Effects of Vitamin D₃ Injection on Activity of Thyroid Parafollicular Cells in Pregnant Rats

Jong-Han WON, Kazuyuki TANIGUCHI, Reeko SATO, and Yoshihisa NAITO
Departments of Veterinary Internal Medicine and Veterinary Anatomy, Faculty of Agriculture, Iwate University, 3–18–8 Ueda, Morioka, Iwate 020, Japan
(Received 21 February 1995/Accepted 1 September 1995)

ABSTRACT. Effects of vitamin D₃ (VD₃) injection on the activity of thyroid parafollicular cells (C cells) and calcium (Ca) metabolism were examined in rats of non-pregnancy (NP), middle (MP) and late pregnancy (LP). At 3 days after injection, the average area of a C cell was significantly wider (P<0.01) in VD₃ groups than that in their control groups in NP, MP and LP. On the other hand, the plasma Ca concentration in the VD₃ groups decreased significantly (P<0.05) in comparison with that in their control groups in NP and LP, and tended to decrease in MP. These results suggest that injection of VD₃ may accelerate the activity of C cells, which may result in the decrease of plasma Ca concentration in both non-pregnant and pregnant rats. — KEY WORDS: Ca, thyroid C cell, vitamin D₃.


The calcium (Ca) homeostasis in the body fluid is controlled by interaction of parathyroid hormone (PTH), 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃] and calcitonin (CT) [1]. The homeostasis changes dramatically during pregnancy [8]. CT secretion particularly increases to maintain maternal bone store of minerals during pregnancy [1]. On the other hand, administration of vitamin D₃ (VD₃) sometimes caused an increase in plasma CT level at least partially due to hypercalcemia [9], but sometimes had no effect [3]. Effects of injected VD₃ on the Ca metabolism and activity of parafollicular C cells (C cells) of the thyroid gland during pregnancy remain unclear. The objective of this study, therefore, was to evaluate the influence of VD₃ injection on the activity of thyroid C cells and the plasma levels of PTH, Ca and inorganic phosphorus (Pi) in pregnant rats.

Twenty-five female Sprague-Dawley rats, 10–12 weeks of age, were used in the present study. They were given a commercial diet and water ad libitum, and maintained in a 12:12 hr photoperiod at 22–24°C. They were mated overnight and day 0 of pregnancy was determined by the presence of sperm in the vaginal smear. They were divided into three groups, the non-pregnancy (NP) (10 weeks of age), middle pregnancy (MP) (13 days of pregnancy) and late pregnancy (LP) (18 days of pregnancy). Thirteen and 18 days of pregnancy were arbitrarily categorized as representatives of NP and LP, respectively, NP, MP and LP rats were subdivided into VD₃ and control groups. Rats in the VD₃ groups received a single intramuscular injection of 20,000 IU of VD₃/kg (Duphafarld VD₃ 600, Duphar, Netherlands) suspended in 250 μl of olive oil, while rats in the control groups received the same amount of olive oil without VD₃.

At 3 days after injection, blood samples were collected by intracardiac puncture from rats under ether anesthesia, and succumbed to death. The thyroid glands were removed from animals, fixed in Bouin’s solution for 24–48 hr, embedded in paraffin and sectioned serially at 4 μm. Immunohistochemical stainings were performed by the avidin biotin peroxidase complex (ABC) method using an antiserum to CT (Inestar, U.S.A.).

For morphometric analysis, 100 of the CT-immunopositive thyroid C cells containing nucleus were selected from several sections in each group. The area of a C cell was measured mechanically by an image analyzing system (SPICCA-II, Japan Avionics, Japan) [6]. Plasma biochemical parameters were measured as follows; intact PTH with radioimmunoassay method, Ca with atomic absorption spectrophotometric method, and Pi with molybdenum method. Statistical analysis of the data was determined by the Student’s t test. The significant differences between groups were taken at the P<0.05 level.

At 3 days after injection, the average area of a C cell was significantly wider (P<0.01) in the VD₃ groups than that in their control groups in NP, MP and LP (Table 1). The plasma PTH concentration of the VD₃ group decreased significantly (P<0.05) at 3 days after injection in NP in comparison with that of the control, but difference was not significant in MP and LP from that of the control (Table 1). At 3 days after injection, the plasma Ca concentration of the VD₃ groups decreased significantly (P<0.05) in NP and LP in comparison with that in their control groups, and tended to decrease in MP (Table 1). The plasma Pi concentration in the VD₃ groups did not differ significantly in NP, MP and LP at 3 days after injection from that in their control groups (Table 1).

CT released from thyroid C cells mainly inhibits bone resorption, regardless of the particular agents inducing bone resorption, and increases urinary Ca excretion. Hiruma et al. [4] reported that the increase in the area of cells was in parallel with the expansion of the Golgi area and with the distension of cisternae of the rough endoplasmic reticulum. Therefore, the significant increase in the average area of C cell in VD₃ groups suggests the activation of release and synthesis of CT. Okada et al. [7], and Capen and Young [2] reported that chronic hypercalcemia following long term administration of VD₃ (3 to 30 days of duration) promotes hyperplasia and hypertrophy of thyroid C cells in the sheep and cows, respectively. Thus, the increase in the area of thyroid C cells in the present study might be influenced by VD₃ injection. On the other hand, the plasma PTH concentration of the VD₃ group decreased significantly in NP in comparison with that of the control group at 3 days.
Table 1. Area of a C cell, and concentrations of PTH, Ca and Pi in the plasma at 3 days after vitamin D$_3$ injection in pregnant and non-pregnant rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of rats</th>
<th>Average area of a C cell (µm$^2$)</th>
<th>PTH (pg/ml)</th>
<th>Ca (mg/dl)</th>
<th>Pi (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>Control 4</td>
<td>102.8 ± 25.6</td>
<td>770 ± 40</td>
<td>9.7 ± 0.2</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>VD$_3$ 4</td>
<td>120.4 ± 34.6$^*$</td>
<td>570 ± 130$^*$</td>
<td>8.7 ± 0.3$^*$</td>
<td>6.8 ± 0.7$^*$</td>
</tr>
<tr>
<td>MP</td>
<td>Control 4</td>
<td>97.9 ± 19.7</td>
<td>960 ± 190</td>
<td>10.3 ± 0.5</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>VD$_3$ 4</td>
<td>119.2 ± 30.7$^*$</td>
<td>770 ± 40</td>
<td>9.0 ± 1.2</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>LP</td>
<td>Control 5</td>
<td>101.0 ± 27.0</td>
<td>930 ± 200</td>
<td>8.6 ± 0.6</td>
<td>5.9 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>VD$_3$ 4</td>
<td>121.9 ± 32.0$^*$</td>
<td>1020 ± 100</td>
<td>7.8 ± 0.2$^*$</td>
<td>5.0 ± 0.8$^*$</td>
</tr>
</tbody>
</table>

NP: non-pregnancy, MP: middle pregnancy, LP: late pregnancy. Data represent the mean ± SD. * P<0.01, $^*$ P<0.05 as compared with the age-matched control group.

after injection. The plasma Ca concentration in the VD$_3$ groups decreased in NP, MP and LP in comparison with that in their control groups at 3 days after injection. Such decrease in the plasma concentrations of PTH and Ca might result from the increase of CT secretion caused by the activation of thyroid C cells. These results might also be caused by the suppression of 1,25-(OH)$_2$D production and the activation of 24,25-dihydroxyvitamin D production following the administration of large doses of VD$_3$ [5, 10]. Therefore, it is suggested that the VD$_3$ injection may accelerate the activity of thyroid C cells, which may decrease the plasma Ca concentration at 3 days after injection regardless of pregnancy.

REFERENCES