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Culture serum-induced conversion from agonist to antagonist of a Vitamin D analog, TEI-9647

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Abstract

The nuclear receptor for Vitamin D (VDR) mediates many of the effects of Vitamin D in target tissues by regulating gene expression. The transactivation function of ligand-bound VDR in target tissues is thought to depend on the tissue-type and the cellular-environment, but the molecular basis for these differences has not been fully understood. In this study, during characterization of TEI-9647 as a synthetic ligand for the VDR, we found that depletion of serum from the culture medium converted TEI-9647 from an antagonist to an agonist of VDR-mediated transactivation, whereas it retained antagonistic activity in the presence of serum. Consistent with these results, using a mammalian two-hybrid system, we found that TEI-9647 recruited different coactivators to the VDR in the presence and absence of serum. These findings suggest that an unknown serum factor modulates the transactivation function of the VDR.

Keywords: Vitamin D receptor; Tissue-specificity; Ligand; Cofactor; Structural alteration; Transcription

1. Introduction

Many of the actions of Vitamin D in calcium homeostasis, bone formation, and cellular differentiation and proliferation are thought to be mediated by its nuclear receptor, the Vitamin D receptor (VDR) [1]. The VDR belongs to the nuclear receptor gene superfamily, a large family that acts as ligand-inducible transcriptional factors [2,3]. The VDR binds to Vitamin D-responsive elements (VDREs) as heterodimer with the retinoid X receptor α (RXRα), another member of the nuclear receptor superfamily, modifying the transcription of VDR target genes in a Vitamin D-dependent manner. This transcriptional regulation requires the recruitment of a number of coactivator and corepressor complexes [4]. Such coregulatory complexes have been recently shown to contain enzymes that covalently modify histones, including their acetylation, methylation, and phosphorylation. Coactivation through histones acetylation by histone acetyltransferase-contained coactivator complexes and corepression through histones deacetylation by histone deacetylase-contained corepressor complexes in ligand-dependent transcriptional regulation of nuclear receptors are well demonstrated to reflect the state of chromatin for gene regulations.

The physiological and pharmacological actions of 1α,25-Vitamin D_3, the biologically active form of Vitamin D, and its synthetic analogs have indicated the application of VDR ligands in osteoporosis, dermatological indications, cancers, inflammation, and autoimmune diseases [5]. However, 1α,25-Vitamin D_3 causes major side effects from its hypercalcemic potency at the pharmacological doses for these applications. This is the reason why great efforts have been made to obtain a compound with dissociation between efficacy and calcemic effects in its design and synthesis. Although a number of Vitamin D analogs have been syn-

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thesized, most are agonists. Only two prototypical VDR antagonists, TEI-9647 and ZK159222, have been generated, and their functions and the mode of action as antagonists has been examined by several approaches [6–10]. There have also been recent advances in the development of selective receptor modulators (SRMs), which often act as both agonists and antagonists, depending on the promoter, cellular, and tissue context [11]. Tamoxifen, a representative selective estrogen receptor modulator, is designated and widely used for the prevention and treatment of breast cancer due to its antagonistic action, but it is also known to be beneficial for bone and lipid metabolism. Because tamoxifen alters the conformation of the ligand-binding domain of estrogen receptors, it alters their functional association with coregulators, thereby exhibiting tissue-specific actions [12,13].

Therefore, cell or tissue-specific modulation of transactivation is thought to contribute to the tissue-selective action of synthetic VDR ligands [14,15]. However, the molecular mechanism underlying such selective actions of the SRMs remains largely unknown. In the present study, we characterized TEI-9647 as a synthetic VDR ligand, and found that its function as an antagonist or agonist depends on the species of the VDR and on the concentration of serum in the culture medium. Thus, tissue-specific actions of TEI-9647 are modulated by an unknown serum factor.

2. Materials and methods

2.1. Compounds

1α,25(OH)$_2$D$_3$, TEI-9647, and ZK159222 were synthesized at the Teijin Institute for Bio-Medical Research. Each compound was dissolved in absolute ethanol.

2.2. Plasmids

Expression vectors for full-length rat VDR, rat RXRα and the reporter plasmid for human 24-hydroxylase gene, were as previously described [16]. Full-length human VDR and human RXRα cDNA were inserted into pTracer and pcDNA3 (Invitrogen), respectively.

2.3. Cell culture and transient transfection assay

HOS (human osteosarcoma) or 293T (human kidney) cells were maintained in DMEM supplemented with 5% FBS (GIBCO BRL) at 37 °C in 5% CO_2. For transfection, cells were plated in phenol-red-free DMEM supplemented with 5% charcoal-stripped FBS in 12-well plates 1 day before transfection. Cells at 40–50% confluence were transfected with the indicated plasmids (0.3 μg human 24OHase promoter-Luc, 0.1 μg VDR, and RXRα were transfected) using Lipofectamin Plus (GIBCO BRL). Total amounts of cDNA were adjusted by supplementing with empty vector up to 1.0 μg. After 3 h, cells were incubated in DMEM with or without 5% FBS, and 1α,25(OH)$_2$D$_3$ (1×10^{-8} M) and/or Vitamin D analogs were added to the culture medium. Then the cells were incubated continuously at 37 °C for 24 h. Luciferase activity was determined with the Luciferase Assay System (Promega). As a reference plasmid to normalize transfection efficiency, 2 ng pRL-CMV plasmid (Promega) was co-transfected in all experiments.

2.4. Mammalian two-hybrid assay

HOS cells were co-transfected with 17mer × 8-Luc reporter plasmid, pVP-human VDR(DEF) or -rat VDR-(DEF), and pM-SRC-1(RID), -TIF2(RID), -AIB-1(RID) or -TRAP220(RID) [4]. After 3 h, cells were incubated in DMEM with or without 5% FBS, and 1α,25(OH)$_2$D$_3$ (1×10^{-8} M) and/or Vitamin D analogs were added to the culture medium. Luciferase assays were performed as described above.

2.5. ABCD precipitation

Avidin resin (Promega) was incubated with biotin-conjugated consensus VDRE (DR3) oligonucleotides, followed by incubation with HOS cell lysates in lysis buffer (20 mM HEPES, 100 mM KCl, 0.5 mM EDTA, 0.1% Triton X-100 and 1 mM dithiothreitol) for 30 min [17]. The subsequent VDRE–protein complexes trapped on the resin were then eluted and western blotted [18].

3. Results

3.1. The VDR antagonist, TEI-9647, antagonizes Vitamin D-induced transactivation of the human VDR but not the rat VDR

Based on comparisons with ZK159222, which acts as a pure antagonist in several experimental conditions, TEI-9647 has been suggested to show tissue-dependent antagonism [19]. To investigate this in greater detail, we monitored the selective activity of both compounds against 1α,25-Vitamin D$_3$-induced transactivation using cells transfected with reporter plasmids encoding the human 24 (OH)ase promoter. Consistent with previous reports [10], TEI-9647 was as potent as ZK159222 at antagonizing human VDR-mediated Vitamin D action (Fig. 1, left panel). However, unlike ZK159222, TEI-9647 did not antagonize the Vitamin D-induced transactivation of rat VDR (Fig. 1). We suspect that this is due to slight differences between the rat and human VDR binding pockets, and TEI-9647 causes a structural alteration of VDR in a different way from 1α,25(OH)$_2$D$_3$ raising the possibility that TEI-9647 can act as a tissue-selective VDR ligand.