Effects of phylloquinone and magnesium on ATDC5 prechondrocytes

A. Raimann1, A. Javanmardi1, S. Sagmeister S, D.A. Ertl1, C. Hochsmann1, M. Egerbacher2, G. Haeseler1

1Univ. Clinic of Pediatrics and Adolescent Medicine, Department of Endocrinology, Vienna, Austria
2Institute of Anatomy, Histology and Embryology, University of Veterinary Medicine Vienna, Vienna, Austria.

Introduction

The balance between mineral deposition and resorption is essential for function of a multitude of tissues. Pathologic conditions such as osteoporosis or arteriosclerosis feature an imbalance of mineralization leading to pathologic conditions.

The regulated acquisition of mineralized matrix is essential for both maintenance and formation of bone. Cell-mediated initiation of enchondral ossification represent a key role for growth plate maturation. Thus, the deposition of mineral components within the cartilaginous matrix of the hypertrophic zone of the growth plate is an important regulatory step in the process of ossification. To control the movement of mineral components such as calcium, phosphate and magnesium to the extracellular matrix, regulatory proteins are secreted to the extracellular space by growth plate chondrocytes. Expression control of these genes by minerals themselves such as Magnesium show the complexity of mineralization physiology.

Two main regulators of mineralization, matrix gla protein (MGP) and osteocalcin (OC), are dependent on gamma-carboxylation by members of the Vitamin K family, such as phylloquinone (K1) and menaquinone (K2). Both MGP and OC contain glutamic acid groups which are modified by K-dependent γ-carboxylase to promote binding of Ca and P ions. The impact of the Vitamin K family on bone strength has been demonstrated by BMD increases under Vitamin K supplementation (Knappen 2007). In contrast, Vitamin K antagonists such as Warfarin was associated with lower bone mass in children (Barnes 2005). Knowledge of the effects of Vitamin K administration and depletion on enchondral ossification and chondrocyte maturation remains limited so far (Tab 1)

Study aims

This study aims to characterize Vitamin K2 dependent effects on growth plate chondrocyte differentiation and proliferation. Both K1 administration as well as combined treatments with the Vitamin K antagonist Warfarin are performed. Secondary aim of the study is to identify potentially clinically relevant modifiers of K1 dependent effects on chondrocytes. Both cell line and primary cell experiments are planned to be performed (Fig 1)

Fig 1: Study plan

Materials and Methods

Chondrogenic ATDC5 cell in alginate bead culture in DMEM + 10%FBS are treated with 1, 10 or 100μM K1 with or without 2.5μM MgCl₂ for 14d. Harvests are performed at day 1, 7 and 14 of treatments to cover time points until maximum chondrogenic differentiation.

To exclude alginate specific effects, monolayer experiments are run for comparison.Chondrocyte differentiation marker expression is investigated by RT-PCR. BrdU and E24U assays are used for cell proliferation and metabolic activity determination.

Preliminary results

Fig 2: ATDC5 3D culture

ATDC5 cells seeded in gelation (GL) coated compared to monolayer (ML) cells in similar media. A) ATDC5 GL:Pre GL:pre mRNA expression of COL1a1, COL2a1 and COL Xa1 mRNA expression of GL cells. B) ALP activity, C) Alizarin red staining, D) TGFβ expression, E) collagen type 1 mRNA expression, F) collagen type 10 mRNA expression.

Fig 2: mRNA expression of ATDC5 chondrocytes

Collagen type 1, 2 and 10 mRNA expression after 1-3 and 14 days of culture. Expression data are shown related to PPA expression by ddCT calculation. (N=3, error bars bSEM (NTC control), K1 = phylloquinone, 1: 100 = 1μM = 10μM, M = 2.5μM MgCl₂).

Results RT-PCR

To study the effects of phylloquinone (K1) on ATDC5 differentiation mRNA expression analyses of collagen expression. ATDC5 cells under K1 treatments exhibited a reduction in collagen mRNA levels. Low dosage K1 treatment reduced Col1 and Col2 expression after 7 days of culture but increased to similar levels at day 14 in comparison with controls. High K1 dosage treatments with 100μM K1 inhibited the raise of Col1 and Col2 expression (Fig 3a, c). Gain of Col3 levels, as seen until terminal chondrocyte differentiation, were halted by 10-100μM K1 (Fig 3e). Co-treatment with 2.5μM Magnesium partly reversed the observed effects on K1 chondrocytes. Col1 and Col2 expression patterns during differentiation revealed more similar patterns in cultures with combined K1 and Magnesium treatments in comparison to K1 alone (Fig 3b, d). CoL1 mRNA levels of co-treated chondrocytes displayed a partial rescue of the diminished expression with high dosage K1 alone. (Fig 3f)

Fig 3: Pathologic literature

The authors declare no actual or potential conflict of interest related to this poster.

References

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