Effects of Large Amounts of Vitamin D₃ Injection on Plasma Vitamin D₃ Metabolites in Lactating Cows

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ABSTRACT. Effects of injection of large amounts of vitamin D₃ (VD₃) on the concentrations of plasma 25-hydroxyvitamin D (25OHD), 1α,25-dihydroxyvitamin D (1,25(OH)₂D), calcium (Ca) and inorganic phosphorus (Pi) in the lactating Holstein-Friesian cows were examined. The cows were divided into two groups, an untreated control group consisting of 4 cows and a treatment group (VD₃ group) consisting of 5 cows. Each cow in the VD₃ group received 10 million I.U. of VD₃ intramuscularly at 7 days after parturition. Blood and milk samples were taken from 0 to 15 days after the VD₃ injection. The plasma 25OHD concentration was significantly higher in the VD₃ group than that in the control group throughout the experimental period, whereas the plasma 1,25(OH)₂D concentration rose only transiently after the VD₃ injection. The plasma Ca concentration was also significantly higher in the VD₃ group than that in the control group throughout the experiment, while the plasma Pi concentration was higher in the VD₃ group only from the 3rd to 6th day of the experiment. These results suggest that the initial increase in the plasma Ca concentration may be due to the increase in the 1,25(OH)₂D level whereas the substained increase in the Ca concentration may be due to the increase in the plasma 25OHD concentration. Further, it seems likely that the plasma 1,25(OH)₂D concentration is suppressed by the increase in the plasma Ca concentration in the later stage of experiment and also by the negative feedback of plasma 1,25(OH)₂D concentration.—KEY WORDS: lactating cow, parturient paresis, vitamin D₃.


Parturient paresis (milk fever) is a metabolic disorder occurring in association with parturition in dairy cows. This disease is closely related to the initiation of lactation and the intestinal absorption of calcium (Ca), and is characterized by a rapid decline in the concentrations of serum Ca and inorganic phosphorus (Pi) [1, 14]. An injection of large amounts of vitamin D₃ (VD₃) has extensively been adopted as one of the effective methods to prevent this disease [4, 15] because VD₃ is expected to stimulate the intestinal absorption of Ca and Pi [10, 11, 15]. VD₃ is hydroxylated in the liver to 25-hydroxyvitamin D₃ (25OHD₃), which is then converted in the kidney to 1α, 25-dihydroxyvitamin D₃ (1,25(OH)₂D₃); 1,25(OH)₂D₃ is known to be a terminal metabolite most potent in stimulating the absorption of Ca from the gut [10, 11].

The purpose of the present study is to determine the effects of injection of large amounts of VD₃ on the plasma concentrations of 25OHD₃ and 1,25(OH)₂D₃ in the lactating cows.

MATERIALS AND METHODS

Nine lactating Holstein-Friesian cows, aged 2 to 4 years, were divided into two groups, an untreated control consisting of 4 cows and a test group (VD₃ group) consisting of 5 cows. They were fed 8 kg alfalfa hay and 4 kg commercial concentrated diet per day from one week before the expected parturition till the end of the experiment. Each cow in the VD₃ group once received intramuscularly 10 million I.U. of VD₃.
(Duphafra D3 1000; Duphar Co., Amsterdam) at 7 days after parturition.

Blood samples were taken with heparinized syringes from the external jugular vein at 0, 1, 2, 3, 4, 6, 8 and 15 days after the VD3 injection, and immediately centrifuged to separate plasma which was frozen at −20°C until assayed. Milk samples were taken from the mixture of a whole day milk every day from 0 to 8 days after the VD3 injection and frozen at −20°C until assayed. The plasma concentration of 25-hydroxyvitamin D (25OHD) was measured by high pressure liquid chromatography using 0.4×30 cm μ-porasil columns (Waters Associates, Inc., Milford, Mass.) according to the method of Horst et al. [9], and that of 1α, 25-dihydroxyvitamin D (1,25(OH)2D) was measured by a modified sensitive ligand binding assay method of Lambert et al. [12, 20]. Because 1,25(OH)2D3 (or 25OHD3) cannot be separated from 1α, 25-dihydroxyvitamin D2 (1,25(OH)2D2) (or 25-hydroxyvitamin D2(25OHD2)) using conventional chromatography [21], total concentrations of 1,25(OH)2D2 (or 25OHD2) and 1,25(OH)2D3 (or 25OHD3) were expressed as the concentrations of 1,25(OH)2D (or 25OHD) in the present study. Plasma and milk concentrations of Ca were determined with a Hitachi Atomic Absorption Spectrophotometer Model 170–10 [23]. Concentrations of inorganic phosphorus (Pi) were measured by spectrophotometry according to the method of Chen et al. [2]. Results were shown by mean±S.D., and statistic significances of the difference were analyzed using Student’s t-test.

RESULTS

Changes in the plasma concentrations of 25OHD, 1,25(OH)2D, Ca and Pi following the injection of VD3 are shown in Figs. 1–4, respectively. As shown in Fig. 1, the plasma 25OHD concentration in the VD3 group was significantly higher than that in the control group throughout the experiment (P<0.05). The level of 25OHD began to increase immediately after the injection of VD3, reaching 174.4 ng/ml or approximately 3.5 times of the pretreatment level at the 1st day of VD3 injection, and reached the maximum of 345.1 ng/ml at the 8th day of the injection. In contrast to this, the plasma 25OHD concentration in the control group remained in the range from 32.7 to 41.3 ng/ml (37.8±13.6 ng/ml) during the experimental period (Fig. 1).

As shown in Fig. 2, the plasma 1,25(OH)2D concentration in the VD3 group was significantly higher than that in the control group for the first 2 days of the injection, reaching the maximum of 121.6
EFFECTS OF VD₃ INJECTION ON LACTATING COWS

Fig. 3. Changes in the plasma Ca concentrations in the VD₃ and control groups.

Fig. 4. Changes in the plasma Pi concentrations in the VD₃ and control groups.

Table 1. Correlations between each value measured in the VD₃ and control groups

<table>
<thead>
<tr>
<th>Plasma concentration</th>
<th>Control group</th>
<th>VD₃ group</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X)</td>
<td>(Y)</td>
<td>n</td>
</tr>
<tr>
<td>25OHD × 1,25(OH)₂D</td>
<td>31</td>
<td>0.730***</td>
</tr>
<tr>
<td>25OHD × Ca</td>
<td>32</td>
<td>0.690***</td>
</tr>
<tr>
<td>25OHD × Pi</td>
<td>32</td>
<td>0.184</td>
</tr>
<tr>
<td>1,25(OH)₂D × Ca</td>
<td>31</td>
<td>0.514**</td>
</tr>
<tr>
<td>1,25(OH)₂D × Pi</td>
<td>31</td>
<td>0.274</td>
</tr>
</tbody>
</table>

** P<0.01, *** P<0.001.

pg/ml at the 1st day. This was followed by a gradual decrease, reaching a level below control at the 15th day of the injection (P<0.05). In the control group, however, the concentration of 1,25(OH)₂D stayed the level between 48.4 and 62.7 pg/ml (55.5±20.1 pg/ml) during the experiment (Fig. 2).

As shown in Fig. 3, the plasma Ca concentration in the VD₃ group was significantly higher than that in the control group throughout the experiment expect the 1st day of the VD₃ injection (P<0.05). It reached the maximum of 12.33 mg/dl at the 4th day of injection, slightly decreased thereafter, but remained higher than that in the control group. Plasma Ca concentration in the control group remained in the range of 10.05–10.50 mg/dl (Fig. 3).

As shown in Fig. 4, the plasma Pi concentration in the VD₃ group became significantly higher than that in the control group at the 3rd day of the VD₃ injection, reached the maximum of 8.50 mg/dl at the 6th day and decreased thereafter. In the control group, the plasma Pi concentration showed almost no change for the first 8 days, and a slight decrease thereafter (Fig. 4).

Correlations between the above values are shown in Table 1. In the control group, positive correlation was found between the concentrations of plasma Ca and 25OHD, 25OHD and 1,25(OH)₂D, and Ca and 1,25(OH)₂D. In the VD₃ group, positive correlation was found between plasma Ca and 25OHD, and plasma Pi and 25OHD.

The amounts of Ca (Fig. 5) and Pi (Fig. 6) in the milk of both VD₃ and control groups did not differ significantly between both groups, respectively, and tended to decrease in both groups.
DISCUSSION

In the present experiments, the plasma 25OHD concentration rapidly increased to approximately 3.5 times at the 1st day of the VD₃ injection, reached the maximum of 345.1 ng/ml at the 8th day and remained to be high until 15 days after the VD₃ injection. The rapid increase of plasma 25OHD₃ was thought to be caused by the acceleration of the production of 25OHD₃ in the liver, because VD₃ is converted into 25OHD₃ by microsomal and mitochondrial enzymes in the liver [8]. The microsomal enzyme shows a rapid response and high affinity to VD₃ but is rather less in quantity. Therefore, this enzyme responds to the substrate (VD₃) in a physiological range and produces a limited amount of 25OHD₃. On the other hand, the mitochondrial enzyme shows a slow response and low affinity to the substrate but is immense in quantity. Because of this immensity, this enzyme can hydroxylate VD₃ to 25OHD₃ under excess concentration of substrate, such as in the VD₃ toxicity [13]. It may be concluded, therefore, the increase of plasma 25OHD in the VD₃ group is mainly caused by the increase of the mitochondrial 25-hydroxylase enzyme in the liver of cows.

Hollis et al. [7] gave 15 million I.U. of VD₃ intramuscularly to non-lactating and pregnant cows to examine the change of plasma 25OHD₃ concentration. According to their results, plasma 25OHD₃ concentration did not change until 7 days after administration and then increased significantly at 28 days. In our experiment, however, plasma 25OHD concentration rose immediately after VD₃ injection. This discrepancy may be due to the large demand of Ca, which is caused by lactation, pregnancy or parturition in the cows used in the present study. The large demand of Ca is thought to cause the increase of 25OHD₃ hydroxylase in the liver, which may be reflected in the rise of plasma 25OHD₃ concentration [5, 10].

In the present study, the plasma 1,25(OH)₂D concentration in the VD₃ group began to rise immediately after injection and reached the maximum of 121.6 pg/ml or approximately 2 times of the pre-injection level. The 1,25(OH)₂D concentration then gradually decreased to the level lower than that in the control group at 15 days after the injection. Reinhard and Conrad [19] administered 10 million I.U. of VD₃ to cows fed high Ca or high phosphorus diet at 5–7 days before parturition and observed the changes of plasma 1,25(OH)₂D concentration. They reported the rise of the plasma 1,25(OH)₂D concentration to 1.5–2 times of that before treat-
EFFECTS OF VD₃ INJECTION ON LACTATING COWS

ment at the 1st day after treatment, and its restoration to the pre-treatment level of 80 pg/ml at 4–6 days after the treatment (or 1 day before the parturition). The rapid increase of plasma 1,25(OH)₂D concentration was also observed in our experiment. This increase was interpreted as a result of the rapid increase in the amount of the substrate (25OHD) at the 1st day after treatment. The amount of 1,25(OH)₂D was thought to increase under the physiological conditions of 1α-hydroxylase reaction in the kidney.

The 1α-hydroxylase reaction is strictly regulated by serum Ca and Pi concentrations and Ca regulating hormones such as parathyroid hormone (PTH), 1,25(OH)₂D₃ and calcitonin. Increase of 1,25(OH)₂D concentration suppresses the 1α-hydroxylase reaction in the kidney, activates directly the 24-hydroxylase reaction and suppresses indirectly the secretion of PTH [5]. These effects result in the decrease in the production of 1,25(OH)₂D [5]. Therefore, the decrease of plasma 1,25(OH)₂D concentration from 3 days after injection in our experiment was thought to be caused by the suppression of the 1α-hydroxylase reaction via negative feedback of high plasma 1,25(OH)₂D concentration itself. In addition, the plasma 1,25(OH)₂D concentration decreased rapidly [10] because of short half life (12 hr) of 1,25(OH)₂D. In our experiment, the plasma 1,25(OH)₂D concentration was lower in the VD₃ group than in the control group at 15 days after treatment. This might be the result of suppression of PTH secretion and decrease in 1α-hydroxylase reaction.

In our experiment, the plasma Ca concentration in VD₃ group increased to 11.80 mg/dl at 2 days after the VD₃ injection, stayed at the level of 11.86–12.24 mg/dl from 3 to 8 days and then decreased to 11.31 mg/dl at the 15th day. Similarly, the plasma Pi concentration increased from 3 days after the treatment, reached the maximum at the 6th day and then decreased. The 1,25(OH)₂D₃ among metabolites of VD₃ has the highest activity to accelerate the intestinal absorption and bone resorption of Ca and Pi [5, 10]. Consequently, the increase of plasma 1,25(OH)₂D concentration at the 1st and the 2nd days after injection accelerated the intestinal absorption and bone resorption of Ca and Pi, and resulted in the increase of plasma Ca and Pi concentrations. Also, because the increase of plasma Ca concentration accelerates the secretion of calcitonin, and because the bone resorption is suppressed by the calcitonin [7], the continuous increase of plasma Ca and Pi concentrations may be maintained only by the facilitation of the intestinal absorption.

In the present study, plasma 25OHD concentration was positively correlated with plasma Ca concentration in both control and VD₃ groups, and with plasma Pi concentration in the VD₃ group. While, plasma 1,25(OH)₂D concentration was positively correlated with plasma Ca concentration in the control group, but not correlated with plasma Pi concentration in the control and VD₃ groups. Although 1,25(OH)₂D₃ is generally said to increase the plasma Ca and Pi levels more intensely than 25OHD₃ [5, 10], 1,25(OH)₂D did not correlate so highly with the plasma Ca or Pi concentration as 25OHD in the present study. This may suggest that the plasma 1,25(OH)₂D concentration is suppressed by the increase of plasma Ca concentration and the negative feedback of plasma 1,25(OH)₂D concentration itself.

Hidiroglou and Proulx [6] gave VD₃ or 25OHD₃ intramuscularly to preparturient beef cattle and reported that the amounts of Ca and Pi in the milk of 25OHD₃ group were higher than those of control or VD₃ group at 2 to 3 days after parturition. On the other hand, Naito et al. [15] administered 10 million I.U. of VD₃ intramuscularly to
preparturient cows and reported that the amount of colostral Ca in VD3 group tended to decrease below the level in the control group. Tamura and Oku [22] reported that Ca-binding protein in the milk of cows resembled to that in the duodenum. Recently, the receptors of 1,25(OH)2D3 was reported in the milk glands of mice[3], rats [16] and cows [18]. These reports suggested that the amounts of Ca and Pi in the milk may be affected by VD3 and its metabolites. In the present study, however, the amounts of Ca and Pi in the milk were not significantly altered by the administration of VD3 to lactating cows. Therefore, it seems almost impossible at present to give a conclusion to whether the amounts of Ca and Pi in the milk are affected by the administration of VD3 or its metabolites to lactating cows. Further studies are needed to elucidate the effect of VD3 and its metabolites on Ca and Pi in the milk.

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EFFECTS OF VD₃ INJECTION ON LACTATING COWS


要　約

ビタミンD₃の大量投与が泌乳牛の血漿ビタミンD₃代謝産物におよぼす影響：内藤善久・渡辺純次1)・佐藤源子・村上大浩（岩手大学農学部家畜内科学教室，1)山形県農業共済連合会）--乳頭子防に用いられるビタミンD₃（VD₃）大量投与の乳牛VD₃代謝への影響を知るため、ホルスタイン総泌乳牛5頭に分娩後7日目にVD₃1,000万U.（VD₃群）を筋注し、血漿中のVD₃代謝産物25OHDおよび1,25(OH)₂Dを中心に、Caと無機P濃度の推移をも投与後15日まで調べ、4頭の無処置対照群と比較した。乳中のCaおよび無機P量におよぼす影響も同時に調べた。VD₃群における血漿25OHD濃度は持続的に上昇したが、1,25(OH)₂D濃度は投与初期のみ一過性の著明な上昇がみられた。VD₃群の血漿Ca濃度は投与後2日から対照群に比較して有意に高かったが、血漿無機P濃度は投与中にみ上げ、VD₃群の投与初期における血漿Ca濃度の上昇は、血漿1,25(OH)₂D濃度の上昇によって、その後は25OHD濃度の持続的上昇によって維持されているものと考えられた。また、VD₃の大量投与にかかわらず血漿1,25(OH)₂D濃度については厳密な制御機構が働いていることが示唆された。