Effects of phylloquinone and magnesium on ATDC5 prechondrocytes

A. Raimann[1], A. Javanmardi[1], S. Sagmeister S, D.A. Ertl [1], C. Hochsmann[2], M. Egerbacher[2], G.Haeusler[1]

[1]*Univ. Clinic of Paediatrics and Adolescent Medicine, Department of Endocrinology, Vienna, Austria*

[2]*Institute 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 cartilagous 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. Expressional control of these genes by minerals themselves such as Magnesium show the complexity of mineralisation 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 phylloquinon (K1) and menaquinone (K2).

Both MGP and OC contain glutamyl groups which are modified by K-depended y-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 (Knapen 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 K1 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 clinical relevant modificators of K1 dependent effects on chondrocytes. Both cell line and primary cell experiments are planned to be performed (Fig 1)

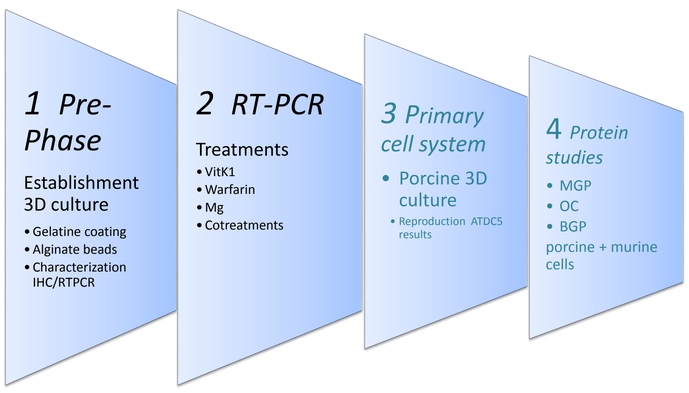

Fig 1: Study plan

Materials and Methods

Chondrogenic ATDC5 cell in alginate bead culture in DMEM + 10%FBS are treated with 1, 10 or 100uM K1 with or without 2.5mM MgCl 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 EZ4U assays are used for cell proliferation and metabolic activity determination.

Preliminary results

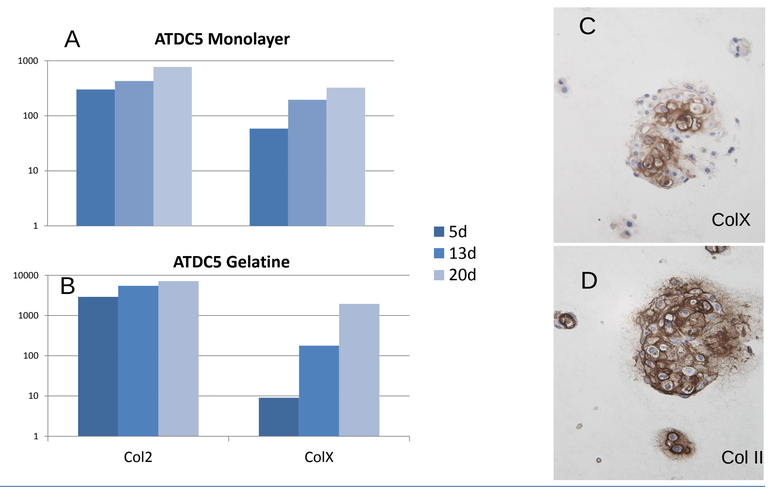

Fig 2: ATDC5 3D culture

ATDC5 cells seeded in gelatine (GL) coated compared to monolayer (ML) cells in similar media. A) Col2A1 and Col10A1 mRNA expression of ML cells. B) A) Col2A1 and Col10A1 mRNA expression of GL cells C) Alginate bead culture: IHC for Collagen 2 D) IHC Collagen 10

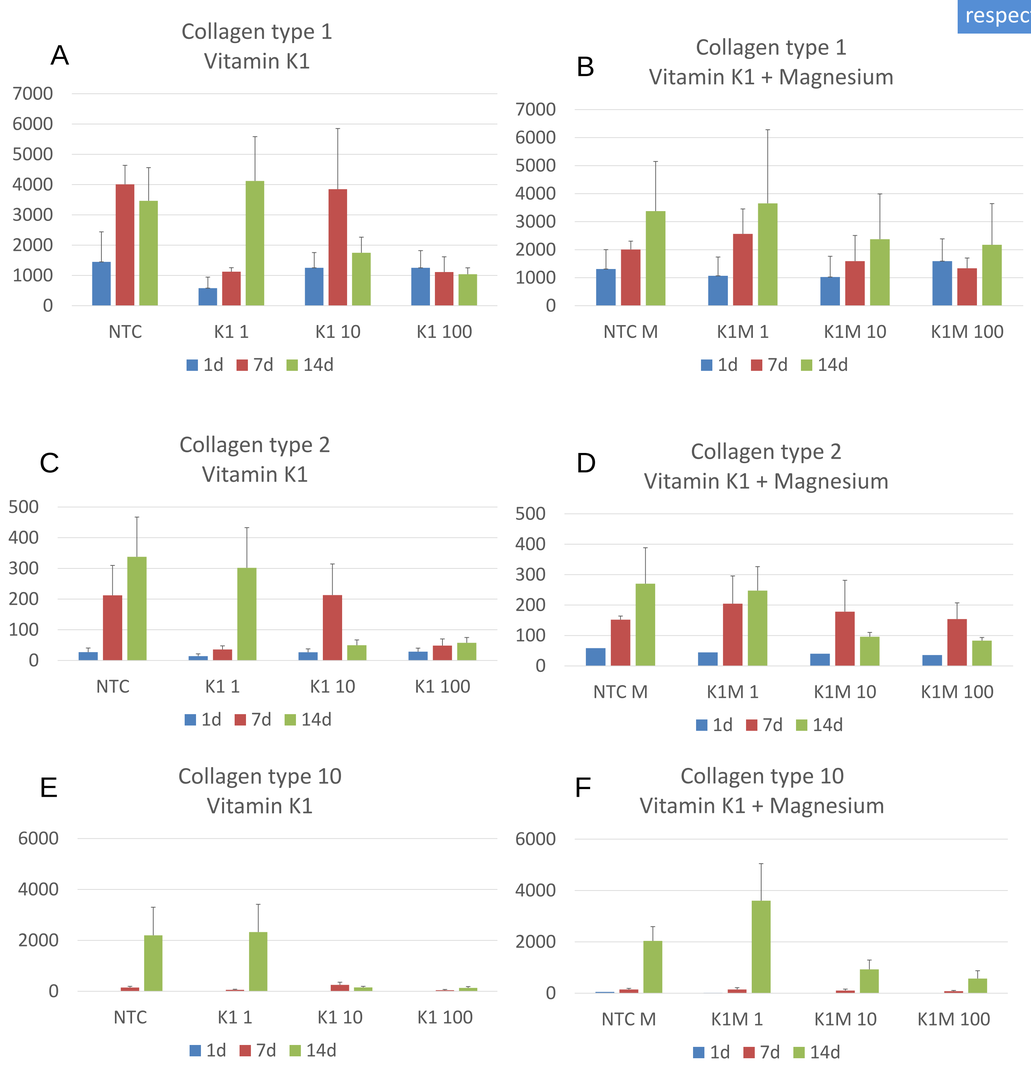

Fig 2: mRNA expression of ATDC5 chondrocytes

Collagen type 1, 2 and 10 mRNA expression after 1, 7 and 14 days of culture. Expression data are shown related to PPIA expression by ddCT calculation. N=3, error bars +SEM (NTC= control, K1 = phylloquinone, 1 - 100 = 1uM – 100uM, M = 2,5mM MgCl).

Results RT-PCR

To study the effects of phylloquinone (K1) on ATCD5 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 dosage K1 treatments with 100uM inhibited the raise of Col1 and Col2 expression (Fig 3a, c).

Gain of ColX levels, as seen until terminal chondrocyte differentiation, were halted by 10-100uM K1 (Fig 3e). Co-treatment with 2.5mM Magnesium partly reversed the observed effects of K1 on 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). ColX mRNA levels of co-treated chondrocytes displayed a partial rescue of the diminished expression with high dosage K1 alone. (Fig 3f)

Author, Date	Model system	Treatment	
Yagami et al 1999	Immature + hypertrophic chick embryo chondrocytes	10uM Vit K1	-
		10mM Warfarin	Excessive matrix mineralization
Barone et al 1994	fetal rats epiphyseal chondrocytes	1-5pg Warfarin	Proliferation↑ Col1A1 ↑No effect on MGP
	fetal rat calvarial osteoblasts	1-5pg Warfarin	Osteocalcin, Col1, ALP ↓ MGP, OPN ↑

Tab 1: Literature on K1/ warfarin on chondrocytes

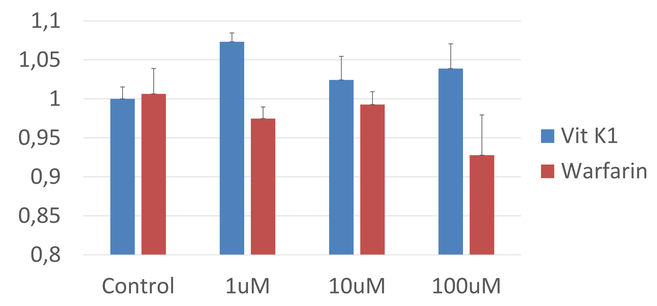

Fig 4: Proliferation rates

BrdU incorporation rates after treatment with K1/warfarin, respectively. All values related to control, error bars + SEM

Results BrdU

Proliferation rates and metabolic activity of ATDC5 chondrocytes has been investigated by BrdU incorporation and by TTC reduction assays respectively. Cell proliferation showed a trend towards increased rates in K1 treated cells , while BrdU incorporation was slightly reduced in comparsion to controls (Fig 4). Magnesium co-treatments led to comparable results (data not shown). Metabolic activity remained unaltered under both treatments (data not shown).

Discussion

Regulation of mineral deposition plays a key role in enchondral ossification. Our preliminary data show effects of K1, an activator of mineralization inhibitors, on differentiating chondrocytes. While most data in literature focused on the K1 antagonist Warfarin, our data supports a K1-induced downregulation of collagen synthesis and inhibition of chondrocyte differentiation.

Interestingly, Magnesium represent both component as well as regulator of mineralization inhibitor expression (Nakati 2006). In our hands, Magnesium partly reversed the inhibiting effects of K1 on collagen synthesis. Both the effects of K1 and Magnesium on chondrocyte differentiation might put these two factors in a more central light regarding disorders of mineralization and pharmacologic interventions on Vitamin K action.

Conclusions:

We found K1 and Magnesium, both modifiers of MGP and OC activity, to affect chondrocyte differentiation inversely.

While K1 downregulates collagen expression in ATDC5 prechondrocytes, Magnesium reverses these effects. Our data point to a possible counter regulation of matrix mineralization and growth plate maturation by Magnesium and K1.

References

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Adalbert Raimann

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