Chronological Changes of $^3$H-1,25(OH)$_2$D$_3$ in Tissue and Serum after Consecutive Oral Doses of Active Vitamin D in rats: Comparative Study of 1αOHD$_3$ and 1,25(OH)$_2$D$_3$

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ABSTRACT

To determine the effect of consecutive oral administration of 1αOHD$_3$ or 1,25(OH)$_2$D$_3$ on the metabolism of 1,25(OH)$_2$D$_3$, seven-month-old female rats were given 1αOHD$_3$ (0.4 μg/kg/day) or 1,25(OH)$_2$D$_3$ (0.2 μg/kg/day) for 14 days. After the oral administration of 2 μCi of $^3$H-1αOHD$_3$ or $^3$H-1,25(OH)$_2$D$_3$, the rats were sacrificed at 2, 6 or 24 h, and the distribution of these tracers and their metabolites in the serum, intestines, liver, kidneys and bone were studied. The consecutive treatment with 1,25(OH)$_2$D$_3$ or 1αOHD$_3$ did not basically alter the elution patterns of $^3$H labeled metabolites on HPLC. The tissue levels of $^3$H-1,25(OH)$_2$D$_3$, administered or converted from $^3$H-1αOHD$_3$, were lower in the treated rats than those in the controls at 24 h, indicating the accelerated disappearance of $^3$H-1,25(OH)$_2$D$_3$ following the treatment with 1αOHD$_3$ or 1,25(OH)$_2$D$_3$. The degree of acceleration, however, was less following the treatment with 1αOHD$_3$ than that after treatment with 1,25(OH)$_2$D$_3$. This finding might indicate that, when 1αOHD$_3$ or 1,25(OH)$_2$D$_3$ is consecutively administered, the 1,25(OH)$_2$D$_3$ converted from 1αOHD$_3$ by the liver remains longer in the tissues including bone than 1,25(OH)$_2$D$_3$ absorbed directly from the intestine.

Key Words
1αOHD$_3$, 1,25(OH)$_2$D$_3$, consecutive oral treatment, metabolism of 1,25(OH)$_2$D$_3$

Introduction

1,25(OH)$_2$D$_3$ is one of the major hormones that regulate bone and calcium metabolism. It is physiologically synthesized from 25OHD$_3$ in the kidney and transported to target tissues where it exerts its biological activity. In recent years 1,25(OH)$_2$D$_3$ and its synthetic precursor, 1αOHD$_3$, have been used to treat several metabolic bone diseases. However, Walters et al.\(^{12}\) reported that long-term oral administration of 1,25(OH)$_2$D$_3$ reduced its inductive effects on calcium binding protein, and Frolick et al.\(^{13}\) has indicated that the reduced effectiveness of 1,25(OH)$_2$D$_3$ in con-
secutive administration is due to an increase in its polar metabolites. Mawer et al. found that oral administration of 1,25(OH)2D3 caused a transient elevation of intestinal calcium absorption, and could cause a quite different effect than in vivo biosynthesized or intravenously administered 1,25(OH)2D3. On the other hand, it has been reported that long-term oral administration of 1αOHD3 is more successful than 1,25(OH)2D3 in preventing rickets in the chick. In this paper, to clarify the difference between the effectiveness of 1,25(OH)2D3 and 1αOHD3 in consecutive oral treatment, the metabolism of 1,25(OH)2D3 in rats was studied.

**Materials and Methods**

Vitamin D3 analogues

1αOHD3, 1,25(OH)2D3 and 1,24,25(OH)3D3 were supplied by Chugai Pharmaceutical Co. (Japan). 1α-hydroxy[1,2H]vitamin D3 (3H-1αOHD3, 9.2Ci/mmol, and 1α,25-dihydroxy[26,27-methyl3H]cholecalciferol (3H-1,25(OH)2D3, 176Ci/mmol) were purchased from Amersham Int. plc (UK).

Administration of vitamin D3 analogues to rats.

Forty-eight female Fischer rats, seven months old, were fed a normal diet (Oriental Yeast Co., Japan) and were divided into 4 groups. The 1st group was placed on daily oral doses of 1αOHD3 (0.4 µg/kg of body weight in 200µl of propylene glycol) for 14 days. The 2nd group, the control, was untreated. Both groups were given an oral dose of 3H-1αOHD3 (0.4 µg/kg, ca 2 pCi in 200µl of propylene glycol) after overnight fasting. The 3rd group was treated with daily oral doses of 1,25(OH)2D3 (0.2 µg/kg in 200µl of propylene glycol) for 14 days. The 4th group, the control, was untreated. Both groups were given an oral dose of 3H-1,25(OH)2D3 (0.2 µg/kg, ca 2 pCi). All cold and radiolabeled vitamin D analogues were given through a stomach tube.

Preparation of tissue homogenate

At 2, 6 and 24 h after the administration of 3H-1αOH D3 or 3H-1,25(OH)2D3, tested rats were sacrificed, and blood was collected from the abdominal vein. The upper 1/3 of the small intestine was removed and rinsed with 10 ml of distilled water. The bones of both hind legs were cleared of muscle and bone marrow. The kidneys and liver were also excised. Each tissue was weighed and then homogenized with 4 volumes of distilled water with a Polytron (Kinematika, Switzerland). A 0.5 g portion of the bone homogenate was dissolved in 1 ml of conc. HCl. A 100 µl portion of serum and a 0.5 g sample of each tissue homogenate except bone were solubilized with NCS (Amersham, UK), and the radioactivity was counted by a liquid scintillation counter with an external standardization system. The distribution of 3H-radioactivity in the tissues was expressed as a percentage of the administered dose per gram of tissue.

Extraction and purification

One gram of (NH4)2CO3 was added to the homogenates and sera, and was extracted 3 times with a total of 22 ml of ethyl acetate/methanol (3:1). The extract was mixed vigorously with 4 ml of chloroform and 1 ml of water, after which the water layer was removed. The sample was evaporated under N2 stream, redissolved in 10 ml of ethyl acetate, and filtered through a silicic mini-column (Disposil-SL, Nakarai Chemicals, Japan). Each sample was evaporated again under N2 and dissolved in n-hexane/iso-propanol (9:1). The 3H-radioactivity in a small part of the sample was counted to calculate the recovery rate.

High pressure liquid chromatography (HPLC)

Each sample was applied to a normal phase HPLC column (Shim-pack CLC-SIL 15 x 0.6 cm, Shimazu, Japan) and eluted with n-hexane/iso-propanol (9:1) at a flow rate of 1.5 ml/min. Each fraction (1.5 ml/min) was collected, and the radioactivity was counted. The authentic 1αOHD3, 1,25(OH)2D3 and 1,24,25(OH)3D3 were also co-eluted, and the results were expressed as fractions of the administered dose per gram of tissue.

**Results**

Elution profiles on HPLC

Figure 1 shows the representative elution profiles of the serum extracts at 2, 6 and 24 h after the administration of 3H-1αOHD3 in the 1αOHD3-treated rats (A) and in the controls (B). Among the peaks observed, 1αOHD3, 1,25(OH)2D3 and 1,24,25(OH)3D3 were...