

THE BIOLOGICAL ACTIVITY OF 26-HYDROXY-DERIVATIVES OF CHOLECALCIFEROL IN VITAMIN D-DEFICIENT RATS

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1. Introduction

In the course of our work investigating the stereochemistry and biological activity of the metabolite of vitamin D₃, 25,26-dihydroxycholecalciferol 25,26(OH)₂D₃, isolated from porcine [1] and human [2] plasma, we have synthesized [3] the C-25 epimers of the latter and established [4] their absolute configuration. One of these epimers, 25R,26- or 25S,26(OH)₂D₃, must be identical with the natural product.

The biological activity of 25R,26- and 25S,26(OH)₂D₃ was examined [3] in rachitic rats and the latter epimer was found superior to the former in elevating serum phosphorus and in the cure of rickets. These results led us to undertake the synthesis [5] of the unknown epimeric 26-hydroxy-derivatives of vitamin D₃, (25R)- and (25S)-26-OH-D₃, which may be helpful in defining more clearly the relation between the 26-hydroxyl and biological activity.

This paper reports the biological activity of (25R)- and (25S)-26-OH-D₃ on bone and gut in vitamin D-deficient rats. We describe also the effects of 25R,26- and 25S,26(OH)₂D₃ on intestinal calcium transport, not reported in [3]. These results make comparisons between the 25R and 25S epimers of either 26-OH-D₃ or 25,26(OH)₂D₃ possible.

2. Materials and methods

Biological methods were detailed in [6].

2.1. Chemical compounds

2.1.1. 25R,26- and 25S,26(OH)₂D₃

These compounds were prepared as in [3,4].

2.1.2. (25R)- and (25S)-26-OH-D₃

These compounds were obtained by chemical synthesis from (25R)- and (25S)-26-hydroxycholesterol [5,7].

2.2. Animals and diets

Weanling Sprague-Dawley male rats were fed for 4 weeks on a synthetic vitamin D-free diet containing 0.47% Ca²⁺ and 0.30% P. Then the animals were kept for a further week either on a low Ca²⁺ (<0.02%) vitamin D-free diet for experiments measuring intestinal Ca²⁺ transport and bone Ca²⁺ mobilization, or a low phosphorus (<0.02%) vitamin D-free diet for experiments investigating bone calcification and serum phosphorus variation.

2.3. Analytical procedures

Calcium was measured by atomic absorption (Perkin-Elmer 303) in presence of 1% of LaCl₃. P_i was determined according to [8]. Radioactivity was measured using a liquid scintillation counter (Inter technique SL 40) and ⁴⁵Ca²⁺ was counted in Instagel solution (Packard).

2.4. In vitro intestinal Ca²⁺ transport

This was measured by the everted gut sac technique [9] and the results expressed as the ⁴⁵Ca²⁺ concentration inside/outside ratio.

2.5. Bone Ca^{2+} mobilization

The increase of serum Ca^{2+} 24 h after injection is regarded as resulting from the mobilization of bone Ca^{2+} .

2.6. Calcification of bone

This was measured by the line-test [10] 1 week after the administration of compounds.

2.7. Serum phosphorus

This was determined 1 week after the administration of compounds.

2.8. Administration of compounds

Epimers of either 26-OH- D_3 or 25,26(OH) $_2\text{D}_3$ (250 ng) were dissolved in ethanol (50 μl) and injected intravenously. Controls were tested with ethanol (50 μl).

2.9. Statistical analysis

Analysis for statistical significance was performed by Wilcoxon's nonparametric test.

3. Results

Table 1 shows the effects of 250 ng (corresponding to 10 IU) of (25R)-26-OH- D_3 and (25S)-26-OH- D_3 on bone. In vitamin D- and phosphorus-deficient rats only the 25R epimer is effective in elevating serum phosphorus and in stimulating bone calcification, while in vitamin D- and Ca^{2+} -deprived animals neither 25R nor 25S induce bone resorption.

Tables 2 and 3 illustrate the effects of 250 ng C-25 epimers of either 26-OH- D_3 or 25,26(OH) $_2\text{D}_3$ on intestinal Ca^{2+} transport in vitamin D- and Ca^{2+} -deficient rats. While 25R,26- and 25S,26(OH) $_2\text{D}_3$ are both

Table 1
Effects of the epimers of 26-OH- D_3 on bone Ca^{2+} mobilization, serum phosphorus and bone calcification

Compound	Bone Ca^{2+} mobilization (serum calcium mg/l) Mean \pm SD	Serum phosphorus (mg/l) Mean \pm SD	Bone calcification (Epiphyseal calcification score) Mean \pm SD
25-OH- D_3 (125 ng)	75.75 \pm 4.79 (4) $W_4^4 = 10$ $p = 0.05$	38.57 \pm 2.99 (7) $W_7^7 = 28$ $p < 0.01$	3.12 \pm 0.05 (8) $W_8^8 = 28$ $p < 0.01$
(25R)-26-OH- D_3 (250 ng)	48.00 \pm 3.40 (6) $W_6^4 = 14$ NS	32.00 \pm 3.00 (7) $W_7^7 = 31$ $p < 0.01$	1.87 \pm 0.95 (8) $W_8^7 = 29$ $p < 0.01$
(25S)-26-OH- D_3 (250 ng)	50.33 \pm 2.87 (6) $W_6^4 = 21$ NS	26.14 \pm 2.79 (7) $W_7^7 = 47.5$ NS	0 \pm 0 (8)
Control (50 μl ethanol)	47.23 \pm 2.22 (4)	26.86 \pm 2.48 (7)	0.07 \pm 0.19 (7)

Table 2
Effects of the epimers of 26-OH- D_3 on intestinal Ca^{2+} transport

Control (50 μl ethanol)	Inside/outside ratio. Mean \pm SD		
	25-OH- D_3 (250 ng)	(25R)-26-OH- D_3 (250 ng)	(25S)-26-OH- D_3 (250 ng)
0.87 \pm 0.05 (4)	1.93 \pm 0.08 (4) $W_4^4 = 10$ $p = 0.05$	1.03 \pm 0.03 (6) $W_6^4 = 10$ $p = 0.01$	0.95 \pm 0.07 (6) $W_6^4 = 15$ NS

Table 3
Effects of the epimers of 25,26(OH)₂D₃ on intestinal Ca²⁺ transport

Control (50 μ l ethanol)	Inside/outside ratio. Mean \pm SD		
	25-OH-D ₃ (250 ng)	25R,26(OH) ₂ D ₃ (250 ng)	25S,26(OH) ₂ D ₃ (250 ng)
0.9 \pm 0.01 (4)	1.90 \pm 0.11 (4) $W_4^4 = 10$	0.99 \pm 0.05 ^a (6) $W_6^4 = 11.5$	1.15 \pm 0.14 ^a (6) $W_6^4 = 10$
	$p = 0.05$	$p < 0.05$	$p = 0.01$

^a Difference between 25R,26- and 25S,26(OH)₂D₃ was significant ($p = 0.05$)

effective (the latter significantly ($p = 0.05$) more than the former) only (25R)-26-OH-D₃ stimulates Ca²⁺ absorption.

4. Discussion

Our results demonstrate that among the 26-hydroxy-derivatives of vitamin D₃ the biological activity depends upon the configuration at C-25 and the 25R epimer: (25R)-26-OH-D₃ is more effective on bone and gut than the 25S one: (25S)-26-OH-D₃. We have investigated [3] the relation between C-25 configuration and biological response with respect to the 25,26-dihydroxy-derivatives of vitamin D₃: 25R,26- and 25S,26(OH)₂D₃ and found the latter biologically superior to the former.

The fact that the more effective epimers of either 26-OH-D₃ or 25,26(OH)₂D₃: (25R)-26-OH-D₃ and 25S,26(OH)₂D₃, respectively, bear opposite signs of conventional configuration at C-25, can be probably explained by their metabolic relationship and the rules of nomenclature [11].

Indeed, 26-hydroxy-derivatives of vitamin D₃ are very likely to be converted by a hepatic hydroxylase system [12] into 25,26-dihydroxy-derivatives (similar metabolic hydroxylation at C-25 has been reported for 24-hydroxyvitamin D₃ [13]) and this reaction generally proceeds with retention [14] of spatial configuration. But introduction of the hydroxyl into the asymmetric centre C-25 induces inversion of conventional signs (R or S) in accordance with the rules of nomenclature [11].

From the foregoing it may be concluded that metabolic hydroxylation of (25R)- and (25S)-26-OH-D₃ should yield 25S,26- and 25R,26(OH)₂D₃, respectively.

In other words, (25R)-26-OH-D₃ and 25S,26(OH)₂D₃ on the one hand, (25S)-26-OH-D₃ and 25R,26(OH)₂D₃ on the other hand, should have similar spatial configuration and activity.

Our biological results support this conclusion.

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