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Conversion of vitamin D_3 to hormonally active 1α,25-dihydroxyvitamin D_3 in cultured keratinocytes: Relevance to cell growth and differentiation☆

Bodo Lehmann*, Katrin Schättiger, Michael Meurer

Department of Dermatology, Carl Gustav Carus Medical School, Dresden University of Technology, D-01307 Dresden, Fetscherstrasse 74, Germany

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ABSTRACT

The regulatory potential of intracellularly generated calcitriol on growth and differentiation of cultured keratinocytes is determined by the degree of cell confluence and availability of the highly lipophilic substrate vitamin D_3 to these cells. The enzymatic conversion of vitamin D_3 to calcitriol is considerably elevated in the presence of the nontoxic surfactant copolymer pluronic® F127 (120 μg/ml medium) compared to the control without this agent. We found a positive correlation between the formation rate of calcitriol and inhibition of the ^{3}H-thymidine incorporation rate into the DNA of keratinocytes. Intracellularly generated calcitriol causes a clear increase of the cell diameter, and thus has a prodifferentiating effect on keratinocytes *in vitro*. These findings corroborate the hypothesis that UVB-induced production of vitamin D_3 in human skin results in formation of substantial amounts of calcitriol in keratinocytes which suppress the growth and initiate differentiation of these cells.

1. Introduction

The skin is the only tissue yet known in which the complete UVB-induced pathway from 7-DHC to hormonally active calcitriol occurs under physiological conditions [1–3]. From the therapeutic point of view both calcitriol [4–6] and UVB radiation [7,8] exert potent antipsoriatic effects. One should expect that the therapeutic effect of UVB radiation is causally related to UVB-triggered cutaneous synthesis of calcitriol. We have investigated in this study whether metabolisation of biologically inactive vitamin D_3 to hormonally active calcitriol in keratinocytes is associated with suppressed cell growth and increased differentiation of these cells.

2. Materials and methods

2.1. Cell culture and incubation conditions

Human keratinocytes from neonatal foreskin (tebu-bio GmbH, Offenbach, Germany)) were grown to different degrees of confluence (40–80%) in a 24-well plate, preincubated with several concentrations of vitamin D3 (0.25, 0.50, 1.00, and 2.00 μM) for 24 h or 48 h. The nontoxic surfactant copolymer pluronic® F127 (Sigma, Germany) (120 μg/ml medium) was used to improve the solubility of the highly lipophilic vitamin D3 in aqueous culture medium. The production of calcitriol was determined after liquid/liquid extraction with methanol/chloroform by a ^{125}I-RIA method (IDS, Frankfurt a. M., Germany). ^{3}H-thymidine incorporation into the DNA of keratinocytes was measured to evaluate the growth-inhibitory effect of calcitriol. The CASY cell counter TT (Schärfe System, Reutlingen, Germany) was used for cell counting and determination of cell size/cell volume, aggregation and viability.

2.2. Statistical analysis

Results are presented as mean or mean ± SD.

3. Results and discussion

Our results demonstrate that pluronic® F127 (120 μg/ml medium) markedly increases the conversion rate of vitamin D3 to calcitriol compared to controls without this additive (Fig. 1). Consequently, the ^{3}H-thymidine incorporation into keratinocytes DNA was stronger inhibited when the conversion of vitamin D3 to calcitriol was facilitated by pluronic® F127 (Fig. 2).

The differentiation of cultured keratinocytes is associated with an increased cell diameter. Both calcitriol generated by intracellular hydroxylation of vitamin D3 (Fig. 3) and synthetic calcitriol (Fig. 4) contribute to magnification of the cell diameter provided the degree cell confluence is sufficiently high. Subconfluent keratinocytes show only little tendency for differentiation.

We conclude from these experiments that conversion of vitamin D3 to calcitriol in cultured keratinocytes is considerably elevated

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* Corresponding author. Tel.: +49 3514582692; fax: +49 3514584338.
E-mail address: Bodo.Lehmann@mailbox.tu-dresden.de (B. Lehmann).

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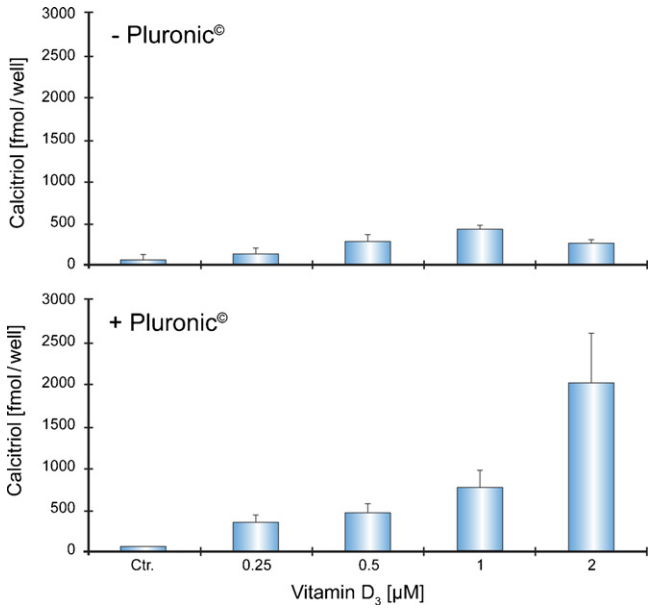

Fig. 1. Influence of pluronic® F127 (120 µg/ml) on the conversion rate of vitamin D3 (0.25–2.00 µM) to calcitriol (mean ± SD, $n = 3$).

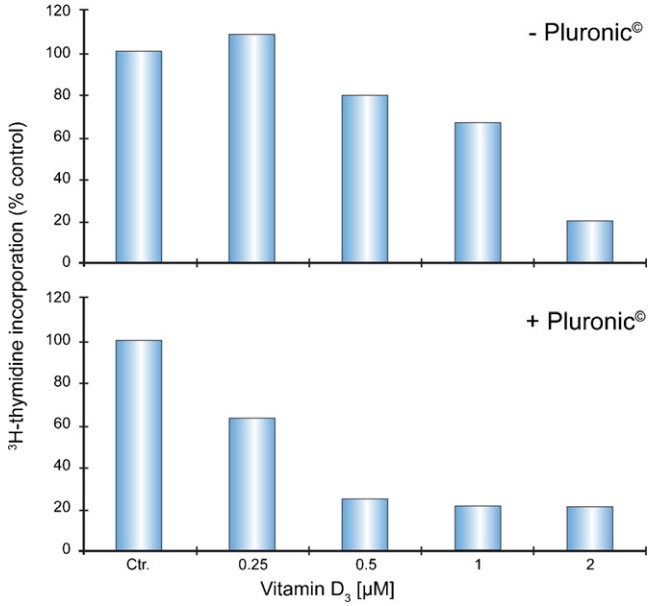

Fig. 2. Dependence of the ^{3}H-thymidine incorporation into the DNA of keratinocytes on the conversion rate of vitamin D3 to calcitriol in presence and absence of pluronic®F127 (mean, $n = 2$).

in the presence of pluronic® F127 compared to the control without this substance. There is an inverse correlation between the formation rate of calcitriol and the 3H-thymidine incorporation into the DNA of keratinocytes. Differentiation of cultured keratinocytes is associated an increase of the cell diameter, and intracellularly generated calcitriol causes a significant increase of the cell diameter, and thus has a prodifferentiating effect on keratinocytes *in vitro*. It can be assumed that UVB-induced production of vitamin D3 in human skin might result in formation of substantial amounts of calcitriol which regulate keratinocytes growth and differentiation.

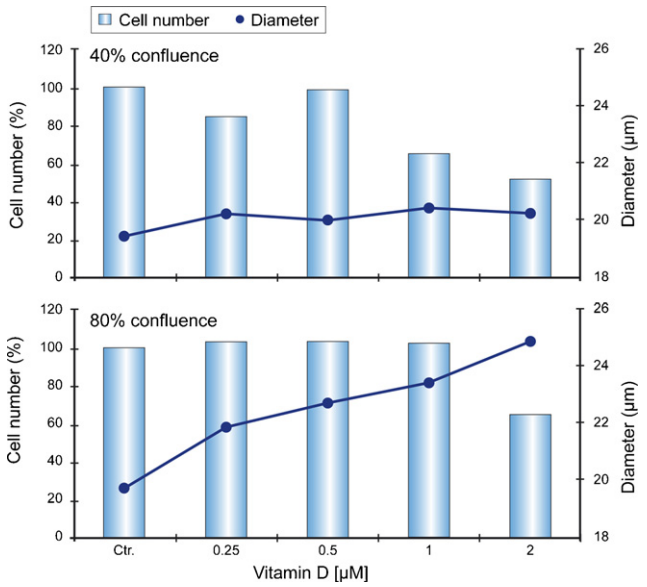

Fig. 3. Influence of the degree of confluence on calcitriol-mediated suppression of the cell number and differentiation-related increase of the cell diameter (mean, $n = 3$).

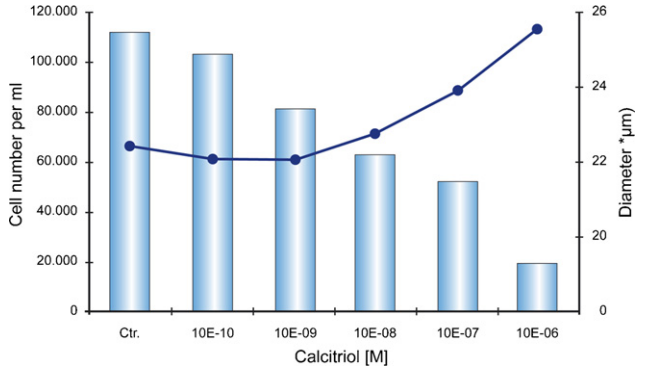

Fig. 4. Inhibitory effect of synthetic calcitriol on growth and enhancement of the cell diameter of keratinocytes (mean, $n = 2$).

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