Preliminary Report

The Role of Vitamin K2 on Osteoblastic Functions by Using Stem Cell Model

Narong Bunyaratavej MD*,
Monipa Sila-asna PhD**, Ahnond Bunyaratavej BSc, PhD***

** Department of Orthopedic Surgery, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand*
*** Cell Engineering and Tissue Growth Laboratory, Institute of Science and Technology for Research and Development, Mahidol University, Phuttamonthon Salaya, Nakhonpathom, Thailand*
**** National Research Council of Thailand (NRCT), Bangkok, Thailand*

Vitamin K2 (MK4) functions were investigated by using the induced skin cell into osteoblast compared with the control media. The real time PCR measured gene expression in both cultures at the fourth, seventh, fourteenth, twenty first, twenty eighth, thirty fifth and forty second days of culture. The gene expressions of osteocalcin, osteonectin, osteopontin , bone sialoprotein, CBFA1, Interleukin-6, Estrogen receptors and collagen type1 were monitored by real time PCR. MK4 had strong power to stimulate gene expression of osteocalcin and osteonectin after one week of culture but MK4 showed weak action on gene of osteopontin, bone sialoprotein and interleukin-6. The gene of estrogens showed the marked expression of estrogen receptor beta at the fourteenth day of culture while estrogen receptor alpha did not respond.MK4 could stimulate genes of RANKL and collagen type 1. This study supported the action of vitamin K2 for enhancing the bone matrix.

Keywords: *Gene expression, Osteocalcin, Osteonectin, Collagen type1, Stem cell, Vitamin K2, Osteoblast*

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Vitamin K2 is recognized as bone forming agent especially bone matrix formation. The synthetic form of vitamin K2 is menatretenone-4 (MK4).MK4 enhances immature osteocalcin (undercarboxylation osteocalcin, UcOC) to become mature osteocalcin. The menopausal women and the elderly are predisposed to deficiency [1] which leads to hip fracture[2,4]. The urbanized elderly had higher deficiency of vitamin K2 than that of the rural elderly[3] comparing with the young adult female.

Material and Method

Imported osteoblast cells (clonetic) were used as a standard for confirmation of the osteoblast phenotype.

Biopsy of skin at forearm was induced to osteoblast by special osteogenic media and confirmed by alkaline phosphatase and osteocalcin including positive von Kossa staining[5]. The induced osteoblast showed the same character as imported clonetic cells. The osteoblastic media was separated into two dishes for controlling and studying. Each contained 10^{-6} millimole concentration of MK4. The gene expression was monitored by real PCR at the fourth, seventh, fourteenth, twenty first, twenty eighth, thirty fifth and forty second days of culture.

Correspondence to: Bunyaratavej N, Department of Orthopaedic Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. Phone: 0-2419-4545, E-mail: todrnarong@yahoo.com

Results

1. MK4 stimulated gene expression of osteocalcin and osteonectin at the seventh day of culture. They gave relative quantitative values (RQ): 7.983, 39.179 respectively (Fig. 1).

The gene of bone sialoprotein, osteopontin did not respond to MK4.

2. Vitamin K2 stimulated gene expression of collagen type 1 and gave a high level of relative quantitative at the fourteenth day of culture (Fig. 2).

3. MK4 enhanced gene of RANKL at a peak of the twenty eighth day of culture.

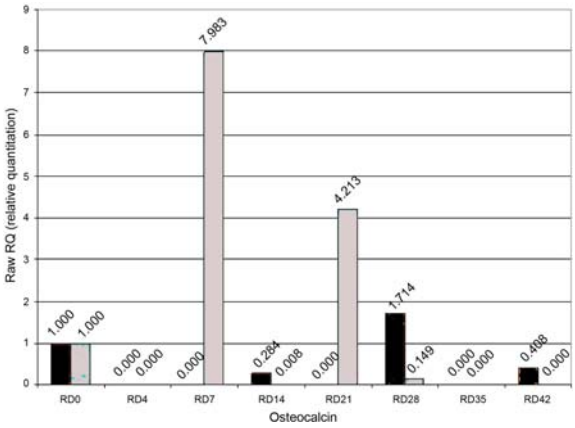

Fig. 1 Amount of the real time PCR related to the days of culture showed gene expression of osteocalcin which was stimulated by MK4 (grey) comparing to the control media

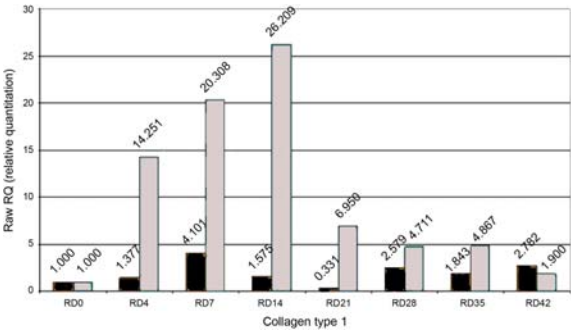

Fig. 2 Gene expression of collagen type 1 in MK4 media (grey) comparing to the control media (black)

4. The gene of CBFA1 was responded by MK4 and gave a high peak at the thirty fifth day.

5. Alpha Estrogen receptor showed weak responses but the beta estrogen receptor gave a high peak at the fourteenth day.

6. Interleukin-6 gene does not prominent response by MK4 (Table 1).

Discussion

This study confirms vitamin K2 has molecular action to genes in osteoblast. The mechanism is still not clear. Some researchers showed vitamin K2 played a significant role in the regular various gene expressions. Ichikawa[6] claimed that vitamin K2 could activate transcription of extracellular matrix-related gene and collagen accumulation in osteoblast-like cells (MG63). However, the outcome of the osteoblastic media with vitamin K2 showed an interesting result that stimulated gene expression in different types. MK4 stimulated not only osteocalcin but also the collagen type 1 which strongly supports that vitamin K2 is the bone forming agent. The action of collagen stimulation is new information especially from human- skin induced cell line ever finding.

Table 1. Various osteoblastic gene expressions showed the day of high peak of relative quantitative amount in the real time polymerase reaction chain (PCR) which was effected by Menatretenone compared with the control dish

Study item	Relative quantitative		Day of peak
	Control	Study	
Osteonectin	2.280	39.179	7
Osteocalcin	0	7.983	7
Collagen type 1	1.570	26.209	14
Interleukin-6	4.885	3.782	42
RANKL	0	10.783	28
ER_{β}	1	7.382	14

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บทบาทของวิตามิน เค สอง ที่มีผลต่อ หน้าที่ของ osteoblast

ณรงค์ บุณยะรัตเวช, มนนิภา ศิลาอาสน์, อานนท์ บุณยะรัตเวช

การศึกษาเปรียบเทียบผลของ วิตามินเค สอง ที่มีต่อ gene expression ใน osteoblast ที่เกิดจากการนำเซลล์ผิวหนังในคนมาเลี้ยงและชักนำให้เป็น เซลล์ osteoblast พบว่าเซลล์ดังกล่าวมีการตอบสนองโดยการแสดงของจีนเด่นชัดในเรื่องการสร้าง osteocalcin, osteonectin, collagen type1, CBFA1, ER beta และ RANKL แต่การแสดงของจีนไม่เด่นในการสร้าง osteopontin, bone sialoprotein, estrogen receptor alpha และ interleukin-6 แสดงให้เห็นว่า วิตามินเค สอง มีบทบาทในการสร้างกระดูกและยังมีส่วนช่วย bone remodeling เป็นไปปกติ โดยการทำให้มีจีนแสดงออกของ ER beta, RANKL