EFFECT AND METABOLISM OF 1α,24-DIHYDROXYVITAMIN D_3 IN HEPATECTOMIZED RATS

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Received 14 May 1979
Revised version received 19 June 1979

1. Introduction

It is well established that vitamin D_3 undergoes metabolic conversion before exerting its biological effects [1–3]. The liver produces 25-hydroxyvitamin D_3 (25-OH-D_3), which the kidney then converts to 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2$-D_3) and 24,25-dihydroxyvitamin D_3 (24,25-$(OH)_2$-D_3). The bulk of experimental evidence suggests that 1α,25-$(OH)_2$-D_3 is a major hormonally active form of vitamin D_3, responsible for increasing intestinal absorption of calcium and phosphorus, enhancing bone resorption, and preventing rickets [1–3].

In contrast, the role of 24-hydroxylation and functions of the resulting metabolites remain still obscure. In a previous report, we demonstrated that 1α,24-dihydroxyvitamin D_3 (1α,24-$(OH)_2$-D_3), a synthetic analog of 1α,25-$(OH)_2$-D_3, has a potent activity in stimulating intestinal calcium absorption and calcium mobilization from bone, and in curing rickets, and also suggested the possibility that 1α,24-$(OH)_2$-D_3 might act without being further metabolized [4]. To examine this, we studied biological activity and metabolism of 1α,24-$(OH)_2$-D_3 in hepatectomized rats and compared the activity with that of 1α,24,25-trihydroxyvitamin D_3 (1α,24,25-$(OH)_3$-D_3) which is a metabolite of 1α,24-$(OH)_2$-D_3. We describe here the biological activity and metabolism of the *R*-isomer of 1α,24-$(OH)_2$-D_3 (1α,24(R)-$(OH)_2$-D_3) only, since the both isomers (*R*- and *S*-isomer) have shown no qualitatively different effects in rats except for the shorter duration of action of the *S*-isomer which might be due to its susceptibility to metabolism [4,5].

Abbreviations: 1α,24-$(OH)_2$-D_3, 1α,24-dihydroxyvitamin D_3; 1α,24(R)-$(OH)_2$-D_3, 1α,24(R)-dihydroxyvitamin D_3; 1α,25-$(OH)_2$-D_3, 1α,25-dihydroxyvitamin D_3; 1α,24,25-$(OH)_3$-D_3, 1α,24,25-trihydroxyvitamin D_3; 1α,24(R),25-$(OH)_3$-D_3, 1α,24(R),25-trihydroxyvitamin D_3; 1α-OH-D_3, 1α-hydroxyvitamin D_3; 24,25-$(OH)_2$-D_3, 24,25-dihydroxyvitamin D_3; 24(S)-^{3}H-1α,24(R)-$(OH)_2$-D_3, 24(S)-^{3}H-1α,24(R)-dihydroxyvitamin D_3; ^{3}H-1α,24(R),25-$(OH)_3$-D_3, ^{3}H-1α,24(R),25-trihydroxyvitamin D_3

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This is the second report on the Biological Activity of 1α,24-Dihydroxyvitamin D_3

2. Materials and methods

Male Wistar rats, either normal or vitamin-D-deficient [4], were used. Groups of five rats, which are either intact or hepatectomized, received vitamin D_3 analogs intraperitoneally (in 95% ethanol) or orally (in corn oil). Rats were killed at the predetermined time after drug administration and intestinal calcium absorption and serum calcium concentration were measured. Intestinal calcium absorption assay was performed by the everted gut sac method [4] and calcium concentration was determined by the orthocresolphthalein complexone method [6]. To examine biological activity of vitamin D_3 analogs in hepatectomized rats, male Wistar rats fed a normal

diet were used, since the vitamin-D-deficient rats were not so strong as to survive several hours after the surgery. Hepatectomy was performed under ether anesthesia removing about 95% of the liver after ligation as near as possible to the blanchings of blood vessels which come into or out of the liver.

To examine metabolism of 1α,24-$(OH)_2$-D_3, 24(S)-^{3}H-1α,24(R)-$(OH)_2$-D_3 (3.0 Ci/mmol) was orally administered (in saline containing 0.1% Triton X-100, 1 μg/head) to either intact or hepatectomized vitamin-D-deficient rats. After 4 h (vitamin-D-deficient rats could not survive longer than 4 h after the surgery) rats were killed by bleeding from abdominal aorta. Plasma was extracted by chloroform–methanol (1/1, v/v) and chloroform layer was applied to Sephadex LH-20 column chromatography. Elution was performed by chloroform–*n*-hexane–methanol (75/23/2, v/v/v) and radioactivity of each fraction (3.5 ml each) was measured by liquid scintillation counter (Model 3330, Packard). The ^{3}H-1α,24(R),25-$(OH)_3$-D_3 fractions were also applied to high pressure liquid chromatography (Hitachi, Model 635; column: Zorbax Sil, Du Pont) comparing with authentic sample.

1α,24(R)-$(OH)_2$-D_3, 24(S)-^{3}H-1α,24(R)-$(OH)_2$-D_3, 1α,24(R),25-$(OH)_3$-D_3 and 1α-OH-D_3 were chemically synthesized, and ^{3}H-1α,24(R),25-$(OH)_3$-D_3 was obtained by the enzymatic conversion with chick kidney homogenates [1].

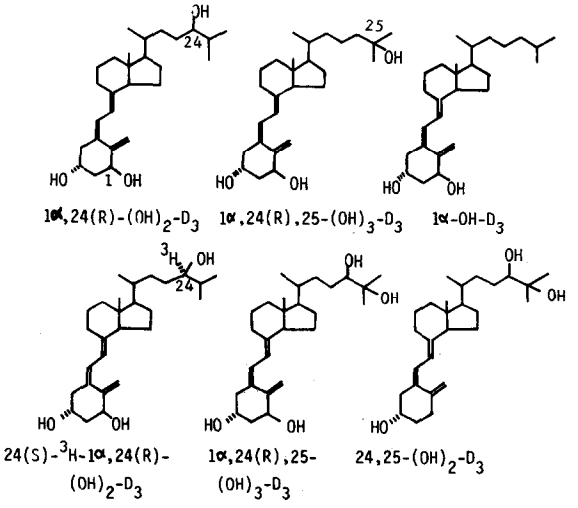

Fig.1. Chemical structures of vitamin D_3 analogs.

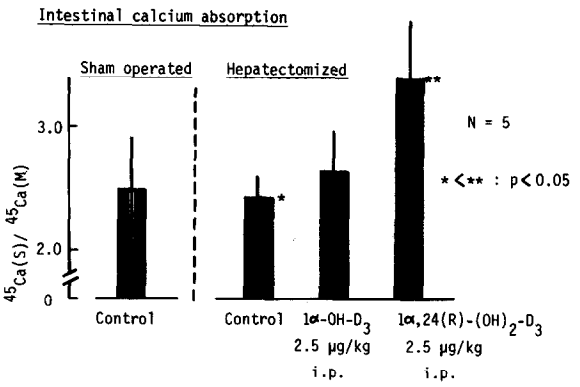

Fig.2. Effects of 1α,24(R)-$(OH)_2$-D_3 and 1α-OH-D_3 on intestinal calcium absorption in hepatectomized rats.

3. Results and discussion

First, biological activity of 1α,24(R)-$(OH)_2$-D_3 and 1α-OH-D_3 were examined. In hepatectomized rats, 2.5 μg/kg of 1α,24(R)-$(OH)_2$-D_3 stimulated intestinal calcium absorption at 8 h after the administration, while 1α-OH-D_3 showed no effect (fig.2). These sterols showed almost equipotent effect in stimulating both intestinal calcium absorption and calcium mobilization from bone in intact rats [4,5].

Second, metabolism of ^{3}H-1α,24(R)-$(OH)_2$-D_3 was studied both in intact and hepatectomized rats. Sephadex LH-20 column chromatograms of lipid extracts of rat plasma demonstrate that ^{3}H-1α,24(R)-$(OH)_2$-D_3 was partly metabolized to ^{3}H-1α,24(R),25-$(OH)_3$-D_3 in intact rats, while no ^{3}H-1α,24(R),25-$(OH)_3$-D_3 was found in hepatectomized rats (fig.3). The ^{3}H-1α,24(R),25-$(OH)_3$-D_3 fraction coincided with authentic 1α,24(R),25-$(OH)_3$-D_3 on the chromatogram of high pressure liquid chromatography [5]. Similar results were obtained in lipid extracts of small intestine and liver (data is not shown).

These results clearly indicate that 1α,24(R)-$(OH)_2$-D_3 functions without being further metabolized and that the liver has a major role, if not all, in the 25-hydroxylation of vitamin D as previously reported by others [8], although other organs such as small intestine have been known to have a similar function of 25-hydroxylation in chicks [9]. The fact that 1α-OH-D_3 did not stimulate intestinal calcium absorption in hepatectomized rats is correspondent to its low affinity to the intestinal receptor for 1α,25-$(OH)_2$-D_3 [1–3].

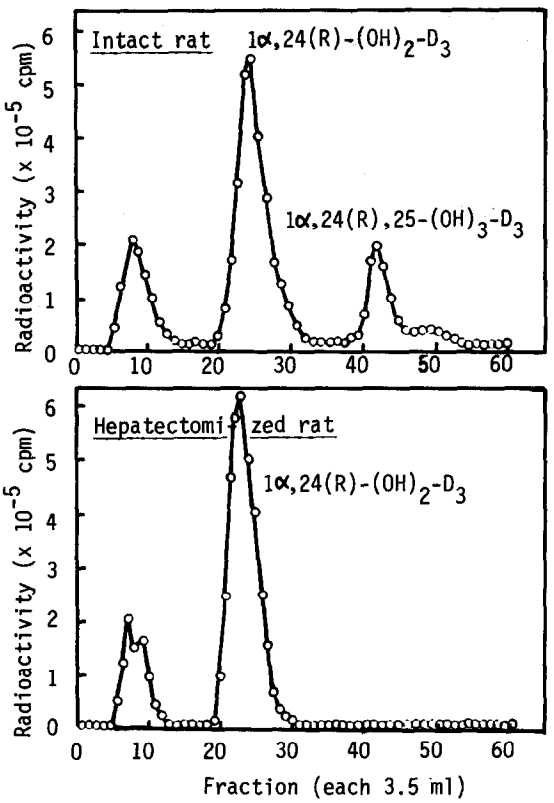

Fig.3. Sephadex LH-20 column chromatograms of lipid extracts of rat plasma after oral administration of ^{3}H-1α,24(R)-$(OH)_2$-D_3.

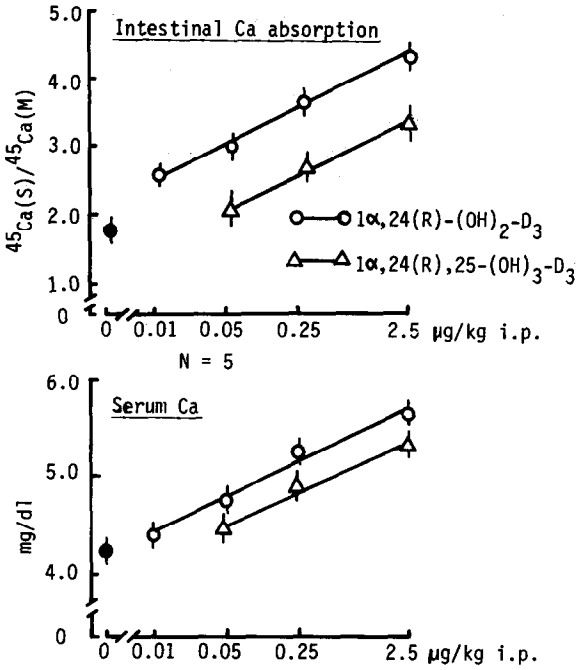

Fig.4. Effects of 1α,24(R)-$(OH)_2$-D_3 and 1α,24(R),25-$(OH)_3$-D_3 on intestinal calcium absorption and calcium mobilization from bone in vitamin-D-deficient rats.

Third, it was shown that 1α,24(R)-$(OH)_2$-D_3 is more potent than 1α,24(R),25-$(OH)_3$-D_3 in stimulating both intestinal calcium absorption and calcium mobilization from bone in vitamin-D-deficient rats (fig.4), the latter is also one of the naturally occurring metabolites of vitamin D_3.

Our results strongly suggest that 24-hydroxyl is a good substitute for 25-hydroxyl concerning the well-known biological functions of 1α,25-$(OH)_2$-D_3 as far as the sterol has a hydroxyl on its C-1 position, and that 24-hydroxylation may not always be the reduction of the biological activity of the sterol. Although it is clear that 1α,24-$(OH)_2$-D_3 is a potent bioisomer of 1α,25-$(OH)_2$-D_3 as described, the characterization of its effect, especially the difference from that of 1α,25-$(OH)_2$-D_3, if any, remains a problem of future investigation. Recent studies on the biological function of 24,25-$(OH)_2$-D_3 suggest that this metabolite has some important roles [9–13].

Considering these reports and our results mentioned above, further investigation on the biological activity and metabolism of 1α,24-$(OH)_2$-D_3 may provide fruitful insight into the physiological role of 24-hydroxylation and the biological activities of the resulting metabolites.

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