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Inhibition of Vitamin D_3 metabolism enhances VDR signalling in androgen-independent prostate cancer cells

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Abstract

Induction of growth arrest and differentiation by 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) occurs in non-malignant cell types but is often reduced in cancer cells. For example, androgen-independent prostate cancer cells, DU-145 and PC-3, are relatively insensitive to the anti-proliferative action of 1,25-$(OH)_2D_3$. This appears to be due to increased 1,25-$(OH)_2D_3$-metabolism, as a result of CYP24 enzyme-induction, which in turn leads to decreased anti-proliferative efficacy. In the in vitro rat kidney mitochondria assay, the 2-(4-hydroxybenzyl)-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**4**) was found to be a potent inhibitor of Vitamin D_3 metabolising enzymes (IC_{50} 3.5 μM), and was shown to be a more potent inhibitor than the broad spectrum P450 inhibitor ketoconazole (IC_{50} 20 μM). The combination of the inhibitor and 1,25-$(OH)_2D_3$ caused a greater inhibition of proliferation in DU-145 cells than when treated with both agents alone. Examination of the regulation of VDR target gene mRNA in DU-145 cells revealed that co-treatment of 1,25-$(OH)_2D_3$ plus inhibitor of Vitamin D_3 metabolising enzymes co-ordinately upregulated CYP24, $p21^{waf1/cip1}$ and GADD45α.

Keywords: Vitamin D_3 metabolising enzymes; Tetralone; Prostate cancer cells

1. Introduction

The majority of the prostate cancer patients demonstrate good initial responses to surgical castration and/or hormonal therapy [1]. Unfortunately, hormonal therapy is not capable of producing durable responses in the majority of the patients who subsequently develop androgen-independent prostate cancer (AIPC). New effective therapies are needed in the management of AIPC patients. One potential therapeutic strategy is to employ a differentiating agent to restore the normal balance of proliferation and differentiation, such as with the biological active metabolite of Vitamin D_3, 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$). Encouragingly 1,25-$(OH)_2D_3$ exerts some pro-differentiating actions and inhibits proliferation of prostate cancer cells in vitro and in vivo [2–4].

In parallel to the well established endocrine synthesis of 1,25-$(OH)_2D_3$ via sequential hydroxylation steps in the liver and kidney, it has become apparent there is local autocrine/paracrine synthesis of 1,25-$(OH)_2D_3$. The principle enzymes in this process are the cytochrome P450 enzymes, CYP27B1 (1α-OHase) and CYP24 (24-OHase). It has been shown that CYP24 and CYP27B1 are also expressed in many target tissues, including prostate-epithelial cells [5,6], supporting the role for local 1,25-$(OH)_2D_3$ synthesis in the prostate. One well established VDR target gene is the CYP24, which is highly inducible by 1,25-$(OH)_2D_3$, resulting in an increase in the metabolism of 1,25-$(OH)_2D_3$. Prostate cancer cells expressed high level of 1,25-$(OH)_2D_3$-induced CYP24 activity, which is inversely proportional to growth inhibition [7]. It has been suggested that in cancer cells the rapid breakdown of 1,25-$(OH)_2D_3$ by over-active CYP24 might be the cause of resistance to 1,25-$(OH)_2D_3$. Reflecting this, P450 inhibitors which are able to inhibit the activities of CYP24, e.g. ketoconazole (**1**), genistein (**2**), and liarozole

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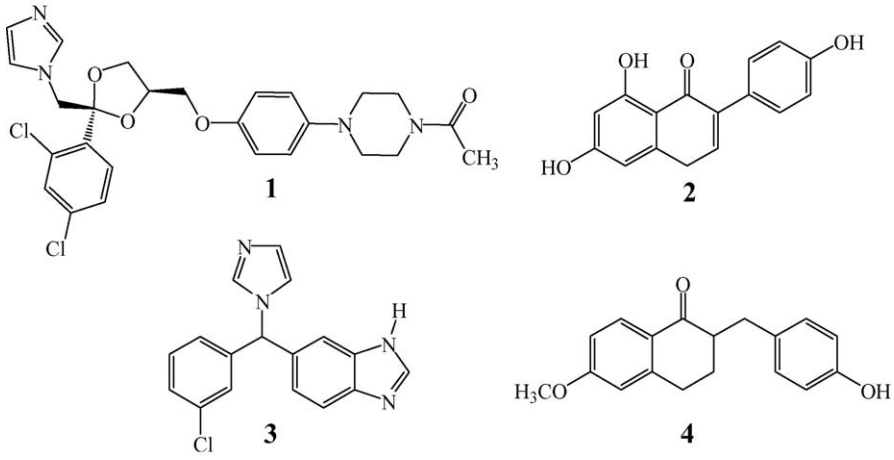

Fig. 1. Inhibitors of Vitamin D_3 metabolising enzymes–ketoconazole, **1**; genistein, **2**; liarozole, **3**; the tetralone derivative, 2-(4-hydroxybenzyl)-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one, **4**.

(**3**), result in increased 1,25-$(OH)_2D_3$ half-life and anti-proliferative effect in DU-145 cells [8–11]. We have demonstrated that 2-substituted-3,4-dihydro-2*H*-naphthalen-1-one (tetralone) derivatives inhibit the activities of Vitamin D_3 and/or retinoic acid metabolising enzymes [13,14]. Among the tetralone derivatives, 2-(4-hydroxybenzyl)-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**4**) (Fig. 1) showed inhibition against the Vitamin D_3 metabolising enzymes in the rat kidney mitochondria in vitro assay ($IC_{50} = 3.5\ \mu M$, compared to ketoconazole, $IC_{50} = 20\ \mu M$) [13].

In the current study, we have examined the anti-proliferative effects of the inhibitors, ketoconazole (**1**) and the tetralone derivative (**4**), both alone and in combination with 1,25-$(OH)_2D_3$ in DU-145 and PC-3 cells. We also examined the effect of the inhibitors alone and in combination with 1,25-$(OH)_2D_3$ on the regulation of VDR target genes, CYP24, $p21^{waf1/cip1}$ and GADD45α.

2. Materials and methods

2.1. Chemicals

1α,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) was a kind gift from Dr. Milan R. Uskokovic (Hoffman-La Roche, Nutley, NJ). 25-Hydroxyvitamin D_3 (25-$(OH)D_3$) (Fluka chemicals, Dorset, UK) and 1,25-$(OH)_2D_3$ compounds were stored at 1 mM in ethanol at −20 °C in the dark. 25-Hydroxy-[26,27-methyl-^{3}H]-vitamin D_3 (30 Ci/mmol) was purchased from Amersham Biosciences (Buckinghamshire, UK). 2-(4-Hydroxybenzyl)-6-methoxy-3,4-dihydro-2*H*-naphthalen-1-one (**4**) was chemically synthesised in the laboratory as described previously [13] and ketoconazole (Sigma, Poole, UK) stored as a 1 mM stock solution in phosphate buffered saline (PBS) pH 7.5 at 4 °C. (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (MTT) for cell-proliferation assay was purchased from Sigma (Poole, UK). All solvents used for HPLC were of HPLC grade and were purchased from Fisher Scientific (Leicestershire, UK).

2.2. Cell culture

The androgen-independent prostate cancer cell lines PC-3 and DU-145 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The cells were maintained in RPMI 1640 medium (Gibco-BRL, Paisley), supplemented with 10% fetal calf serum (Gibco-BRL), 100 units/mL penicillin and 100 μg/mL streptomycin. The cells were passaged by trypsinising with 0.25% trypsin–EDTA (Gibco-BRL). The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2 in air.

2.3. Preparation of rat kidney mitochondria

Mitochondria were isolated from male Wistar rats. Three Wistar rats (250 g each) were fed for 2 weeks with calcium and Vitamin D_3 replete diet (calcium carbonate was added to the feed to achieve a 1% calcium level and Vitamin D_3 was added to achieve 2200 i.u./kg in the feed). The isolated kidneys were washed with ice-cold phosphate buffer (50 mM, pH 7.4) containing 0.25 M sucrose, then resuspended in ice-cold Tris–acetate buffer (15 mM, pH 7.4) containing 0.25 M sucrose. The kidney was cut into smaller pieces using scissors and the tissues were homogenised using an Elvejhm-Potter homogeniser. The nuclei and unbroken cells were pelleted by centrifugation for 20 min at 600 × *g* at 4 °C. The above supernatant was then centrifuged for 20 min at 12 000 × *g* at 4 °C. The pellet containing the mitochondria was washed with the Tris–acetate buffer and resuspended in ice-cold 20% glycerol and 15 mM Tris–acetate pH 7.4, containing 0.6% sodium cholate. This 20% (w/w) homogenate was stirred on ice for 1 h and the homogenate was centrifuged at 12 000 × *g* for 1 h to disrupt the mitochondria pellet. The suspension was then distributed into 1.5 mL capped microcentrifuge tubes, frozen in liquid N_2 and stored at −80 °C until needed.