25-HYDROXYLATION OF 1α-HYDROXYVITAMIN D_3 IN VIVO AND IN PERFUSED RAT LIVER

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1. Introduction

The demonstration that 1α-hydroxyvitamin D_3 (1α-OH-D_3) can replace the metabolically active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2$-D_3) in the treatment of various vitamin D refractory syndromes [1–3] has prompted investigations on the metabolism of this analog. In 1975, we synthesized [2-^{3}H]-1α-OH-D_3 and demonstrated that if was metabolized very rapidly to [^{3}H]-1α,25-$(OH)_2$-D_3 in perfused rat liver [4]. [^{3}H]-1α,25-$(OH)_2$-D_3 was found to be the only metabolite of [^{3}H]-1α-OH-D_3 produced by the liver [4]. Almost simultaneously, Holick et al. synthesized [6-^{3}H]-1α-OH-D_3 and reached the same conclusion from in vivo studies in rats [5]. This paper reports in vivo and liver perfusion studies showing that 25-hydroxylation of 1α-OH-D_3 by the liver is not under metabolic control.

2. Materials and methods

2.1. *Materials*

Crystalline 1α-OH-D_3 and 1α,25-$(OH)_2$-D_3 were kindly donated by Dr I. Matsunaga, Chugai Pharmaceutical Company. [2-^{3}H]-1α-OH-D_3 (specific activity, 4 Ci/mmole) was synthesized in our laboratory (Tohira et al., in preparation).

Weanling male rats (Sprague-Dawley) were maintained for 6–7 weeks on a vitamin D deficient diet [6] before use in in vivo and liver perfusion studies. In some experiments, normal rats fed on a stock diet containing vitamin D were used.

Abbreviations: 25-OH-D_3, 25-hydroxyvitamin D_3; 1α-OH-D_3, 1α-hydroxyvitamin D_3; 1α,25-$(OH)_2$-D_3, 1α,25-dihydroxyvitamin D_3; DHT_3, dihydrotachysterol$_3$; 25-OH-DHT_3, 25-hydroxydihydrotachysterol$_3$.

2.2. *Liver perfusion studies*

The livers of vitamin D deficient rats were carefully removed by the method of Miller et al. [7], and perfused with a mixture of heparinized blood (30 ml) from vitamin D deficient rats and Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4, (45 ml), equilibrated with 95% O_2, 5% CO_2. Livers from normal rats were perfused with a similar mixture containing blood from normal rats. The bile duct was cannulated and bile-flow was maintained during the experiments. When the flow of perfusate through the liver (approx. 6 g wet weight) attained 30 ml/min, 6.25–62 500 pmol of 1α-OH-D_3 containing 0.2 μCi of radiochemically pure [2-^{3}H]-1α-OH-D_3 was added to the perfusate, and perfusion was continued for 2 h. In some experiments, non-radioactive 1α,25-$(OH)_2$-D_3 was added to the perfusate 30 min before addition of [^{3}H]-1α-OH-D_3.

2.3. *In vivo studies*

Vitamin D deficient or normal rats were injected intravenously with 6.25 (4 μCi/nmole) – 62 500 (0.04 μCi/nmole) pmoles of [^{3}H]-1α-OH-D_3 and killed 2 h later.

2.4. *Measurements of 1α,25-(OH)$_2$-D_3 generated in vivo and in perfused rat liver*

The perfusate and liver (perfusion studies), or plasma and liver (in vivo studies) were each extracted by the method of Bligh and Dyer [8]. The chloroform

layers of the extracts were evaporated to dryness and the residues were chromatographed on Sephadex LH-20 columns (1.5 X 30 cm) using a solvent of 65% chloroform–35% hexane [9]. All radioactive determinations were carried out with a Packard Tris-Carb. Model 3385 liquid scintillation spectrometer. [^{3}H]-1α,25-$(OH)_2$-D_3 was identified by co-chromatography with authentic 1α,25-$(OH)_2$-D_3 on Sephadex LH-20 column and Celite column, as described previously [10]. The amount of 1α,25-$(OH)_2$-D_3 found in vivo or produced in perfused liver was calculated from the radioactivity of [^{3}H]-1α,25-$(OH)_2$-D_3 recovered and the specific activity of the isotope used.

3. Results

At all doses examined, [^{3}H]-1α-OH-D_3 was found to be converted to only one radioactive metabolite by perfused rat liver. This metabolite was unequivocally identified as [^{3}H]-1α,25-$(OH)_2$-D_3 by co-chromatography with authentic 1α,25-$(OH)_2$-D_3 on Sephadex LH-20 and Celite columns. At all doses of above 250 pmol tested, about 20% of the [^{3}H]-1α-OH-D_3 added was converted to [^{3}H]-1α,25-$(OH)_2$-D_3 during 2 h perfusion. The conversion rate was slightly higher at a lower dose. Fig.1 shows the relation between the amount of 1α-OH-D_3 added and that of 1α,25-$(OH)_2$-D_3 produced by perfused liver. It can be seen that the amount of 1α,25-$(OH)_2$-D_3 produced increased linearly with increase in the amount of 1α-OH-D_3 added. From table 1 it is clear that proir administration of large amounts (62.5 nmol) of 1α,25-$(OH)_2$-D_3 to the perfusate did not decrease the rate of production of [^{3}H]-1α,25-$(OH)_2$-D_3 by the perfused liver. Livers from normal rats fed on stock diet also produced similar amounts of [^{3}H]-1α,25-$(OH)_2$-D_3 from [^{3}H]-1α-OH-D_3 (table 1).

When vitamin D deficient rats were injected with 6.25, 62.5, 625, 6250 or 62 500 pmol of [^{3}H]-1α-OH-D_3, the rates of appearance of [^{3}H]-1α,25-$(OH)_2$-D_3 in the plasma and liver increased exactly 10-fold for each 10-fold increase in the dose of 1α-OH-D_3 (fig.2). Normal rats fed on stock diet metabolized [^{3}H]-1α-OH-D_3 to [^{3}H]-1α,25-$(OH)_2$-D_3 at the same rate as vitamin D deficient rats.

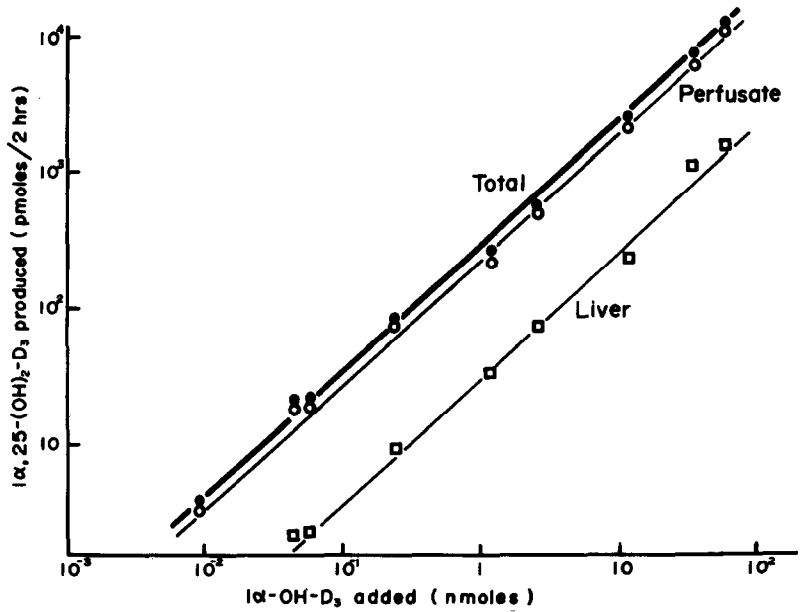

Fig.1. Relation between the amount of 1α-OH-D_3 added to the perfusate and that of 1α,25-$(OH)_2$-D_3 produced by perfused liver from vitamin D deficient rats. 'Total' (—●—) indicates the sum of the amounts of 1α,25-$(OH)_2$-D_3 recovered in extracts of the perfusate (—○—) and liver (—□—).

Table 1
Influence of prior administration of vitamin D_3 or its metabolites in vivo and in vitro on the production of 1α,25-$(OH)_2$-D_3 from 1α-OH-D_3 by perfused rat liver

Rat	Prior additon	[^{3}H]-1α-OH-D_3	1α,25-$(OH)_2$-D_3 generated		
		added (nmoles)	Perfusate (pmoles/h/g of liver)	Liver	Total
D deficient	None	2.7	42.2	5.7	47.9
D deficient	1α,25-$(OH)_2$-D_3[a] (62.5 nmol)	2.7	44.7	18.5	63.2
Normal	None	2.7	52.2	5.7	57.9

[a]The liver was perfused with 62.5 nmol of non-radioactive 1α,25-$(OH)_2$-D_3 for 30 min before addition of [^{3}H]-1α-OH-D_3.

4. Discussion

Bhattacharyya and DeLuca [11] compared the 25-hydroxylations of vitamin D_3 and dihydrotachysterol$_3$ (DHT_3), a reduction product of tachysterol$_3$ and vitamin D_3. They demonstrated that the rate of appearance of 25-hydroxyvitamin D_3 (25-OH-D_3) in the blood only increased about 2-fold with a 100-fold increase in the dose of vitamin D_3, whereas the rate of appearance of 25-hydroxydihydrotachysterol$_3$ (25-OH-DHT_3) increased 100-fold with a 100-fold increase in the dose of DHT_3 [11].

The present results indicate that the 25-hydroxylation of 1α-OH-D_3 by the liver is similar to that of DHT_3, but not to that of vitamin D_3. Thus, the same

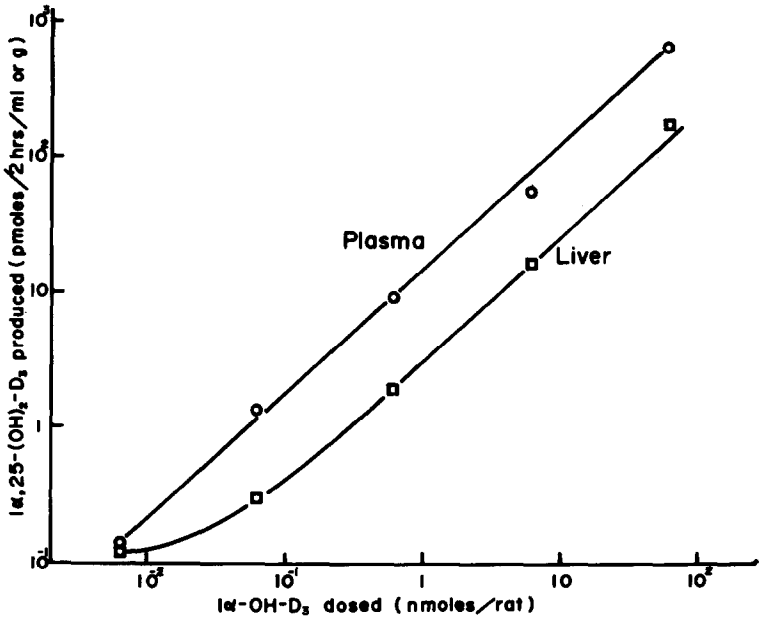

Fig.2. Relation between the amount of 1α-OH-D_3 administered and that of 1α,25-$(OH)_2$-D_3 found in the plasma (——○——) and liver (——□——). 6.25 – 62 500 pmoles of [^{3}H]-1α-OH-D_3 were injected into the jugular vein of vitamin D deficient rats, and animals were killed 2 h later.

liver enzyme may be responsible for the 25-hydroxylations of 1α-OH-D_3 and DHT_3, but probably not for that of vitamin D_3. Bhattacharyya and DeLuca concluded that 25-hydroxylation of DHT_3, unlike that of vitamin D_3, is not under metabolic control [11,12]. The same seems true of the 25-hydroxylation of 1α-OH-D_3.

The present data suggest two important points in connection to the treatment of various vitamin D refractory syndromes by this analog. First, 1α-OH-D_3 administered is metabolized quantitatively to the natural hormone of vitamin D_3, 1α,25-$(OH)_2$-D_3, and this compound is the only metabolite of 1α-OH-D_3 generated by the liver. Second, the 25-hydroxylation of 1α-OH-D_3 is not under metabolic control, unlike the 25-hydroxylation of vitamin D_3 [11]. Therefore, administration of large amounts of 1α-OH-D_3 to human patients would certainly induce hypercalcemia. Thus, as with 1α,25-$(OH)_2$-D_3, care should be taken not to administer excess 1α-OH-D_3 to human patients. Neither 1α-OH-D_3 nor DHT_3 is a naturally occurring form of the D vitamin, but 1α-OH-D_3 is metabolized very quickly and quantitatively to the natural hormone of vitamin D_3. In addition, 1α-OH-D_3 is much easier and cheaper to prepare than 1α,25-$(OH)_2$-D_3. Therefore, 1α-OH-D_3 seems to be the best analog of 1α,25-$(OH)_2$-D_3 for clinical use. Comparative studies on the 25-hydroxylations of vitamin D_3 and 1α-OH-D_3 in perfused rat liver are now in progress.

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