

BIOLOGICAL ACTIVITY OF $1\alpha,24$ -DIHYDROXYCHOLECALCIFEROL; A NEW SYNTHETIC ANALOG OF THE HORMONAL FORM OF VITAMIN D

H. KAWASHIMA, K. HOSHINA, Y. HASHIMOTO, T. TAKESHITA, S. ISHIMOTO and T. NOGUCHI

Teijin Institute for Bio-medical Research, 4-3-2 Asahigaoka, Hino-shi, Tokyo 191

and

N. IKEKAWA and M. MORISAKI

Tokyo Institute of Technology, 2-12-1 Ohokayama, Meguro-ku, Tokyo 152

and

H. ORIMO

Section of Endocrinology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan

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1. Introduction

It is well established [1] that vitamin D_3 must be hydroxylated on C-25 in the liver and subsequently on C-1 in the kidney before it can carry out its functions in the intestine and bone. The resulting metabolite, $1\alpha,25$ -dihydroxycholecalciferol ($1\alpha,25$ -(OH) $_2$ - D_3)² is the presently known most potent and rapidly active analog of vitamin D_3 and is considered to be the hormonal form of the vitamin. Among many synthetic analogs, 1α -hydroxycholecalciferol (1α -OH- D_3) has the approximately equivalent activity of $1\alpha,25$ -(OH) $_2$ - D_3 , in promoting intestinal calcium transport, bone mineral mobilization and curing rickets [2].

However, we have recently found [3] that 1α -OH- D_3 has a very high toxicity. LD₅₀-Values for

1α -OH- D_3 were 476 μ g/kg (p.o.), 71 μ g/kg (i.v.) in male mice and 440 μ g/kg (p.o.), 56 μ g/kg (i.v.) in female mice, respectively. Similar results were obtained in rats. In rats, oral administration of 2.5 μ g/kg of 1α -OH- D_3 for 30 days was considered to be a nontoxic dose. The cause of death in both acute and subacute toxicity experiments might be due to hypercalcemia, considering the toxic signs.

In our continuing search for a less toxic vitamin D analog with enhanced and/or specific activity, we have now chosen $1\alpha,24$ -dihydroxycholecalciferol ($1\alpha,24$ -(OH) $_2$ - D_3 (fig.1) whose chemical synthesis has been described previously [4,5].

The present report describes the biological activity of $1\alpha,24$ -(OH) $_2$ - D_3 in normal and anephric rats. The activities of both stereoisomers of $1\alpha,24$ -(OH) $_2$ - D_3 are also compared with those of 1α -OH- D_3 .

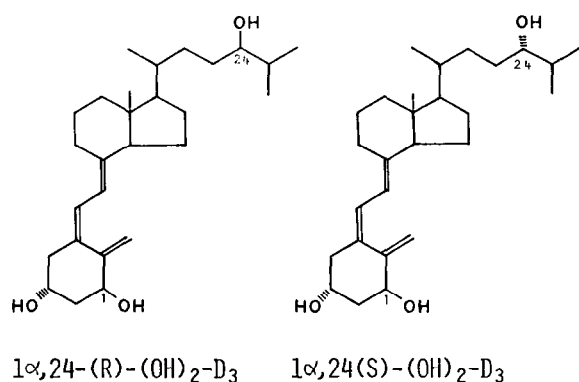
2. Materials and methods

2.1. Intestinal calcium transport and bone mobilization

Weanling male wistar rats were fed for 4 weeks on a vitamin D-deficient low calcium diet (0.003%). At

Abbreviations: $1\alpha,25$ -(OH) $_2$ - D_3 – $1\alpha,25$ -dihydroxycholecalciferol, $1\alpha,24$ -(OH) $_2$ - D_3 – $1\alpha,24$ -dihydroxycholecalciferol, 1α -OH- D_3 – 1α -hydroxycholecalciferol, 24-OH- D_3 – 24-hydroxycholecalciferol, $24,25$ -(OH) $_2$ - D_3 – $24,25$ -dihydroxycholecalciferol, 25-OH- D_3 – 25-hydroxycholecalciferol, $1\alpha,24,25$ -(OH) $_3$ - D_3 – $1\alpha,24,25$ -trihydroxycholecalciferol

Studies on $1\alpha,24$ -dihydroxycholecalciferol, part I

Fig.1. Structures of 1 α ,24-dihydroxycholecalciferol isomers.

the end of the 4th week, groups of 5 rats (weighing about 90 g), which were either intact or bilaterally nephrectomized, received intraperitoneally (in 95% ethanol) 1 α -OH-D₃ or 1 α ,24-(OH)₂-D₃ (0.05 ml/100 g rat). The rats were sacrificed at various time from 4–24 h after drug administration, and intestinal calcium transport and serum calcium concentration were measured. Intestinal calcium-transport assay was performed as described by Martin and DeLuca [6]. Serum calcium concentration was determined by the OCPC (*o*-cresolphthalein complexone) method [7].

2.2. Antirachitic activity

The antirachitic activity of the sterols was examined

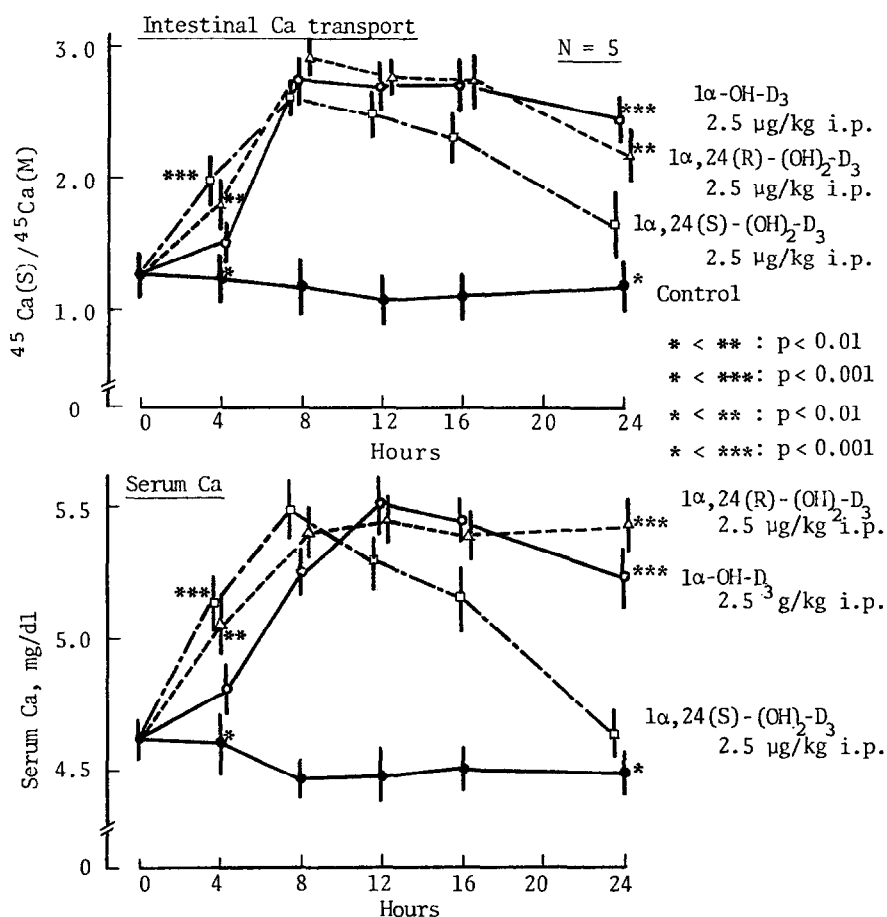


Fig.2. Effect of vitamin D₃ analogs on intestinal calcium transport and serum calcium level in vitamin D deficient rats. 2.5 $\mu\text{g/kg}$ of the sterols dissolved in 95% EtOH was given intraperitoneally to the rats fed the low calcium, vitamin D-deficient diets for 4 weeks. At the indicated times, they were killed for determination of serum calcium concentration and intestinal calcium transport. There were 5 rats in each group. The vertical bars represent the SEM.

by means of the line-test method [8]. Weanling male Wistar rats were maintained in overhanging wire cages and fed on a rachitogenic diet (USP) for 3 weeks and were given either a single dose or a small daily dose of a vitamin D₃ analog for 6 days. Seven days after the first dose, the rats were killed and their radii (distal-end) and ulnae (proximal-end) were removed.

After cleaning of adhering tissues, each bone was cut with a clean sharp blade and a median, longitudinal section through the juncture of the epiphysis and diaphysis was made. The section was rinsed in purified water and immediately immersed in 1.5% silver nitrate solution for 1 min and rinsed again in purified water. The section was exposed, in water, to actinic light until the calcified areas develop a

clearly defined stain. The new calcification epiphyseal plate width) was scored as described in US pharmacopeia using vitamin D₃ as a control [8].

3. Results

Both stereoisomers of 1 α ,24-(OH)₂-D₃ and 1 α -OH-D₃ stimulate intestinal calcium transport and elicit a rise in serum calcium presumably at the expense of bone calcium (bone mineral mobilization) (fig.2). 1 α ,24-(OH)₂-D₃ was active 4 h after administration while 1 α -OH-D₃ was inactive. After 8 h, each sterol caused maximum response and the effects were almost identical to each other. It is noted that

Table 1
Effect of vitamin D₃ analogs on intestinal calcium-transport and serum calcium in vitamin D-deficient rats

Compound	Dose (μ g/kg)	Intestinal calcium-transport ⁴⁵ Ca (S/M)	Serum calcium concentration (mg/dl)
Normal			
(95% Ethanol)		1.73 \pm 0.08 (9)	4.8 \pm 0.12 (9)
1 α -OH-D ₃	0.025 i.p.	1.74 \pm 0.03 (5)	4.9 \pm 0.10 (5)
	0.25 i.p.	2.15 \pm 0.21 (5)	5.3 \pm 0.25 (5)
	2.5 i.p.	2.72 \pm 0.26 (5) ^c	5.5 \pm 0.14 (5) ^b
1 α ,24-(OH) ₂ -D ₃ (24 R Isomer)	0.025 i.p.	1.92 \pm 0.08 (5)	5.3 \pm 0.20 (5) ^a
	0.25 i.p.	2.83 \pm 0.39 (5) ^b	5.5 \pm 0.17 (5) ^b
	2.5 i.p.	3.53 \pm 0.17 (5) ^c	5.9 \pm 0.13 (5) ^c
1 α ,24-(OH) ₂ -D ₃ (24 S Isomer)	0.025 i.p.	1.70 \pm 0.03 (5)	4.6 \pm 0.08 (5)
	0.25 i.p.	2.35 \pm 0.17 (5) ^b	5.0 \pm 0.17 (5)
	2.5 i.p.	2.58 \pm 0.19 (5) ^c	5.5 \pm 0.19 (5) ^b
Anephric			
(95% Ethanol)		1.34 \pm 0.04 (4)	4.6 \pm 0.09 (4)
1 α -OH-D ₃	2.5 i.p.	1.70 \pm 0.06 (4) ^b	5.2 \pm 0.12 (4) ^b
1 α ,24-(OH) ₂ -D ₃ (24 R Isomer)	2.5 i.p.	1.76 \pm 0.10 (4) ^b	5.4 \pm 0.12 (4) ^b
1 α ,24-(OH) ₂ -D ₃ (24 S Isomer)	2.5 i.p.	2.04 \pm 0.18 (4) ^b	5.2 \pm 0.08 (4) ^b

Student's t-test

^a Significantly different from control, $p < 0.05$

^b $p < 0.01$

^c $p < 0.001$

Rats, either intact or bilaterally nephrectomized, were intraperitoneally given the sterols and killed 8 h after administration for determination of serum calcium and intestinal calcium-transport. There were 4–9 rats in each groups (number in parentheses).

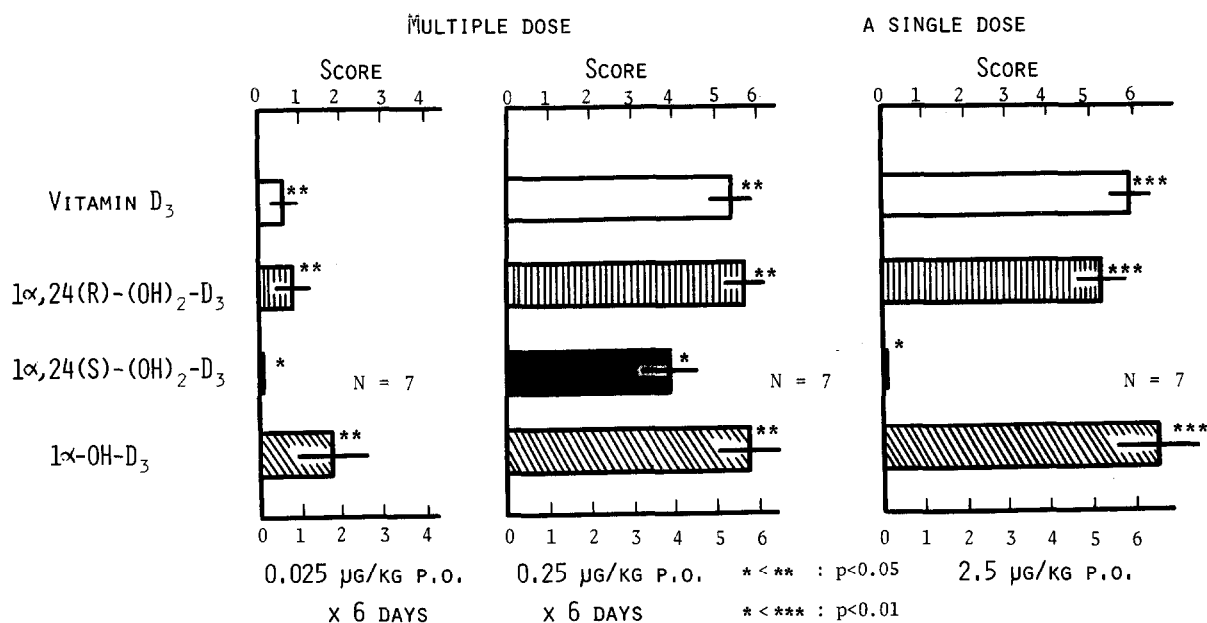
the *S*-isomer of $1\alpha,24-(\text{OH})_2\text{-D}_3$ lost its effect 24 h after administration. This indicates that the *S*-isomer might be rapidly metabolized to an inactive form or excreted. $1\alpha,24-(\text{OH})_2\text{-D}_3$ was also as active as $1\alpha\text{-OH-D}_3$ in anephric rats (table 1).

Fig.3 compares activity of $1\alpha,24-(\text{OH})_2\text{-D}_3$ and $1\alpha\text{-OH-D}_3$ in curing rickets in rats. Both stereoisomers of $1\alpha,24-(\text{OH})_2\text{-D}_3$ and $1\alpha\text{-OH-D}_3$ were active (fig.3). $1\alpha\text{-OH-D}_3$ was most active, given either as a single dose or small daily dose. The *R*-isomer of $1\alpha,24-(\text{OH})_2\text{-D}_3$ was as active as $1\alpha\text{-OH-D}_3$, while the *S*-isomer was less active than vitamin D_3 , and it did not show any activity when it had been given a single dose. This was consistent with the results mentioned above that the *S*-isomer of $1\alpha,24-(\text{OH})_2\text{-D}_3$ had lost its activity 24 h after administration.

These results revealed that $1\alpha,24\text{-DHCC}$ was as active as $1\alpha\text{-OH-D}_3$ on intestine and bone both in intact and anephric rats and that the *S*-isomer had a short duration of action.

4. Discussion

Contrary to 1α -hydroxylation, the physiological meaning of 24-hydroxylation of vitamin D has not yet been clarified, although several factors are known to be involved [1]. 24-Hydroxycholecalciferol (24-OH-D_3) [9] and 24,25-dihydroxycholecalciferol ($24,25-(\text{OH})_2\text{-D}_3$) [10] are as active as 25-hydroxycholecalciferol (25-OH-D_3) in stimulating intestinal calcium transport in vitamin D deficient rats. However, their 24 *S* stereoisomers have little or no activity in the mobilization of calcium from bone, in the elevation of serum phosphorus or in the calcification of bone, while the 24 *R* isomers are almost as active as 25-OH-D_3 in all of these systems [9,10]. Since these sterols were inactive both on intestine and bone in anephric rats [9,10] they presumably function after being hydroxylated on C-1. It has been also demonstrated that $1\alpha,24,25\text{-trihydroxycholecalciferol}$ ($1\alpha,24,25-(\text{OH})_3\text{-D}_3$) has a preferential action on



ANTIRACHITIC ACTIVITY OF VITAMIN D_3 ANALOGS IN RATS.

Fig.3. Antirachitic activity of vitamin D_3 analogs in rats. Weanling male Wistar rats were fed a rachitogenic diet for 3 weeks and were given either a single dose or a small daily dose of vitamin D_3 analogs. Seven days after the first dose, the rats were killed and their radii and ulnae were removed to score epiphyseal plate calcification [8]. There were 7 rats in each group. The horizontal bars represent the SEM.

intestine [11]. As above mentioned, both stereoisomers of $1\alpha,24(\text{OH})_2\text{-D}_3$ have about the same activity both on intestine and bone within 10 h after administration and after 24 h the *S*-isomer has lost activity. Possible explanations for these findings will be the subject of future experiments.

The present report establishes that $1\alpha,24(\text{OH})_2\text{-D}_3$ is as potent as $1\alpha\text{-OH-D}_3$ in the stimulation of intestinal calcium transport and bone mineral mobilization both in normal and anephric rats. In view of these facts and its low toxicity (Kawashima, H. et al. in preparation) $1\alpha,24(\text{OH})_2\text{-D}_3$ is potentially attractive for clinical use in patients with renal failure and other metabolic bone diseases.

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