News and Views

Vitamin D signaling in a mouse allergic sensitization model

Renata Lucas[1], Johanna Mihály[2], Janine Gericke[2], Dániel Törőcsik[1], and Ralph Rühl[2,3]

[1] Department of Dermatology, University of Debrecen, Hungary

[2] Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary

[3] Paprika Bioanalytics BT, Debrecen, Hungary

Abstract: Vitamin D mediated signalling in the skin is discussed controversially for its beneficial or detrimental influence. In this study we examined various factors involved in Vitamin D-mediated signalling in a mouse model for allergic dermatitis with systemic (OVA IP) and systemic plus topical allergic sensitization (OVA IP + EC). We found that the major enzyme responsible for 1,25-Vitamin D3 synthesis, the 1-hydoxylase CYP27B1 (3,6-fold for OVA IP and 2,7-fold for OVA IP + EC), the vitamin D receptor (not altered) and the sensitive Vitamin D-mediated signalling target gene CYP24A1 (65-fold in OVA IP and 726-fold in OVA IP + EC) are upregulated after systemic and systemic plus topical allergic sensitization (OVA IP + EC). In consequence, active Vitamin D-mediated signalling is involved in systemic as well as systemic/topical allergic sensitization in mouse skin.

Keywords: Atopic dermatitis, skin inflammation, retinoid X receptor

Introduction

Vitamin D has been involved in maintenance of normal epidermal barrier, skin homeostasis and a disturbed vitamin D-mediated signaling is associated with inflammatory skin diseases such as atopic dermatitis (AD) [1]. Till now however the available studies are not coherent about the involvement of vitamin D-signaling pathways in allergic diseases [2]. In various studies, reduced serum levels of 25-hydroxy vitamin D3 (25VD3) were observed in patients with AD [3, 4], but the status of vitamin D-mediated signaling is not well studied in skin of AD patients.

The skin is a main target organ of vitamin D synthesis in which biologically active 1,25-dihydroxy-vitamin D3 (1,25VD3) is metabolized starting from 7-dihydrocholesterol, which after exposure to UV is phototransformed to VD3, a substrate for 25-hydoxylation (CYP27A1 and CYP2R1), 1-hydroxylation (CYP27B1) to the active vitamin D metabolite 1,25VD3 [5], which is the potent endogenous activator of the vitamin D receptor (VDR). This active ligand can further be metabolized and thereby inactivated by the VDR-target gene, the 24-hydroxylase (CYP24A1) [5]. A better understanding of the status of cutaneous vitamin D-mediated pathway in AD-skin is crucial for targeted therapy using systemic or topical treatment strategies with vitamin D analogues.

Topical and systemic ovalbumin (OVA)-sensitized mice are widely used for analysis of pathogenesis and background of allergic diseases. In our experiments, we sensitized mice systemic or systemic plus topical using OVA (Figure 1A). Later we examined via qRT-PCR analysis the expression levels of the nuclear hormone receptors RXR, VDR and various previously mentioned vitamin D synthesizing enzymes in skin samples after allergic sensitization.

Materials and methods

Animal experiments; 8–10 weeks old female BALB/c mice (n = 6, per group) were treated intraperitoneally (i.p.) with phosphate-buffered saline (PBS, Figure 1A a) or with 10 µg OVA (Figure 1A b) adsorbed to 1.5 mg $Al(OH)_3$ on days 47, 60, and 67 [6]. A third group of mice (female BALB/c mice (n = 6, per group) were treated intraperitoneally (i.p.) with 10 µg OVA adsorbed to 1.5 mg $Al(OH)_3$ on days 1, 14, and 21 of experiment, following epicutaneous (e.p./topical) application of 100 µg OVA absorbed to 1.5 mg $Al(OH)_3$ in 100 µl PBS (weekly dose) [6]. Animal experiments were approved by the Committee of Animal research of the University of Debrecen, Hungary (Approval number: 25/2006 DEMAB). Shaved dorsal skin was treated with 25 µl OVA solution on every other day of a one-week period. There were three periods of topical OVA treatment with two-week intervals. Mice were always shaved the day before the first and the third topical application of OVA.

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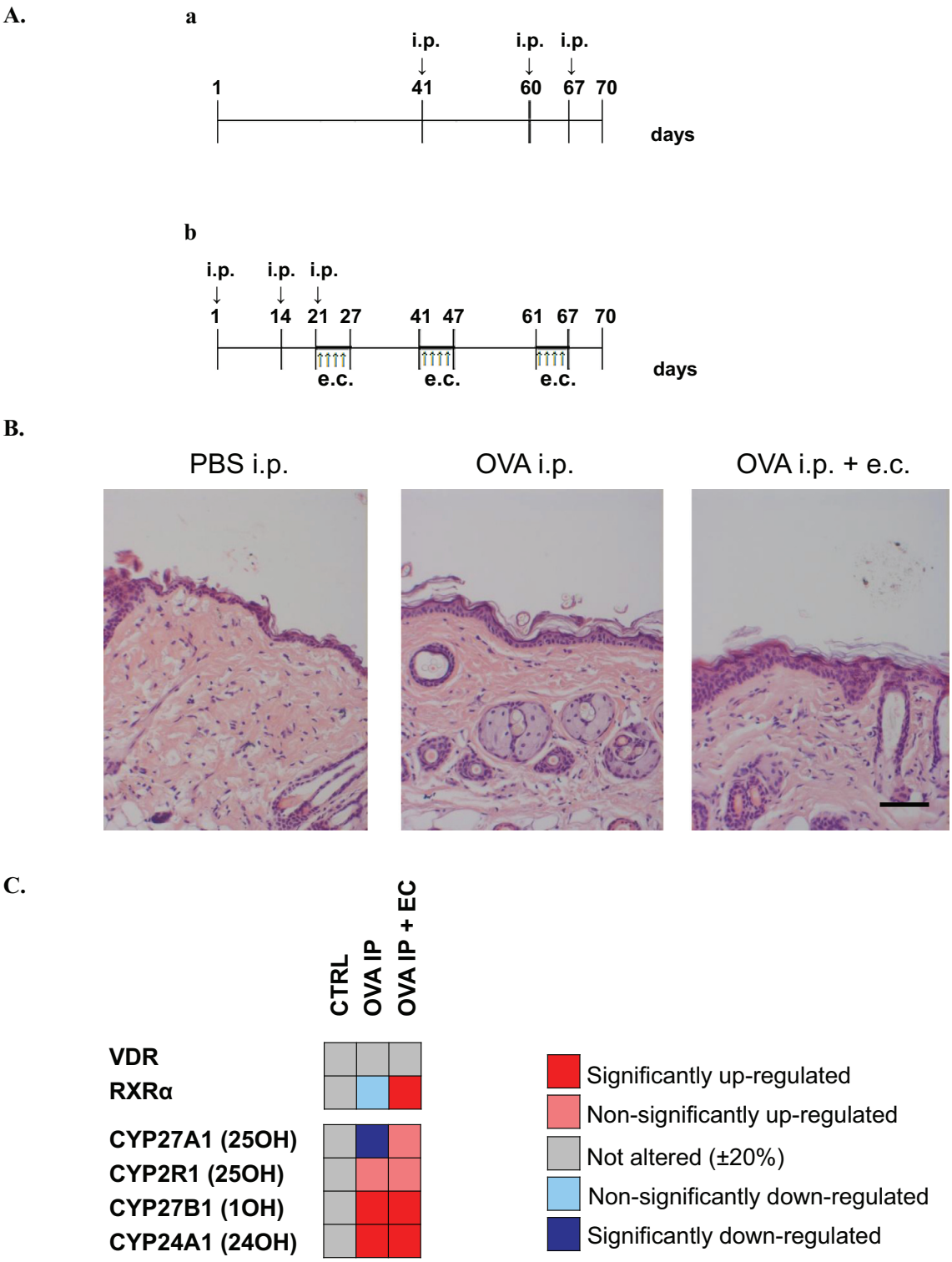

Figure 1. A. Schematic model of the allergic sensitization of 8–10 weeks old female BALB/c mice (n = 6, per group) by repetitive systemic and topical application of OVA. B. Hematoxylin and Eosin staining of five μm skin sections obtained from treated dorsal skin sites. Images were taken at ×10 magnification (scale bar = 50 μm). Figures are reproduced from [6] and the materials and methods used are described in this previous study. C. Induced mRNA expression of genes involved in vitamin D metabolism in i.p. OVA-treated mice with or without e.c. OVA exposure. Heat map shows fold change of gene expression in mice skin treated systemically with OVA followed by or not topical OVA exposure compared to PBS control group. Color codes: dark red – significantly up-regulated; light red – non-significantly up-regulated; grey – not altered (±20%); light blue – non-significantly down-regulated; dark blue – significantly down-regulated.

Three days after the last treatment (day 70) mice were sacrificed; skin and serum samples were collected and kept at –80 °C until analyses. This mouse study was already examined for retinoid signaling [6] and topical TSLP regulation [7].

PCR analysis; was performed like previously described [6] including statistical evaluation using ANOVA and differences were considered significant at $p < 0.05$.

RNA preparation and reverse transcription; Total RNA was isolated from frozen skin using Tri® reagent (Molecular Research Center Inc., Cincinnati, OH) following the manufacturer's instructions. 750 ng of total RNA were reverse transcribed into cDNA in a 30 µl reaction using the High Capacity cDNA Reverse Transcription Kit (Life Technologies, Budapest, H) according to the manufacturer's protocol.

Analysis of mRNA expression; mRNA expression in skin was determined by means of quantitative real time-PCR (qRT-PCR) and TaqMan® Low Density Arrays (TLDA) on an ABI Prism 7900. qRT-PCR measurements were performed in triplicate using pre-designed TaqMan® Gene Expression Assays and reagents; TaqMan® Low Density Array cards were used for duplicate determinations using TaqMan® Gene Expression Master Mix (all Applied Biosystems Applera Hungary, Budapest, H). Relative quantification of mRNA expression was achieved using the comparative CT method and values were normalized to cyclophilin A mRNA. Gene expression values below detection limit were assumed to be zero for the purpose of statistical analysis.

Results

Histological examination was reproduced from a previous study [6]. Sensitization with OVA induced mild focal hyperplasia with a ca. two-fold (OVA i.p. vs. PBS i.p.) or ca. three-fold (OVA i.p. + e.c. vs. PBS i.p. vs. OVA i.p.) increase in epidermal thickness, respectively (Figure 1B). The histological analysis further revealed scaly skin comparable to human atopic dermatitis phenotype in both OVA-sensitized groups (Figure 1B).

The expressions of RXRα, VDR-synthesizing enzymes and a VDR-target gene were mainly increased in systemic and systemic and topical OVA-sensitized mice compared to control group shown as a heat map in Figure 1C and as supplemental table 1. The expression of RXRα and the mitochondrial 25-hydroxylase CYP27A1 were increased only in systemic plus topical OVA-sensitized animals (significantly 1.6-fold for RXR and with tendency 1.4-fold for CYP27A1), while its expression was slightly reduced by systemic application of OVA (with tendency 0.7-fold for RXR and significantly 0.5-fold for CYP27A1, Figure 1B). The expression of the microsomal 25-hydroxylase CYP2R1 and the 1-hydroxylase CYP27B1 were increased both in systemic (with tendency 3.3-fold for CYP2R1 and significantly 2.6-fold for CYP27B1) and systemic plus topical sensitized mice (with tendency 1.5-fold for CYP2R1 and significantly 2.7-fold for CYP27B1) (Figure 1C and supplemental table 1). These results indicate upregulation of 1,25VD3-synthesis in the skin of systemic as well as systemic plus topical sensitized mice. In addition, the expression of the VDR-target gene 24-hydroxylase [5] was also upregulated both in the skin of systemic (significantly 65-fold) and systemic plus topical allergic sensitized animals (significantly 726-fold).

Discussion

In summary, increased expression of RXRα, the key 1,25VD3-synthesizing enzyme (CYP27B1) and the most sensitive vitamin D-target gene CYP24A1 display increased VDR-mediated signaling in the skin of systemic as well as systemic plus topical sensitized mice. In controversy, CYP24A1 also indicates increased catabolism of the active endogenous ligand 1,25VD3 to auto-downregulate VDR-mediated signalling via reduction of available ligand concentrations [5]. This detected increased vitamin D-mediated signaling may favor pro-allergic conditions present in skin of sensitized mice [1]. These results confirm also our and others data where increased vitamin D-mediated signaling is present in affected and non-affected skin of AD-patients (Weise et al. personal communication/submitted). We believe, that in opposite to the major current believe of reduced vitamin D levels in serum because of reduced availability of vitamin D in AD-patients [3, 4], instead increased local vitamin D-mediated signaling at the site of inflammation is present and reduced vitamin D levels are thereby present in AD-patients as a consequence of the systemic and/or topical allergic sensitization, comparable to the situation of vitamin A [8].

We suggest that strategies using systemic vitamin D supplementation to AD-patients with a systemic and/or topical allergic sensitization, like suggested by others [9], are a potential dangerous strategy circumventing important defense strategies of the human organism to prevent increased pro-inflammatory and pro-allergic vitamin D-mediated signaling in AD-patients. We propose that instead topical treatment strategies antagonizing VDR-mediated signaling might be a useful strategy for AD therapy with a topical allergic sensitization background.

Electronic supplementary material

The electronic supplementary material is available with the online version of the article at https://doi.org/10.1024/0300-9831/a000633

ESM 1. Systemic and topical OVA sensitization results in altered VDR-mediated signalling indicated by the Vitamin D receptor (VDR), the retinoid X receptor alpha (RXRα), the vitamin D metabolizing enzymes; CYP27A1, CYP2R1, CYP27B1 and CYP24A1. The CYP24A1 is also the most sensitive marker gene for VDR-mediated signalling. Fold change data are expressed as mean ± SEM (n = 6) and were determined in skin specimen of sensitized mice. Statistical significance (p) was tested using one-way ANOVA followed by Tukey's multiple comparison test.* – indicates statistical significance with $p < 0.05$. e.c., epicutaneous; i.p., intraperitoneal; OVA, ovalbumin (Table E1).

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Conflict of interest
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ORCID
Ralph Rühl
https://orcid.org/0000-0002-8410-6659

Dr. Ralph Rühl
Paprika Bioanalytics BT
Mezögazdász utca 62
4002 Debrecen
Hungary

ralphruehl@web.de

