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Original Research

Vitamin D receptor knockout mice exhibit elongated intestinal microvilli and increased ezrin expression☆

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ABSTRACT

In addition to its principle function as a calcium regulator, vitamin D can affect cell and tissue morphology. The intestine is an important target tissue of vitamin D, as it must ensure the efficient transport of nutrients across the epithelium while excluding the passage of harmful molecules and bacteria into the organism. These functions require a highly organized morphology, which may be modified by vitamin D deficiency. To elucidate the role of vitamin D in gut morphology and barrier function, we compared the enterocyte microstructures, gut permeability, and cytoskeletal and cell junction protein expression in vitamin D receptor (VDR) knockout (KO) and wild-type (WT) mice. We found that the duodenal epithelial cells in the VDR-KO mice had longer microvilli (+19%) than those of the WT mice ($P < .05$). Interestingly, microvilli elongation in the VDR-KO mice was associated with higher messenger RNA and protein expression of ezrin, which is involved in the regulation of microvillus morphogenesis. Intestinal tight junction width and permeability were assessed by measuring the fluorescein isothiocyanate dextran concentrations in plasma; the concentrations were comparable between the 2 groups of mice. We further observed a decrease in the messenger RNA and protein expression of the calcium-transporting tight junction protein claudin-2 in the VDR-KO mice compared with the WT mice ($P < .05$). In conclusion, the mice lacking VDR had longer enterocyte microvilli, likely as a result of increased ezrin expression. However, the morphology of the tight junctions and the intestinal permeability for large molecules were not affected.

Abbreviations: β-Cat, β-catenin; Cld, claudin; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; E-Cad, E-cadherin; FITC-D, fluorescein isothiocyanate dextran; Gapdh, glyceraldehyde 3-phosphate dehydrogenase; Hprt, hypoxanthine-guanine phosphoribosyltransferase; JAM-A, junctional adhesion molecule A; KO, knockout; Ocln, occludin; Ppia, peptidylprolyl isomerase A; PTH, parathyroid hormone; RT-PCR, real-time reverse transcriptase polymerase chain reaction; SCB, sodium acetate buffer; TRPV6, transient receptor potential vanilloid-6; VDR, vitamin D receptor; VitD, vitamin D; WT, wild-type; ZO, zonula occludens protein.

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1. Introduction

Originally designated as a system to facilitate the detoxification of xenobiotics [1], the most prominent function of vitamin D (VitD) and the vitamin D receptor (VDR) in vertebrates is balancing calcium homeostasis [2]. Most of the actions of VitD are mediated by genomic effects via VDR activation. The VDR is a nuclear transcription factor, which, once activated by the biologically active form of vitamin D (1,25-dihydroxycholecalciferol [1,25$(OH)_2$D]), binds to the VitD responsive elements of numerous genes, thereby modifying their expression. Vitamin D receptor is expressed in almost all tissues of the body [3], suggesting that VitD exerts several functions beyond calcium regulation. Some studies have documented an impact of VitD and the VDR on cell size and cell shape. For example, adipocytes [4] and skeletal muscle cells [5,6] were smaller in animal models lacking VitD function. In addition, substantial changes in cellular morphology could also be observed in breast cancer, prostate cancer, and bronchial epithelial and dendritic cells treated with 1,25$(OH)_2$D or its corresponding analogs [7–10]. In general, cellular morphology is determined by the arrangement of intracellular cytoskeletal proteins, and VitD has been identified as a modulator of cytoskeletal regulation. For example, human breast cancer cells incubated with 1,25$(OH)_2$D exhibit rearrangements of cytoskeletal components, such as actin filaments and microtubules, thereby inducing the formation of cytoplasmic projections, filopodia, and lamellipodia [7]. Dendritic cells treated with the VitD analog TX527 started to dedifferentiate and lose their dendritic cell characteristics, including fewer and shorter dendrites and a disturbed F-actin and fascin network in the vicinity of the plasma membrane [10].

The intestine is an important target tissue for VitD, which is reflected by the high number of VDRs and the fundamental role of VitD in intestinal calcium absorption [11]. The main function of the intestine is to ensure the efficient transport of nutrients across the epithelium while excluding the passage of harmful molecules and bacteria into the organism. These functions require absorptive enterocytes with a large surface area for nutrient absorption and the presence of a gastrointestinal barrier that is primarily formed by tight junctions. The cells of the small intestine have a polar structure, with apically located microvilli to increase the absorbing surface and a basement membrane that establishes contact with the blood. In addition, a series of different tight junction proteins localized between 2 enterocytes inhibit paracellular transport of nutrients and potentially hazardous compounds, such as lipopolysaccharides from gram-negative bacteria. Numerous experimental and epidemiologic studies have found that a VitD deficiency is associated with inflammatory bowel diseases [12–14], and Fujita et al [15] were able to demonstrate that the VDR controls the cell-cell contact proteins claudin (Cld)-2 and Cld-12. Both proteins form cation-selective pores, rendering the epithelium more “leaky” [16]. Cellular membrane protrusions, which are necessary to increase the surface area of enterocytes, and intercellular tight junctions require cytoskeletal proteins and correct cytoskeletal assembly. Based on the observation that VitD can affect cell and tissue morphology, we hypothesized that VitD deficiency could affect intestinal morphology and permeability by modulating cytoskeletal and tight junction protein expression. We compared VDR-knockout (KO) mice, an animal model that emulates VitD deficiency [17], and wild-type (WT) mice to elucidate the role of VitD in gut morphology and barrier function. In the first study, the villi length, crypt depth of the duodenum and distal colon, enterocyte microstructures, and tight junction width were analyzed to elucidate the impact of VDR deficiency on gut morphology. In addition, the expression of cytoskeletal and tight junction proteins in the duodenal mucosa and the distal colon was measured to identify the possible mechanisms that may explain the specific differences in gut morphology of the VDR-KO and WT mice. To elucidate the impact of the VDR on the intestinal barrier, we conducted a second study in which VDR-KO and WT mice were orally treated with fluorescein isothiocyanate dextran (FITC-D) and its serum concentrations analyzed.

2. Methods and materials

2.1. *Animals and study design*

Two studies with VDR-KO mice and corresponding WT mice were conducted. In the first study, we compared the gut morphology and the expression of proteins involved in cell structure and cell-cell contact in VDR-KO mice with those in WT mice. In the second study, we assessed the intestinal barrier and permeability of the VDR-KO mice compared with the WT mice. All mice were housed individually on a 12-hour light, 12-hour dark cycle in a room controlled for temperature (22°C ± 2°C) and relative humidity (50%-60%). The experimental procedures described below followed the established guidelines for the care and handling of laboratory animals and were approved by the local government (Landesverwaltungsamt Sachsen-Anhalt, Germany; approval number of study 1: H1-4/T1-12; approval number of study 2: 42502-2-1276MLU G).

The homozygous VDR-KO mice (Boston strain; the mice were generated by a targeted mutation of the second zinc finger of the VDR DNA-binding domain [18]) and the corresponding homozygous WT mice were bred in our facilities by mating heterozygous B6.129S4-VDR^{tm1Mbd} >/J mice (Jackson Laboratories, Bar Harbor, ME, USA). To assess the genotypes of the offspring, tail biopsies were taken and processed with a KAPA mouse genotyping Kit (Peqlab, Erlangen, Germany) using the primer sequences displayed on the Jackson Laboratories Web site (www.jax.org). Sex- and age-matched homozygous littermates were used for both studies. At the age of 3 weeks, all mice were fed a commercial rescue diet containing 20 g/kg calcium, 12.5 g/kg phosphorus, 200 g/kg lactose, and 1100 IU/kg $VitD_3$ (S8852-S010; ssniff Spezialdiäten GmbH, Soest, Germany; Table 1).

In the first study, 8 male VDR-KO and 8 male WT mice received the rescue diet ad libitum for 17 weeks. At the age of 5 months, the mice were euthanized by decapitation under anesthesia with diethyl ether. Food was withdrawn 4 hours prior to sacrifice. The small and large intestine were completely excised, assessed for their lengths, and gently washed with an ice-cold NaCl solution (0.9%). For the light microscopy analyses, samples of the duodenum, jejunum, and ileum were immediately transferred into a 3% paraformaldehyde solution for fixation. A 0.3-cm segment of the duodenum was excised and fixed in 3% glutaraldehyde for electron microscopy analysis.