Effects of 1α -Hydroxyvitamin D_3 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 D_3 (1α OHD₃) on the plasma 1α , 25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$) and calcium (Ca) concentrations in lactating cows, and to determine the efficacy of 1α OHD₃ as prophylactic of parturient hypocalcemia. The cows were divided into two groups, a control group consisting of 6 cows and treatment (1α OHD₃) group consisting of 5 cows. Each cow in the treatment group once received intramuscularly 500 μg of 1α OHD₃ resolved in 3 ml ethanol. The plasma $1,25(\text{OH})_2\text{D}$ concentration in the 1α OHD₃ 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 1α OHD₃ 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 1α OHD₃ intramuscularly within 2 to 5 days before parturition. However, if 1α OHD₃ was injected earlier than 10 days before parturition, this might produce a contrary result because the injection of 1α OHD₃ caused the decrease of plasma $1,25(\text{OH})_2\text{D}$ and ionic Ca concentration at 10 and 14 days, respectively, after injection.—**KEY WORDS:** lactating cow, parturient paresis, 1α OHD₃

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Although the injection of large amounts of vitamin D_3 (VD_3) 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 VD_3 metabolites have not yet been elucidated in detail. In the previous study, therefore, we injected large amounts of VD_3 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 ($1,25(\text{OH})_2\text{D}$) concentration after VD_3 injection [14].

On the other hand, derivatives of VD_3 such as 1α -hydroxyvitamin D_3 ($1\alpha\text{OHD}_3$) and 1α , 25-dihydroxyvitamin D_3 ($1,25$

$(\text{OH})_2\text{D}_3$) 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 $1\alpha\text{OHD}_3$ to nonlactating and nonpregnant cows kept the high level of plasma $1,25(\text{OH})_2\text{D}$ longer than did that of equivalent amount of $1,25(\text{OH})_2\text{D}_3$.

Therefore, the present study has been carried out to compare the effects of the injection of $1\alpha\text{OHD}_3$ on the plasma $1,25(\text{OH})_2\text{D}$ and Ca concentrations in lactating cows with the results obtained in the previous study after VD_3 injection [14], and to determine the efficacy and safety of injection of $1\alpha\text{OHD}_3$ 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 ($1\alpha\text{OHD}_3$) group consisting of 5 cows, and were fed 23 kg corn silage, 9.4 kg concentrated diet, 2.7 kg beat pulp and 1 kg soy bean per day throughout experiment. Each cow in the treatment group once received intramuscularly 500 μg of $1\alpha\text{OHD}_3$ (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 $1,25(\text{OH})_2\text{D}$ 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 $1,25(\text{OH})_2\text{D}_3$ cannot be measured apart from that of 1α , 25-dihydroxyvitamin $\text{D}_2(1,25(\text{OH})_2\text{D}_2)$ by usual chromatography [18], values obtained as the total concentration of $1,25(\text{OH})_2\text{D}_2$ and $1,25(\text{OH})_2\text{D}_3$ were by usage described as the concentration of $1,25(\text{OH})_2\text{D}$ 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

Fig. 1. Changes in the plasma $1,25(\text{OH})_2\text{D}$ concentrations in the $1\alpha\text{OHD}_3$ (—●—) and control (---○---) groups.

investigation were subjected statistical analysis using the *t*-test.

RESULTS

Figs. 1~4 show the changes in the concentration of the plasma $1,25(\text{OH})_2\text{D}$, total and ionic Ca, and Pi.

The plasma $1,25(\text{OH})_2\text{D}$ concentration in the $1\alpha\text{OHD}_3$ 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 $1,25(\text{OH})_2\text{D}$ 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 $1\alpha\text{OHD}_3$ 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

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.

to the normal level of 9.17 mg/dl at 10 days after injection (Fig. 2). The plasma ionic Ca concentration in the $1\alpha\text{OHD}_3$ group became significantly higher than that in the control group at 1 day ($P < 0.05$), i.e., a little earlier than in the plasma total Ca concentration, remained higher until 7 days, then gradually decreased and became significantly lower than that in the control group at 14 days after injection ($P < 0.05$). Its peak in the $1\alpha\text{OHD}_3$ group showed the value of 5.42 mg/dl at 3 days after injection (Fig. 3). The ionic/total ratio of the plasma Ca concentration did not differ significantly between the $1\alpha\text{OHD}_3$ and control groups during the experiment. The mean value and standard deviation of this ratio were $49.5 \pm$

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 25OHD_3 , which is then hydroxylated in the kidney to $1,25(\text{OH})_2\text{D}_3$. The hydroxylation in the kidney is strictly regulated by the Ca or Pi concentration in the plasma and activities of 1α -hydroxylase and parathyroid hormone (PTH). On the other hand, because $1\alpha\text{OHD}_3$ is independent of kidney hydroxylation and converted into $1,25(\text{OH})_2\text{D}_3$ 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 μg of $1\alpha\text{OHD}_3$ intramuscularly to nonlactating and nonpregnant Jersey cows to examine the change of $1,25(\text{OH})_2\text{D}$ concentration in the plasma,

and reported that the plasma $1,25(\text{OH})_2\text{D}$ concentration rapidly increased after injection, reached the peak of 500 pg/ml at 2 days and then gradually decreased. Carstairs *et al.* [3] administered 400 μg of $1\alpha\text{OHD}_3$ intramuscularly to lactating cows and observed that the plasma $1,25(\text{OH})_2\text{D}$ reached the peak of 140 pg/ml during 18 to 72 hr after injection.

In the present study, the plasma $1,25(\text{OH})_2\text{D}$ concentration in the $1\alpha\text{OHD}_3$ 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 $1,25(\text{OH})_2\text{D}$ after $1\alpha\text{OHD}_3$ 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 $1,25(\text{OH})_2\text{D}$ 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 $1,25(\text{OH})_2\text{D}$ at 10 days after injection of $1\alpha\text{OHD}_3$ in the present study was thought to be due to the short duration of biological half life of $1,25(\text{OH})_2\text{D}_3$ [9]. Because its half life is reported to be 1.5 days, $1,25(\text{OH})_2\text{D}_3$ converted from $1\alpha\text{OHD}_3$ in the liver might become depleted at about 7 days after $1\alpha\text{OHD}_3$ injection [9]. Additionally, the decrease of plasma $1,25(\text{OH})_2\text{D}$ in the present study might be correlated with the decrease in the production of $1,25(\text{OH})_2\text{D}_3$ in the kidney, which was directly suppressed by the increase of plasma $1,25(\text{OH})_2\text{D}_3$ concentration, and indirectly by the increase of plasma Ca concentration.

In our previous study [14], 10 million I.U. of VD_3 was injected to lactating cows to examine the change of plasma $1,25(\text{OH})_2\text{D}$ concentration, which reached the peak of 121 pg/ml at 1 day after injection. From the biochemical point of view, the quantity of $1,25(\text{OH})_2\text{D}_3$ converted from 10 million I.U. of VD_3 is explained to be almost equal to that of $1,25(\text{OH})_2\text{D}_3$ converted from 500 μg of $1\alpha\text{OHD}_3$ [15]. In the present study, however, the plasma $1,25(\text{OH})_2\text{D}$ concentration reached higher and decreased more gradually after the injection of 500 μg $1\alpha\text{OHD}_3$ than after that of 10 million I.U. VD_3 . This might be resulted from the differences in the synthesizing process of $1,25(\text{OH})_2\text{D}_3$ from VD_3 and $1\alpha\text{OHD}_3$; 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, $1\alpha\text{OHD}_3$ increased the plasma $1,25(\text{OH})_2\text{D}_3$ concentration to a higher level than did VD_3 [15].

The injection of $1\alpha\text{OHD}_3$ is explained to increase the plasma Ca concentration higher than that after injection of VD_3 or 25OHD_3 [12, 15]. In a report of Hove *et al.* [8], 500 μg of $1\alpha\text{OHD}_3$ 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 $1\alpha\text{OHD}_3$ 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 $1\alpha\text{OHD}_3$ 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 $1\alpha\text{OHD}_3$ 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 $1\alpha\text{OHD}_3$ 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 $1\alpha\text{OHD}_3$ 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 $1\alpha\text{OHD}_3$ 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 decrease of plasma $1,25(\text{OH})_2\text{D}$ concentration at 10 days after injection. The injection of 10 million I.U. VD_3 in the previous study increased total Ca concentration in plasma higher than did that of $500\text{ }\mu\text{g}$ $1\alpha\text{OHD}_3$ in the present study; the plasma Ca concentration continued to be high until 15 days after injection of VD_3 [14]. This difference might be resulted from the continuous high level of plasma 25OHD concentration which was caused by the injection of VD_3 in the previous study [14].

Hove *et al.* [8] reported that the plasma Pi in nonpregnant and nonlactating cows given $500\text{ }\mu\text{g}$ $1\alpha\text{OHD}_3$ 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 $700\text{ }\mu\text{g}$ $1\alpha\text{OHD}_3$ 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 $1,25(\text{OH})_2\text{D}$ concentration between their [2, 8] and our studies; because $1,25(\text{OH})_2\text{D}_3$ was suggested to stimulate Pi absorption dose-dependently from the intestine [2, 8], the significantly low concentration of $1,25(\text{OH})_2\text{D}$ induced the low plasma Pi concentration in the present study.

In conclusion, the intramuscular injection of $500\text{ }\mu\text{g}$ of $1\alpha\text{OHD}_3$ to lactating cows kept high level of plasma concentration of $1,25(\text{OH})_2\text{D}$ 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 D_3 in the prevention of bovine parturient paresis; the effect of a single injection on plasma 1α -hydroxy-

- vitamin D₃, 1,25-dihydroxyvitamin D₃, calcium, and hydroxyproline. *J. Dairy Sci.*, 68: 1952–1958.
2. Bar, A., Sachs, M., and Hurwitz, S. 1980. Observation on the use of 1 α -hydroxycholecalciferol in the prevention of bovine parturient paresis. *Vet. Rec.* 106: 529–532.
 3. Carstairs, J.A., Treutelaar, M.K., Jorgensen, N.A., and DeLuca, H.F. 1981. Potential use of a combination of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) and 1 α -hydroxyvitamin D₃ (1 α -OHD₃) as a milk fever preventive. *J. Dairy Sci.* 64 (Suppl. 1): 156.
 4. Chen, P.S., Toribara, T.Y., and Warner, H. 1956. Microdetermination of phosphorus. *Anal. Chem.* 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 1 α -hydroxycholecalciferol (1 α -OHD₃) in the prevention of milk fever. *Vet. Rec.* 102: 440–442.
 6. Gast, D.R., Marquardt, J.P., Jorgensen, N.A., and DeLuca, H.F. 1977. Efficacy and safety of 1 α -hydroxyvitamin D₃ for prevention of parturient paresis. *J. Dairy Sci.* 60: 1910–1920.
 7. Horst, R.L., and Reinhardt, T.A. 1983. Vitamin D metabolism in ruminants and its relevance to the periparturient cow. *J. Dairy Sci.* 66: 661–678.
 8. Hove, K., Horst, R.L., and Littledike, E.T. 1983. Effects of 1 α -hydroxyvitamin D₃, 1,25-dihydroxyvitamin D₃, 1,24,25-trihydroxyvitamin D₃, and 1,25,26-trihydroxyvitamin D₃ on mineral metabolism and 1,25-dihydroxyvitamin D concentrations in dairy cows. *J. Dairy Sci.* 66: 59–66.
 9. Kanis, J.A. and Russell, R.G.G. 1977. Rate of reversal of hypercalcaemia and hypercalciuria induced by vitamin D and its 1 α -hydroxylated derivatives. *Brit. Med. J.* 1: 78–81.
 10. Kvart, C., Björzell, K.A., and Larsson, L. 1982. 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. *Acta Vet. Scand.* 23: 184–196.
 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.
 12. McMurray, C.H., Rice, D.A., and McBride, P.S. 1980. Milk fever controls: comparison of 1-alpha and vitamin D₃ in conjunction with induced parturition. *Vet. Rec.* 107: 188–191.
 13. Naito, Y., Saito, M., Watanabe, D., and Murakami, D. 1984. Effects of administered vitamin D₃ on calcium metabolism in cows before and after parturition. *J. Jpn. Vet. Med. Assoc.* 37: 297–302 (in Japanese).
 14. Naito, Y., Watanabe, E., Sato, R., and Murakami, D. Effects of large amounts of vitamin D₃ injection on plasma vitamin D₃ metabolites in lactating cows. *Jpn. J. Vet. Sci.* 49: 123–129.
 15. Nishii, Y. 1982. Metabolism of 1 α -hydroxyvitamin D₃ and mechanism of its function. *Vitamin (Japan)* 56: 1–17 (in Japanese).
 16. Sachs, M., Bar, A., Cohen, R., Mazur, Y., Mayer, E., and Hurwitz, S. 1977. Use of 1 α -hydroxycholecalciferol in the prevention of bovine parturient paresis. *Am. J. Vet. Res.* 38: 2039–2041.
 17. Simesen, M.G. 1980. Calcium, phosphorus, and magnesium metabolism. pp. 583–584. In: *Clinical Biochemistry of Domestic Animals*. (Kaneko, J.J. ed.), Academic Press, New York.
 18. Suda, T., Ogata, E., Ogura, Y. and Ishii, Y. 1982. Vitamin D, pp. 104–105., Kodansha, Tokyo (in Japanese).
 19. Vagg, M.J., Allen, W.M., Davies, D.C., Sansom, B.F., Edwards, H.J., Pott, J.M., and Riley, C.J. 1981. Field trial to determine the efficacy of two doses of 1 α -hydroxycholecalciferol in the prevention of milk fever. *Vet. Rec.* 109: 273–275.
 20. Willis, J.B. 1961. Determination of calcium and magnesium in urine by atomic absorption spectroscopy. *Anal. Chem.* 33: 556–559.

要 約

泌乳牛における血漿 $1\alpha, 25\text{-dihydroxyvitamin D}$ 濃度に対する $1\alpha\text{-hydroxyvitamin D}_3$ 投与の影響：内藤善久・佐藤 行¹⁾・佐藤れえ子・谷口和之²⁾・村上大蔵（岩手大学農学部家畜内科学教室・²⁾ 家畜解剖学教室・¹⁾ 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， $1\alpha\text{-hydroxyvitamin D}_3(1\alpha\text{OHD}_3)$ の泌乳牛に対する効果を調べた。分娩後1.5～3.4カ月のホルスタイン種泌乳牛5頭（ $1\alpha\text{OHD}_3$ 群）にエタノール 3 ml に溶解した $1\alpha\text{OHD}_3 500\mu\text{g}$ を筋肉注射し，投与後14日まで血漿 $1,25(\text{OH})_2\text{D}$ 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 $1,25(\text{OH})_2\text{D}$ 濃度は投与後6時間 (123.3 pg/ml) 以降対照群に比較して有意に高く，投与後2日では最高値 (307.1 pg/ml) に達し，その後は低下して投与後10日には 24.2 pg/ml であった。投与群の血漿総 Ca 濃度は，投与後2～7日 ($10.6\sim10.9\text{ mg/dl}$) 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 $1\alpha\text{OHD}_3 500\mu\text{g}$ 1回筋肉注射は，分娩前2～5日に行うのが適当と考えられた。