

MC3T3 - E1

- BB

I. 가 10),
 가
 11),
 - BB - AA - AB
 (polypeptide
 growth factor)가
 1,2). Terranova Wikesjo³⁾가 AA가 13-16)
 , - BB , 16)
 가 5), 2)
 17)
 가 DNA 18)
 4). 가 (platelet - derived 가 19)
 growth factor) 20)
 5). 가
 30kDa 가
 6-8) (,
 - AA, BB) (, 가
 - AB) 9) 14),
 , 가

21). , , , ,

I

31,32). , osteocalcin

33)

22) , Choi 30) MC3T3 - E1

가

23). (his - - BB , MC3T3 - E1

tone, c - fos, c - myc)

(type I collagen, fibronectin, transforming growth factor - 1(TGF - 1)가 가 I osteocalcin, osteopontin, bone sialoprotein mRNA osteopon - tin mRNA

mRNA

osteopontin, alkaline phosphatase, vitamin D inducible osteocalcin mRNA가 ,

II.

alkaline phosphatase, osteopontin, osteocalcin 1. ()

24). I 가 minimum essential medium alpha medium(Gibco Co., USA, - MEM) , fetal bovine serum(Gibco Co., USA, FBS) , trypsin(Sigma Co., USA) - BB(Genzyme Co., USA) RNA TRI REAGENT (Molecular Research Center, INC, USA)

25) osteo -

pontin, osteocalcin

23), osteopontin

26) , 가 ,

27). MC3T3 - E1

MC3T3 - E1 100 mm (Corning Co., USA) 10% FBS, 10 mM - glycerophosphate, 100 U/ml penicilline(,) , 100µg/ml streptomycin(,) , 5µg/ml amphotericin

28 - 30) MC3T3 - E1

B(Sigma Co., USA) - MEM RNA
 37 , 5% CO₂ 10µg total RNA 2.2 M formalde-
 (Sanyo Co., Japan) hyde 1% agarose gel
 0.05% trypsin/ 0.02% EDTA Hybond N plus
 nylon(Amersham, Arlington Heights, IL)
 [- ³²P] dCTP labeling type
 I collagen cDNA Hf677, osteopontin
 cDNA hybridization . filter 2
 × SSC, 0.05 % SDS 20
 , 0.1 × SSC, 0.1 % SDS 20 50
 -70 film
 . autoradiography filter 10 0.5 %
 SDS
 cDNA .

3. RNA Northern Blot
 Hybridization

MC3T3 - E1 5 × 10⁵ 가 2 -70 film
 100 mm 3, 7, SDS
 14, 21, 28 48

buffered saline, PBS (phosphate
) III.

FBS가 가 - MEM 1. I mRNA
 24 0.1, 1, 10 ng/ml , 3
 -BB 가 7 가
 24 가 14 , 21
 가 PBS 2 TRI 가 28
 REAGENT (Molecular Research Center, 3
 INC, USA) Chomczynski³³)
 total RNA -BB 3
 1 ml TRI 0.1 ng/ml
 REAGENT 가 가 , 1 ng/ml , 10 ng/ml
 centrifuge tube 5 가 가
 0.2 ml chloroform 4 , 가 , 0.1 ng/ml
 12,000g 15 14 0.1 ng/ml
 centrifuge tube 0.5 ml , 1, 10 ng/ml
 isopropanol 가 5 - 10 가 가 ,
 4 , 12,000g 8 21 0.1 ng/ml
 . RNA 1 ml 가 1 ng/ml
 75% 2 , 28
 diethyl pyrocarbonate(DEPC) 0.1, 1 ng/ml

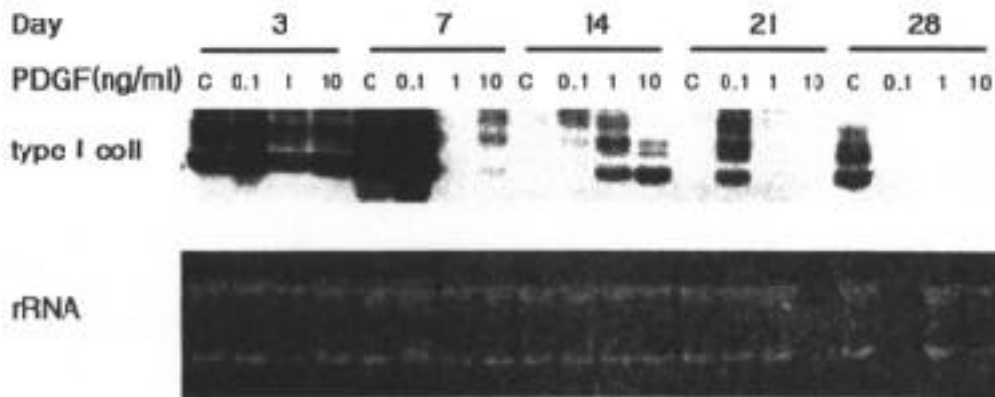


Figure 1. Time - and dose - response effect of 0.1, 1, 10 ng/ml of platelet - derived growth factor - BB on the expression of type I collagen mRNA in MC3T3 - E1 cell culture. Cells were seeded at 5×10^5 cells in 10 ml of minimum essential medium alpha medium containing 10% fetal bovine serum, 10 mM β -glycerophosphate and cultured for 3, 7, 14, 21, 28 days. Before 48 hours of indicated time, media were changed to serum free media. After incubation for 24 hours, indicated amount of platelet - derived growth factor - BB were added. Northern

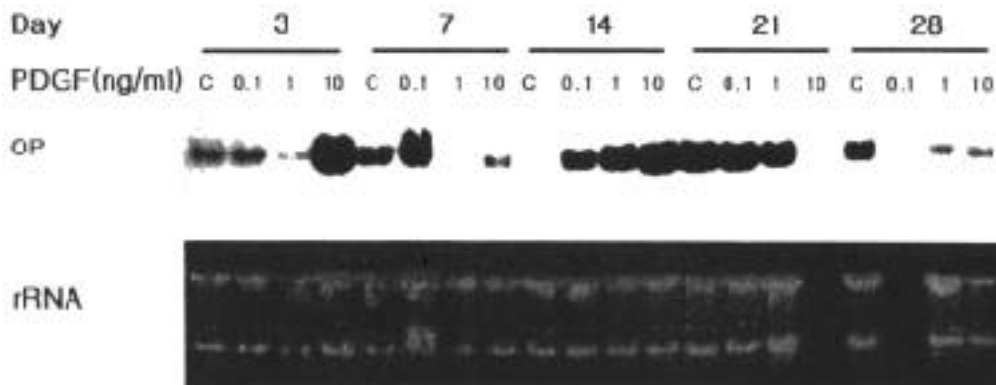


Figure 2. Time - and dose - response effect of 0.1, 1, 10 ng/ml of platelet - derived growth factor - BB on the expression of osteopontin mRNA in MC3T3 - E1 cell culture. Cells were seeded at 5×10^5 cells in 10 ml of minimum essential medium alpha medium containing 10% fetal bovine serum, 10 mM β -glycerophosphate and cultured for 3, 7, 14, 21, 28 days. Before 48 hours of indicated time, media were changed to serum free media. After incubation for 24 hours, indicated amount of platelet - derived growth factor - BB were added. Northern

2. osteopontin mRNA

가 21 28 가

가 21

3 - BB 3

7 가 mRNA 가 7 가 0.1, 1 ng/ml

가 14 가 - BB

가 10 ng/ml
 가 0.1 ng/ml
 BB ng/ml
 histone H3, H4, ribosomal protein S6
 가 1
 4 - 10
 가
 10 - 16
 /
 , fibronectin,
 TGF - 1, osteonectin 16
 mRNA
 가 21
 16 - 30
 osteocalcin
 28
 nodule
 osteocalcin
 가
 MC3T3 - E1

IV.

growth factor)가
 1,2),
 (polypeptide
 31)
 , Osteocalcin
 가 가
 29,33), MC3T3 - E1
 - BB가
 가
 MC3T3 - E1
 가
 MC3T3 - E1
 30) 1
 0.1, 1, 10 ng/ml
 - BB
 1,2)
 가
 osteopontin cDNA
 30kDa
 6,8)
 (- AA,
 (- AB)
 Quarles 29) MC3T3 - E1 가
 1 - 9
 가
 DNA ,
 10).
 ,
 , 9
 , fibronectin,
 ALP ,
 가 , 16 가
 30) MC3T3 - 18)

E1

가 fos - Jun 가 type
 I collagen , alkaline phosphatase
 21). , osteopontin
 osteocalcin 가
 가 가
 , osteopontin
 , , hydroxyapatite 44kD phos -
 18,19,34) 2), 17,35) phosphorylated glycoprotein
 36) 44)
 가 45).
 37). 10, 100
 ng/ml - BB 가 47)
 DNA
 Canalis 14) - BB 46) tibial osteotomy
 0.01 - 100 ng/ml
 10 ng/ml DNA , 47), beagle
 , MC3T3 - E1 , dog
 가 Kasperk 38) (IGF) - I 가
 Rutherford 39), Davidai 40) , 48) - BB 가
 5 ng/ml
 - BB 가 가
 Cassiede 41) 가 가
 - BB , 36).
 10 ng/ml Hock 50) 가
 0.1, 1 ng/ml 가
 . 가
 I 가
 42). mRNA - BB 가
 osteopontin, alkaline phos - - BB AA
 phatase, vitamin D inducible osteocalcin
 mRNA osteocalcin
 - BB
 24). Marcus 43)
 51).

Canalis ¹⁴⁾ 0, 10, 50, 250, 500
 ng/ml - BB
 I mRNA 가
 Tanaka ⁵⁴⁾ 가
 IGF 가
 가
⁵²⁾ 가
 IGF - I mRNA 14 가 , 28
 ,
 - BB AB가
 가 ⁵³⁾ - BB가
 가 MC3T3 - E1
 .
 osteopontin mRNA
 21
 Stein ²³⁾ ,
 - BB osteopontin
 mRNA 3 ,
 0.1, 1 ng/ml - BB
 ,
 MC3T3 - E1
 Quarles ²⁹⁾ 가 ,
 가 10 ng/ml 가
 RNA loading
 , 14
³⁰⁾ mRNA 가
 21 10 ng/ml , 28
 0.1 ng/ml RNA
 , 21 ,
 mRNA
 28
 . 250 ng/ml osteo -
 pontin mRNA Tanaka ⁵⁴⁾
 ,
 . 가
 osteopontin mRNA
 14
 가 가
 mRNA 21 , 28

osteopontin mRNA

0.1 ng/ml - BB

3, 7, 14, 21, 28

3, 7, 10, 14, 21, 28

0.1 ng/ml - BB

3, 7, 14, 21, 28

V.

Osteopontin mRNA

MC3T3 - E1

northern blot hybridization

- BB

osteopontin mRNA

14, 21, 28

3, 7, 14, 21, 28

0.1, 1, 10 ng/ml

- BB

1, 3, 7, 14, 21, 28

0.1, 1, 10 ng/ml

- BB

10 ng/ml

- BB

3, 7, 14, 21, 28

0.1 ng/ml - BB

1, 3, 7, 14, 21, 28

0.1, 1, 10 ng/ml

- BB

VI.

BB 24

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- Abstract -

The Effects of Platelet - Derived Growth Factor - BB on the Expression of Bone Matrix Protein in the MC3T3 - E1 Cells

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Bone remodeling results from the combined process of bone resorption and new bone formation which is regulated in part by some of the polypeptide growth factors such as platelet derived growth factor (PDGF), which has been known to be an important local regulator of bone cell activity and participate in normal bone remodeling. This process includes strictly regulated gene expression of several bone matrix proteins such as type I collagen and osteopontin, a 44 kDa phosphorylated glycoprotein, which has important roles in bone formation. The purpose of this study is to evaluate the effects of PDGF - BB on the mRNA expression of bone matrix protein, type I collagen and osteopontin, in MC3T3 - E1 cell culture.

Cells were seeded at 5×10^5 cells in 10 ml of minimum essential medium

alpha(- MEM) containing 10% fetal bovine serum, 10 mM beta glycerophosphate. 0.1, 1, 10 ng/ml PDGF - BB were added to the cells for the day 3, 7, 14, 21, 28 and cultured for 24 hours. Type I collagen cDNA, Hf677, and osteopontin cDNA were used as probes for northern blot analysis. Total cellular RNA was purified at indicated day and northern blot analysis was performed.

The results were as follows :

Type I collagen mRNA expressions were

higher at the day 3 and 7, and lower in the day 14, 21 in the control groups. In the experimental groups, mRNA expressions were increased when 0.1 ng/ml PDGF - BB were added on the day 3, 7, 21, and decreased in dose - dependent manner on the day 14, decreased at all added dose on the day 28.

Osteopontin mRNA expressions were highest in the day 21 groups and lowest in the day 14 groups in the control groups. Interesting results were shown in the day 14 and 21 groups. We found that osteopontin mRNA level was increased in dose dependent manner in the day 14 groups, and decreased dose dependent manner in the day 21 groups. In conclusion, PDGF - BB may have various control effects on type I mRNA expression in the growth and differentiation process of MC3T3 - E1 cells and may have contrary regulatory effects on osteopontin mRNA expression. For examples, when the baseline level of osteopontin mRNA was low, as in the day 14, PDGF - BB up - regulated osteopontin mRNA expression in dose dependent manner, and when the baseline level was high as in the day 21, PDGF - BB down - regulated dose dependent manner. Thus, it may be useful for clinical application in periodontal regeneration procedure if further study were performed.