of partu-
metabolism in ruminants and its relevance to the
Effects of 1α-hydroxyvitamin D₃, 1,25-dihydroxy-
vitamin D₃, 1,24,25-trihydroxyvitamin D₃, and
1,25,26-trihydroxyvitamin D₃ on mineral metab-
olism and 1,25-dihydroxyvitamin D concentra-
reversal of hypercalcemia and hypercalcuria
induced by vitamin D and its 1α-hydroxylated
Parturient paresis in the cow. Serum ionized
calcium concentrations before and after treatment
with different calcium solutions—classification of
different degrees of hypo- and hypercalcemia.
11. Lambert, P.W., Toft, D.O., Hodgson, S.F.,
Lindmark, E.A., Wittrak, B.J., and Roos, B.A.
1978. An improved method for the measurement
of 1,25(OH)₂D₃ in human plasma. Endocrine
Res. Commun. 5: 293–310.
and vitamin D₃ in conjunction with induced
13. Naito, Y., Saito, M., Watanabe, D., and Muraka-
mi, D. 1984. Effects of administrated vitamin D₃
on calcium metabolism in cows before and after
(in Japanese).
14. Naito, Y., Watanabe, E., Saito, R., and Muraka-
mi, D. Effects of large amounts of vitamin D₃
injection on plasma vitamin D₃ metabolites in
15. Nishii, Y. 1982. Metabolism of 1α-
hydroxyvitamin D₃ and mechanism of its func-
16. Sachs, M., Bar, A., Cohen, R., Mazur, Y.,
Mayer, E., and Hurwitz, S. 1977. Use of 1α-
hydroxycholecalciferol in the prevention of
bovine parturient paresis. Am. J. Vet. Res. 38:
2039–2041.
17. Simesen, M.G. 1980. Calcium, phosphorus, and
Biochemistry of Domestic Animals. (Kaneko, J.J.
Vitamin D, pp. 104–105., Kodansha, Tokyo (in
Japanese).
19. Vagg, M.J., Allen, W.M., Davies, D.C., Sansom,
1981. Field trial to determine the efficacy of two
doses of 1α-hydroxycholecalciferol in the preven-
20. Willis, J.B. 1961. Determination of calcium and
magnesium in urine by atomic absorption spec-
要約

泌乳牛における血漿1α, 25-dihydroxyvitamin D濃度に対する1α-hydroxyvitamin D₃投与の影響：内藤善久・佐藤行・佐藤れえ子・谷口和之・村上大蔵（岩手大学農学部家畜内科学教室・家畜解剖学教室・秋田県北部家畜保健衛生所）——乳牛の分娩性低Ca血症の予防に関して。1α-hydroxyvitamin D₃(1αOHD₃)の泌乳牛に対する効果を調べた。分娩後1.5〜3.4か月のホルスタイン種泌乳牛5頭（1αOHD₃群）にエタノール3mlに溶解した1αOHD₃500μgを筋肉注射し、投与後14日まで血漿1,25(OH)₂D濃度と無機成分の推移を6頭の対照群と比較観察した。投与群の1,25(OH)₂D濃度は投与後6時間(123.3pg/ml)に降対照群に比較して有意に高く、投与後2日では最高値(307.1pg/ml)に達し、その後は低下して投与後10日には24.2pg/mlであった。投与群の血漿総Ca濃度は、投与後2〜7日(10.6〜10.9mg/dl)まで対照群に比較して有意に高かった。投与群のイオン化Ca濃度は、対照群のそれに比較して投与翌日から上昇し、投与後3日に最高値に達し、14日には低下した。これらの成績から、分娩性低Ca血症の予防のための1αOHD₃500μg1回筋肉注射は、分娩前に0〜5日に行うのが適当と考えられた。