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Peritonitis induces the synthesis of 1α,25-dihydroxyvitamin D_3 in macrophages from CAPD patients

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Metabolism of 25-[^{3}H]hydroxyvitamin D_3 was studied in peritoneal macrophages from renal failure patients on continuous ambulatory peritoneal dialysis (CAPD). Cells from 5 out of 8 patients with a history of peritonitis produced significant amounts of a metabolite chromatographically identical to 1α,25$(OH)_2D_3$; but none was produced by cells from non-infected patients. The evidence strongly suggests that peritoneal macrophages stimulated by infection can metabolise 25OHD_3 to the active vitamin D_3 metabolite, 1α,25$(OH)_2D_3$, when maintained in short-term primary culture.

Renal failure; Vitamin D metabolism; Continuous ambulatory peritoneal dialysis; (Peritoneal macrophage)

1. INTRODUCTION

The hormonally active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], which promotes intestinal absorption of calcium, is normally synthesized exclusively in the kidney from its precursor 25-hydroxyvitamin D_3 (25OHD_3) [1]. However, patients with sarcoidosis who are hypercalcaemic have elevated plasma levels of 1α,25$(OH)_2D_3$ [2] thought to result from extra-renal synthesis by activated macrophages (Mφ) [3]. Further studies on cultured cells have shown that normal Mφ activated by γ-interferon [4] or bacterial lipopolysaccharides [5] also develop the ability to synthesise 1α,25$(OH)_2D_3$.

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Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; Mφ, macrophage(s); MN, monocytes; HPLC, high-performance liquid chromatography; 25OHD_3, 25-hydroxycholecalciferol; 1α,25$(OH)_2D_3$, 1α,25-dihydroxycholecalciferol

We have addressed the problem as to whether activated Mφ in patients on continuous ambulatory peritoneal dialysis (CAPD) with a history of peritonitis resulting from bacterial infection may produce 1α,25$(OH)_2D_3$. Extra-renal synthesis could prove significant in these patients in whom renal synthesis of the active metabolite is likely to be severely impaired [6].

2. MATERIALS AND METHODS

2.1. *Isolation of peritoneal Mφ from CAPD fluids*

Overnight CAPD fluid drains were collected from 8 patients with a history of peritonitis and 6 patients who had no history of infection. Cells, predominantly Mφ and monocytes (MN) with some neutrophils and lymphocytes, were harvested by centrifugation in 50-ml aliquots [7] and resuspended in 4 ml RPMI-1640 medium supplemented with 10% fetal calf serum (Flow). These were incubated in 20-ml glass vials for 2 h at 36°C in an atmosphere of 95% air/5% CO_2. Adherent Mφ and non-adherent cells were separated, resuspended in 2 ml serum-free RPMI-1640 medium containing 2 mM glutamine, 50 U/ml penicillin and

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50 μg/ml streptomycin and examined for the ability to metabolise [^{3}H]25OHD$_3$. In a separate experiment adherent cells from patient no. 5 were maintained in culture for 7 days before incubation with [^{3}H]25OHD$_3$. The morphology of the harvested cells was examined by light microscopy in all patients and also following Giemsa and non-specific esterase staining of cytocentrifuge preparations for selected patients.

2.2. *Metabolism of 25OHD$_3$ and chromatography of lipid extracts*

Approx. 100 pg [^{3}H]25OHD$_3$ (10^5 dpm; 176 Ci/mmol) in 20 μl ethanol were added to $1–20 \times 10^6$ adherent, non-adherent or mixed cells from each patient. Incubations were carried out at 37°C in an atmosphere of 95% air/5% CO_2 overnight (17 h) and were terminated by adding 3 ml acetonitrile. The acetonitrile extracts of each sample underwent preliminary purification on silica Sep-pak cartridges (Waters Associates) [8] followed by analysis on HPLC using a Zorbax-SIL column developed with *n*-hexane, propan-2-ol, methanol (110:6:4, v/v) at 1.5 ml/min. 30-s fractions were collected and counted for ^{3}H radioactivity. 25OHD$_3$ and 1α,25(OH)$_2$D$_3$ standards (kindly donated by Dr M. Uskokovic) were also chromatographed and their retention times determined by UV absorption at 265 nm. The quantity of the labelled metabolite chromatographing with the same retention time as standard 1α,25(OH)$_2$D$_3$ was expressed as pg/10^6 cells. The identify of this metabolite was confirmed in a pooled sample of cell extracts by rechromatography on the above HPLC system and then by straight-phase (Zorbax SIL) and reverse-phase (Zorbax ODS) HPLC with mobile phases of 2.5% methanol in dichloromethane (1 ml/min) and 15% water in methanol (2 ml/min), respectively.

3. RESULTS AND DISCUSSION

The formation of a vitamin D metabolite, which chromatographs on HPLC with authentic 1α,25(OH)$_2$D$_3$, is clearly demonstrated in cells isolated from CAPD fluid drains in 5 out of 8

Table 1

Metabolism of [26,27-^{3}H]25-hydroxyvitamin D$_3$ in peritoneal macrophages from patients with or without a history of peritonitis

Patient	1,25(OH)$_2$D$_3$ formation (pg/10^6 cells)		Days since last bout of peritonitis
	Adherent macrophages	Non-adherent cells	
1	22.90	0	65 (6)
2	11.36	0.1	10 (3)
3	2.82	0	12 (1)
4	2.62	0.07	85
5	1.19	0.26	7
6	0	0	28 (1)
7	0	0	22 (2)
8	0	0	206
5 1 week in culture	9.52	0	7
9	0	0	no history
10	0	0	of
11	0	0	peritonitis
12	0	0	
13	0	0	
14	0	0	

The number of previous episodes of peritonitis is given in parentheses

CAPD patients with a history of peritonitis (table 1). This metabolic activity was further demonstrated to occur only in glass-adherent cells which have typical Mϕ morphology. By contrast, adherent cells from 6 CAPD patients with no history of peritonitis did not synthesise 1α,25 $(OH)_2D_3$. A product of [^{3}H]25OHD_3 metabolism less polar than 1α,25$(OH)_2D_3$, with HPLC retention similar to that of (5Z)-19-nor-10-oxo-25OHD_3 [9], appeared to be produced by non-adherent cells, but this may be formed by non-specific oxidation of 25OHD_3 as it was also formed under cell-free control incubations (fig.1).

A pooled sample of the [^{3}H]1α,25$(OH)_2D_3$-like material produced by the adherent Mϕ co-chromatographed with the authentic metabolite in three different HPLC systems thus providing good evidence for its identity (fig.2).

Previous studies on the metabolism of [^{3}H]25OHD_3 by Mϕ have used cells maintained in cell culture for 4–10 days [3–5]. However, the cell culture conditions may themselves alter the phenotype of the cells and perhaps influence their ability

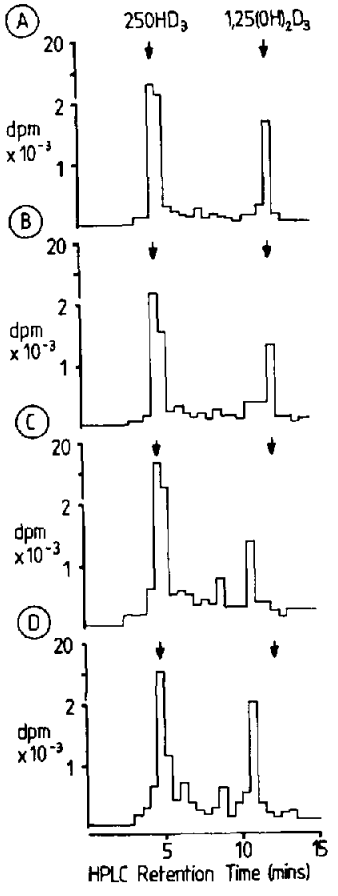

Fig.1. Metabolism in vitro of [^{3}H]25OHD_3 by cells harvested from the CAPD fluid of a peritonitis patient. (A) Mixed cells, (B) adherent cells with Mϕ morphology, (C) non-adherent cells, (D) cell-free incubation. Bars represent the amount of tritiated material in 30-s fractions obtained by HPLC analysis of cell extracts on a Zorbax-SIL column developed at 1.5 ml/min with *n*-hexane, propan-2-ol, methanol (110:6:4). Arrows indicate retention times of standard 25OHD_3 and 1α,25$(OH)_2D_3$ determined by UV absorbance (265 nm).

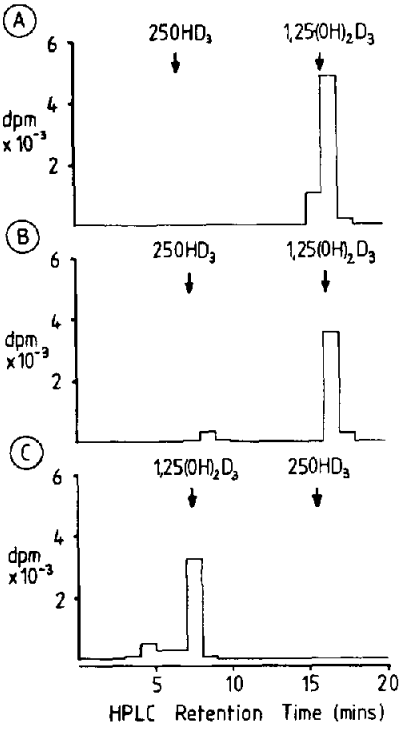

Fig.2. Rechromatography of pooled [^{3}H]1,25$(OH)_2D_3$ produced by peritoneal Mϕ, following initial chromatography on HPLC system (A), from patients with a history of peritonitis on 3 HPLC systems. (A) Zorbax-SIL column eluted at 1.5 ml/min with *n*-hexane, propan-2-ol, methanol (110:6:4), (B) Zorbax-SIL column eluted at 1 ml/min with 2.5% methanol in dichloromethane, (C) Zorbax-ODS reverse-phase column eluted at 2 ml/min with 15% water in methanol. Bars represent the amount of tritiated material in 1-ml fractions and the arrows indicate retention times of standard 25OHD_3 and 1α,25$(OH)_2D_3$ determined by UV absorbance (265 nm).

to metabolise [^{3}H]25OHD$_3$. We have examined cells immediately after isolation using only a 2 h period to select glass-adherent Mϕ and have demonstrated clearly the ability of such cells from CAPD patients with a history of peritonitis to form 1α,25(OH)$_2$D$_3$. After 7 days in culture cells from patient no. 5 formed increased amounts of 1α,25(OH)$_2$D$_3$ (table 1).

The Mϕ appear to retain the capacity to form 1α,25(OH)$_2$D$_3$ many days after an episode of peritonitis (table 1). This could be explained if there were a static peritoneal population of ageing activated Mϕ. This, however, is unlikely because 10^7–10^8 MN and Mϕ are lost daily in CAPD fluid drains. An alternative explanation is that low-grade infection, resulting from bacterial colonisation of the intraperitoneal catheters [10] or sequestration of viable bacteria within MN and Mϕ [11], may persist within the peritoneal cavity in the absence of clinical peritonitis and may provide continuous stimulation of Mϕ. Variations in the degree of bacterial colonisation or sequestration may in part explain why only some of the peritonitis patients had Mϕ that could form 1α,25(OH)$_2$D$_3$.

Renal failure patients have an impaired ability to synthesise 1α,25(OH)$_2$D$_3$ [6] which may be exaggerated by the loss of protein-bound 25OHD$_3$ in CAPD fluid; it is thus possible that extra-renal formation of this metabolite might be significant in maintaining calcium homeostasis in CAPD patients. Reported values for plasma 1α,25(OH)$_2$D$_3$ in CAPD patients are higher (mean 32.1, range 6–81 pg/ml) [12] than those reported by different authors for patients treated by haemodialysis (mean 17.7, range 0–35 pg/ml) [13]. The significance of extra-renal 1α,25(OH)$_2$D$_3$ synthesis may be even greater when its role in haematolymphopoietic tissue is considered. In vitro, 1α,25 (OH)$_2$D$_3$ has been shown to promote the differentiation of MN towards the Mϕ phenotype. It also enhances Mϕ function in non-specific immune processes such as phagocytosis and inhibits interleukin-2 production by activated lymphocytes [14]. Thus, within the micro-environment of the immune response, 1α,25(OH)$_2$D$_3$ secreted by 'activated' peritoneal Mϕ may influence the function of 1α,25(OH)$_2$D$_3$-responsive cells.

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