Extra-renal 25-hydroxyvitamin D3-1α-hydroxylase in human health and disease

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Abstract

Although ectopic expression of 25-hydroxyvitamin D3-1α-hydroxylase (1α-OHase) has been recognized for many years, the precise function of this enzyme outside the kidney remains open to debate. Three specific aspects of extra-renal 1α-OHase have attracted most attention: (i) expression and regulation in non-classical tissues during normal physiology; (ii) effects on the immune system and inflammatory disease; (iii) expression and function in tumors. The most well-recognized manifestation of extra-renal 1α-OHase activity remains that found in some patients with granulomatous diseases where locally synthesized 1α,25(OH)2D3 has the potential to spill-over into the general circulation. However, immunohistochemistry and mRNA analyses suggest that 1α-OHase is also expressed by a variety of normal human tissues including the gastrointestinal tract, skin, vasculature and placenta. This has promoted the idea that autocrine/paracrine synthesis of 1,25(OH)2D3 contributes to normal physiology, particularly in mediating the potent effects of vitamin D on innate (macrophage) and acquired (dendritic cell) immunity. We have assessed the capacity for synthesis of 1,25(OH)2D3 in these cells and the functional significance of autocrine responses to 1α-hydroxylase. Data suggest that local synthesis of 1,25(OH)2D3 may be a preferred mode of response to antigenic challenge in many tissues.

Keywords: Vitamin D; Extra-renal; 1α-Hydroxylase; 24-Hydroxylase; Granulomatous; Macrophage; Dendritic cell; Crohn’s disease; Tuberculosis; Cancer

1. Introduction

Synthesis of the active form of vitamin D, 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), from its precursor form, 25-hydroxyvitamin D3 (25(OH)D3) is a pivotal step in calcium homeostasis and bone metabolism. Not only does this represent the point at which the major circulating form of vitamin D (25(OH)D3) becomes a hormone, but it is also the point at which peptide caiciotropic factors such as parathyroid hormone interact with vitamin D physiology [1]. As a consequence, caiciotropic synthesis of 1,25(OH)2D3 by the kidney, catalyzed by the enzyme 25-hydroxyvitamin D3-1α-hydroxylase (1α-OHase) is tightly regulated with feedback control mechanisms that include a dedicated catabolic enzyme, 24-hydroxylase (24-OHase) [2,3]. As our awareness of the pluripotency of 1,25(OH)2D3 has increased, a key question that has arisen concerns the role of 1α-OHase outside the kidney and the extent to which this represents part of normal physiology. Here we have used in vitro models to assess the relative functional efficacy of exogenous versus endogenous 1,25(OH)2D3 in modulating immune responses. We also discuss the significance of this with respect to other models of extra-renal 1α-hydroxylase.
activity and diseases in which there is dysregulated extra-
renal synthesis of 1,25(OH)2D3.

2. Materials and methods

2.1. Generation of monocyte-derived dendritic cells and maxcrophages

Monocyte-derived dendritic cells (DCs) and macrophages were derived from buffy coats of healthy donors by density centrifugation over Lymphoprep (Robbins Scientific, Sunnyvale, CA, USA), resuspended in RPMI supplemented with 5% human AB serum (HD supplies, Buckinghamshire, UK) and allowed to adhere to plastic at 37 °C for 2 h. Non-adherent cells were then removed and monocytes cultured in RPMI supplemented with the following: 5% AB serum; 2 mM l-glutamine; 100 μg/ml streptomycin; 100 μg/ml penicillin; 800 μg/ml GM-CSF (Sandoz Pharmaceuticals). Dendritic cells cultures also received 1000 μg/ml IL-4 (R&D systems, Abingdon UK) in 75 cm2 flasks. 1, 25(OH)2D3 (100 nM) or 25(OH)D3 (5–150 nM) (a kind gift from Dr. Lise Binderup, Leo Pharmaceuticals, Ballerup, Denmark), as well as keto-
conazole (5 μM) (Sigma, Poole, UK) were added on day 5 of DC/macrophage culture and analysis of vitamin D metabo-
lites and flow cytometry carried out two days later.

2.2. Analysis of 1,25(OH)2D3 in DC and macrophage cell cultures

The accumulation of 1,25(OH)2D3 in cultures of DCs or macrophages incubated with precursor 25OHD3 was carried out using a specific radioimmunoassay kit as described by the manufacturers (IDS, Boldon, UK). Data were reported as concentration of 1,25(OH)2D3 (pM) ± standard deviation (n = 3).

2.3. Analysis of DC and macrophage marker expression by fluorescence-activated cell sorting (FACS)

Expression of CD83, CD86, HLA-DR (DCs) and CD14 (macrophages) was carried out as described previously [4]. Data were reported as the percentage change in marker expression relative to vehicle-treated control cells ± standard deviation (n = 3).

3. Results

Human monocyte-derived DCs and macrophages actively synthesise 1,25(OH)2D3 in vitro. To assess the magnitude of this with respect to autocrine responses to 25OHD3 in these cells we measured the accumulated levels of 1,25(OH)2D3 in day 5 cultures of DC or macrophages incubated for 48 h with varying levels of 25OHD3. Preliminary studies indicated that levels of 25OHD3 in cultures containing 5% AB serum were approximately 5 nM (data not shown). We therefore supplemented DC and macrophage cultures with vehicle, or 45–145 nM 25OHD3 to adjust final culture concentrations to 5, 50 and 150 nM.

These values were chosen to represent conditions of vita-
mim D deficiency, vitamin D insufficiency and vitamin D sufficiency, respectively. Results shown in Fig. 1 indicated that unsupplemented DCs and macrophages were exposed to between 20 and 40 nM 1,25(OH)2D3. At least some of this was due to cellular metabolism of 25OHD3 rather than endogenous hormone as co-culture with the 1α-OHase inhibitor ketoconazole suppressed 1,25(OH)2D3 levels. Supple-
mental 25OHD3 increased levels of 1,25(OH)2D3 in both DC and macrophage cultures in a dose-dependent fashion with the 150 nM 25OHD3 generating levels of 1,25(OH)2D3 between 2 and 3 nM. Once again this was suppressed by co-
culture with ketoconazole implicating DCs or macrophages as the primary source of 1,25(OH)2D3.

DCs and macrophages cultured in the presence of added 25OHD3 were then assayed to determine whether or not the 1,25(OH)2D3 generated in Fig. 1 was sufficient to elicit autocrine responses. Data in Fig. 2A showed that even at 50 nM the added 25OHD3 was able to significantly increase CD14 expression in macrophages and this effect was dimin-
ished in the presence of ketoconazole endorsing an autocrine mode of action. Cells were also treated with 1,25(OH)2D3 to compare responses between endogenously synthesized and exogenously added hormone. Data indicated that 150 nM 25OHD3 was as effective as 100 nM 1,25(OH)2D3 in stim-
ulating CD14 expression despite the fact that this mode of suplementation generated less than 5 nM 1,25(OH)2D3 in either DC or macrophage cultures (Fig. 1). A similar pat-
tern was also observed for the DC markers CD83, CD86 and
4. Discussion

Extra-renal synthesis of 1,25(OH)₂D₃ was first documented more than a quarter of a century ago following studies of vitamin D metabolism in human pregnancy [5] and the granulomatous disease sarcoidosis [6]. This raised three important questions: (i) Is extra-renal 1α-HOase a different enzyme to that found in the kidney? (ii) Is extra-renal 1α-HOase primarily a feature of human disease rather than normal physiology? (iii) Does extra-renal 1α-HOase manifest itself by changes in systemic 1,25(OH)₂D₃ as occurs in the kidney, or does this instead reflect the autocrine/paracrine actions of vitamin D? The first of these questions was resolved following the cloning of the human gene for 1α-HOase (CYP27b1) which revealed identical cDNA sequences in renal [7] and extra-renal tissues [8]. The key issue concerning the extent to which extra-renal expression of 1α-HOase is a component of normal vitamin D physiology has been harder to resolve but has recently attracted much attention with the recognition that vitamin D status may influence susceptibility to many common human diseases [9]. Specifically, it has been proposed that individual vitamin D status is most readily reflected by circulating levels of 25OHD₃ rather than 1,25(OH)₂D₃, and this in turn will have a greater impact on extra-renal vitamin D metabolism. Three other lines of evidence support the proposed role for extra-renal 1α-HOase in mediating the protective effects of 1,25(OH)₂D₃ on human disease: (i) the dysregulation of 1,25(OH)₂D₃ production with human disease in vivo; (ii) the tissue distribution of 1α-HOase; (iii) the autocrine actions of 1α-HOase in vitro. These issues will be highlighted in the remainder of the discussion.

4.1. Extra-renal 1α-hydroxylase and granulomatous disease

Following the initial reports of increased 1,25(OH)₂D₃ production in patients with sarcoidosis, similar evidence for dysregulated extra-renal 1α-HOase activity has been presented for more than twenty other granulomatous diseases [10]. In most cases the enhanced synthesis of 1,25(OH)₂D₃ is associated with varying degrees of hypercalcemia or hypercalciuria but may also have additional complications. For example, we recently described elevated serum 1,25(OH)₂D₃ levels in patients with Crohn’s disease and enhanced expression of 1α-HOase in affected tissue [11]. Here circulating levels of the hormone were not as great as observed in many sarcoidosis patients but were still significantly higher than...
control populations, and were associated with hypercalciuria rather than hypercalcemia. Importantly, analysis of bone mineral density showed that the bone loss which is a prevalent complication of Crohn’s disease was independently associated with serum 1,25(OH)2D3 levels [11]. Further analysis of vitamin D metabolism in inflammatory bowel disease may play a key role in clarifying the physiological relevance of extra-renal 1α-OHase. This is because although patients with Crohn’s disease synthesize more extra-renal 1,25(OH)2D3, it is clear that many are also vitamin D deficient [12]. Further studies are required to determine whether or not this paradox is a consequence of gastrointestinal abnormality or whether vitamin D deficiency contributes to the onset of disease. However, as extra-renal 1α-OHase contributes to some of the complications of Crohn’s disease, it is not unreasonable to hypothesize that it may also contribute to disease susceptibility via a similar mechanism. It also seems likely that this may also occur with related diseases such as tuberculosis, where vitamin D deficiency is associated with the onset of disease [13], but where hypercalcemia and elevated 1,25(OH)2D3 may be a feature of advanced disease [14].

4.2. Extra-renal 1α-hydroxylase and cancer

In addition to granulomatous diseases, we have also reported dysregulated extra-renal 1α-OHase expression and activity associated with B-cell lymphoma, dysgerminomas and breast cancer [15–17]. In lymphomas and dysgerminomas, the increased 1,25(OH)2D3 production was associated with hypercalcemia in a similar fashion to that seen in sarcoidosis patients [16,17]. Increased capacity for synthesis of 1,25(OH)2D3 was also observed in breast tumors, but was accompanied in this case by enhanced 24-OHase activity suggesting that in this type of neoplasm there is attenuation of 1α-OHase by 24-OHase cDNA clone. Further studies are required to determine whether or not this is a true reflection of 1α-OHase expression, particularly as the reporter LacZ gene used in the model is incorporated into the genome at the expense of the mouse CYP27b1 gene itself, and can only be detected in animals on vitamin D and calcium-depleted diets. The latter may be particularly important as in some tissues 1α-OHase expression appears to be more closely linked to expression of immune activators such as TLRs rather than calcitropic factors [26]. Consequently, in the absence of an appropriate TLR signal, expression of 1α-OHase may be limited.

4.4. Functional impact of extra-renal 1,25(OH)2D3 production

The presence of 1α-OHase in extra-renal tissues has been proposed as a mechanism by which vitamin D may exert a raft of protective actions within these tissues. We have previously referred to this as ‘barrier function’ to include possible antiproliferative, barrier integrity and immunomodulatory effects by both epithelial, endothelial and immune cells [27]. Within this broad grouping of cells and effects, the most robust functional response to extra-renal 1α-OHase activity is seen with macrophages and DCs which both show potent upregulation of 1α-OHase expression and activity as they differentiate towards a mature phenotype [4,28]. Thus, both DCs and macrophages are readily able to synthesize 1,25(OH)2D3, with potential autocrine and paracrine consequences. In DCs localized production of 1,25(OH)2D3 may affect proliferation and phenotype of adjacent T-cells but it is also able to exert autocrine effects by suppressing DC maturation and maintaining these cells in a more tolerogenic state [27]. Like DCs, macrophages are also able to present antigen and influence acquired immune responses but we have recently shown that the most likely function of 1α-hydroxylase in these cells is to stimulate innate immunity [29]. In this study expression of 1α-OHase by

4.3. Distribution of extra-renal 1α-hydroxylase in normal human tissue

Although the expression, activity and pathophysiological effects of extra-renal 1α-OHase have been well characterized for human diseases, the function of the enzyme in extra-renal tissues during normal physiology remains less clear. Previously reported in situ hybridization and immunohistochemical analyses by our group have suggest that 1α-OHase is more widely distributed than previously thought, with the enzyme detectable in skin, placenta, colon, pancreas, vasculature and parts of the brain [22–24]. Although many of these observations have been endorsed by other laboratories, recent studies using a CYP27b1 promoter-reporter mouse have sounded a note of caution [25]. In this model CYP27b1 promoter activity was only detectable in the renal cortex and placenta and was notably absent from keratinocytes, the source of mRNA for the original human 1α-OHase cDNA clone. Further studies are required to determine whether or not this is a true reflection of 1α-OHase expression, particularly as the reporter LacZ gene used in the model is incorporated into the genome at the expense of the mouse CYP27b1 gene itself, and can only be detected in animals on vitamin D and calcium-depleted diets. The latter may be particularly important as in some tissues 1α-OHase expression appears to be more closely linked to expression of immune activators such as TLRs rather than calcitropic factors [26]. Consequently, in the absence of an appropriate TLR signal, expression of 1α-OHase may be limited.
monocytes was induced as a result of ligand binding to TLR2 but the enzyme was only functional if sufficient substrate (25OHD3) was available for conversion to 1,25(OH)2D3. This was further emphasized by experiments which showed that macrophages cultured in serum from vitamin D-deficient African-Americans were less able to induce the antimicrobial peptide cathelicidin than cells grown with Caucasian serum, and that this could be corrected by supplementation of the African-American serum with 25OHD3.

There are many questions concerning extra-renal 1α-OHase that remain unanswered. These include the key issue of why extra-renal 1α-OHase becomes dysregulated in granulomatous diseases and some cancers. Is this due to initial vitamin D deficiency leading to abnormal TLR signaling and persistence of inflammation or tumor development? One recent study has shown that 1,25(OH)2D3 suppresses expression of TLR2 and TLR4, raising the exciting possibility that in addition to its antibacterial effects, 1,25(OH)2D3 plays a role in feedback regulation of TLR signaling [30].

Another important question concerns the apparent resistance to 1,25(OH)2D3 in tumors and affected inflammatory tissue. In the case of the latter this may be due to dysregulation of vitamin D signaling in the presence of specific inflammatory cytokines [31]. However, we have also recently shown that regulation of vitamin D responses via expression of the catabolic enzyme 24-OHase is different in macrophages, being associated primarily with a splice-variant form of the enzyme that appears to act as a non-functional decoy with the capacity to bind both 25OHD3 and 1,25(OH)2D3 [32]. Finally, a question that is fundamental to all tissues that express 1α-OHase is the extent to which this is reflected by actual synthesis of 1,25(OH)2D3 in vivo. In the kidney the acquisition of substrate 25OHD3 for conversion to 1,25(OH)2D3 is facilitated by a specific interaction between the vitamin D binding protein and the LDL-like receptor megalin [33]. In future studies it will be interesting to determine whether similar mechanisms are required at an in vivo level to achieve efficient extra-renal synthesis of 1,25(OH)2D3.

References


