

Transforming Growth Factor- 1 Bone Morphogenetic Protein-2

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Transforming Growth Factor- 1 and Bone Morphogenetic Protein-2 Upregulates Matrix Synthesis and Chondrogenic Phenotype in Intervertebral Disc Cells

Dong-Jun Kim, M.D.*, **Seong-Hwan Moon, M.D.**, **Hyang Kim, M.S.**, **Eun-Hae Kwon, B.S.**,
Soo-Bong Hahn, M.D., **Yong-Min Cheon, M.D.**, **Hak-Sun Kim, M.D.**,
Kyeong-Jin Han, M.D.**, **Moon-Soo Park, M.D.**, **Hwan-Mo Lee, M.D.**

Department of Orthopaedic Surgery, Yonsei University, Ewha Women University,
Aju University** College of Medicine, Seoul, Korea*

– Abstract –

Objectives : To determine effect of transforming growth factor- 1 and bone morphogenetic protein- 2 in matrix synthesis and expression of chondrogenic phenotype in human intervertebral disc cells.

Materials and Methods : The intervertebral disc cells were harvested and cultured from the surgical patients for the degenerative disc disease. TGF- 1 was purchased from R&D and BMP- 2 was produced by transfection of pcDNA3.1/Hygro/BMP- 2 to CHO cell using Lipofectamine 2000. rhBMP- 2 was separated by Heparin- Sepharose A chromatography. TGF- 1 and BMP- 2 were administered to culture. Proteoglycan synthesis was assessed by 35S incorporation and expression of matrix mRNA was analyzed by RT- PCR for collagen I, collagen II, aggrecan, and osteocalcin.

Results : TGF- 1 and BMP- 2 showed increased proteoglycan synthesis and expression of collagen I, collagen II and aggrecan mRNA in dose dependent manner respectively. There was no recognizable synergistic effect in matrix synthesis and matrix mRNA expression. Throughout dosage, expression of osteogenic phenotype (osteocalcin mRNA) was not noted.

Conclusion : TGF- 1 and BMP- 2 proved to be effective anabolic agent for maximizing matrix synthesis without evidence of osteogenesis.

Key Words : TGF- 1, BMP- 2, Aggrecan, Collagen I, Collagen II, Osteocalcin

Address reprint requests to

Hwan-Mo Lee, M.D.

Department of Orthopaedic Surgery, College of Medicine, Yonsei University

#134 Shinchon-Dong, Soedaemun-ku, Seoul 120-752, Korea

Tel : 82-2-361-5649, Fax : 82-2-363-1139, E-mail : hwanlee@yumc.yonsei.ac.kr

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(# 2001-3),

, Brain Korea 21

2

24)

BMP-2

가

23)

BMP-2

BMP-2

가 (proteoglycan)

14,15)

TGF- 1

가

BMP-2

BMP-2 TGF- 1

BMP-2 TGF- 1

1.

6 (23 -45)

가

19) transforming growth factor- β 1

5% heat-inactivated fetal bovine serum(FBS, GIBCO-BRL, Grand Island, NY), 0.2% trypsin 0.1% EDTA(Calbiochem, La Jolla, CA) Hams F-12 medium and Dulbeccos Modified Eagle Medium(F12/DMEM, GIBCO-BRL, Grand Island, NY) 37°C 60

(TGF- β 1)

TGF- β 1

가

F12/DMEM

clostridial collagenase type II (Sigma, St. Louis, MO)

2 37°C 4

F12/DMEM

Nylon (pore size 75 um)

(Maldonado and

Oegema 1992) 5 × 10⁵ /ml

24 well plate(Falcon, Franklin Lakes, NJ)

10% FBS, 1% v/v penicillin,

streptomycin, nystatin(all antibiotics from GIBCO-BRL, Grand Island, NY) F12/DMEM

3 37°C 5% CO₂

2.

3

TGF- β 1, Insulin like growth factor-1, Osteogenic protein-1, Fibroblast growth factor, Epidermal growth factor 7,20,25,26) Bone morphogenetic protein-2(BMP-2) 가

. BMP-2

2,11,12)

BMP-

0.15M NaCl

1.2%

low viscosity alginate gel

(Kelco, Chicago, IL)

algininate gel 102mM CaCl₂ .22 gauge algininate gel

CaCl₂ 10 .0.15M NaCl

F12/DMEM 3 (chiba et al 1997). Algininate bead 24well culture plate well 10 10% FBS, 1% v/v penicillin, streptomycin, nystatin F12/DMEM 48 37°C 5% CO₂

3. TGF- 1 BMP-2

TGF- 1 R&D BMP-2 plasmid DNA BMP-2A pcDNA3.1/hygro vector , Chinese hamster ovary(CHO-K1) lipofectamine(Gibco BRL) pcDNA3.1/hygro/BMP-2A CHO-K1 hygromycin (CHO-K1/BMP-2A)

BMP-2A , Heparin sepharose A Chromatography , YM10 -70

4.

35S(20 uCi/ml) (serumless medium, Newman-Tytell) 4 가 algininate beads Na Citrate 37 °C gel HCl Guanitidine 4 °C 48

Sephadex G-25M PD-10 column(Pharmacia Biotech, Uppsala, Sweden) scintillation mixture(Ultima Gold, Packard, Meriden, CT) 가 PD-10 column 2, 3, 4 scintillation

5. Aggrecan, I , II osteocalcin mRNA

Alginate bead , 28 mM EDTA/0.15 M NaCl chelation . D-PBS RNA TRIzol reagent(Life Technologies) RNA 1 µg Oligo d(T)16 primer 2.5 µM, dNTP mixture 20 mM, 10x buffer 2 µl, 20 U/ µl RNase inhibitor, 25 mM MgCl₂ 4 µl, MuLV Reverse transcriptase 2.5 U/ µl 20 µl , 42 30 , 95 5 , 4 5 cDNA 20 µl . 20 µl 3 µl aggrecan, I , II , osteocalcin sense primer antisense primer 10 pmole, 25 mM MgCl₂ 1 µl, 10x PCR buffer 2.5 u dNTP mixture 20 mM, Taq polymerase 2.5 u PCR 94 2 94 30 , 55 30 , 72 1 72 3 .

6.

BMP-2 BMP-2(2.5, 5, 10, 50, 100, 500, 1000, 1500, and 2000 ng/ml) BMP-2 TGF- 1 aggrecan, I , II , osteocalcin mRNA . RT-PCR -actin .

7.

SPSS(SPSS, Chicago, IL) t-test ANOVA p<0.05 .

1. TGF- 1 TGF- 1

ng/ml 가가 , 2
 200%, 10 ng/ml 250%
 가 (p<0.05)(Fig. 1).

2. TGF- 1 , II , osteocalcin mRNA
 Aggrecan, 1 , 2 mRNA
 RT-PCR densitometry TGF- 1 가
 osteocalcin mRNA TGF- 1 가

3. BMP-2
 BMP-2(2.5, 5, 10, 50, 100, 500, 1000, 1500, and 2000 ng/ml)

50 ng/ml 가가
 , 300 ng/ml 50%, 100 ng/ml
 100%, 1500 ng/ml 250%
 가 (p<0.05) (Fig. 1).

4. BMP-2 , II , osteocalcin mRNA
 Aggrecan, 1 , 2 mRNA
 RT-PCR densitometry BMP-2 가

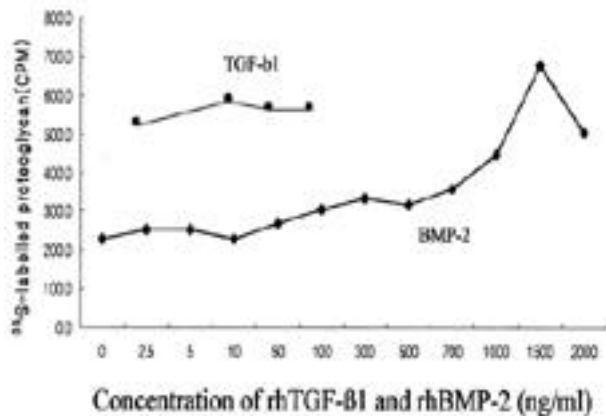


Fig. 1. Newly synthesized proteoglycan as measured by incorporation 35S-sulfate according to the dose of TGF-β1 and BMP-2. In given doses, proteoglycan synthesis was upregulated compared to control culture (p<0.05).

aggrecan mRNA 1500 ng/ml
 BMP-2 400% 가가 I
 mRNA BMP-2 100 ng/ml
 70% 가가 2
 mRNA BMP-2
 50% 가가 osteocalcin
 mRNA BMP-2
 가 (Fig. 2).

5. TGF- 1 BMP-2
 TGF- 1 BMP-2

6. TGF- 1 BMP-2
 aggrecan, 1 , II , osteocalcin mRNA
 TGF- 1 BMP-2 mRNA
 , II mRNA
 (Fig. 3).

(bone morphogenetic protein: BMP)

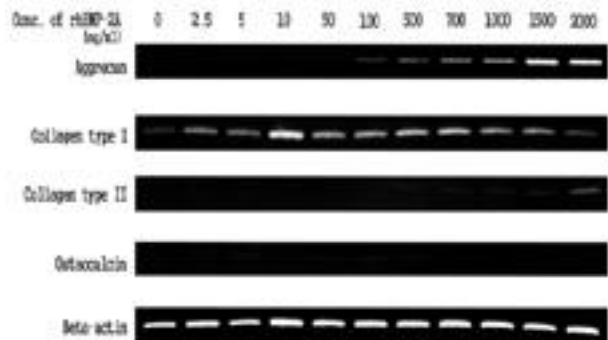


Fig. 2. Dose response of BMP-2 in mRNA expression of collagen type I, collagen type II, aggrecan, and osteocalcin as measured by reverse transcriptase-polymerase chain reaction. Collagen type I, collagen type II, and aggrecan mRNA expression were upregulated, while osteocalcin mRNA showed no recognizable upregulation.

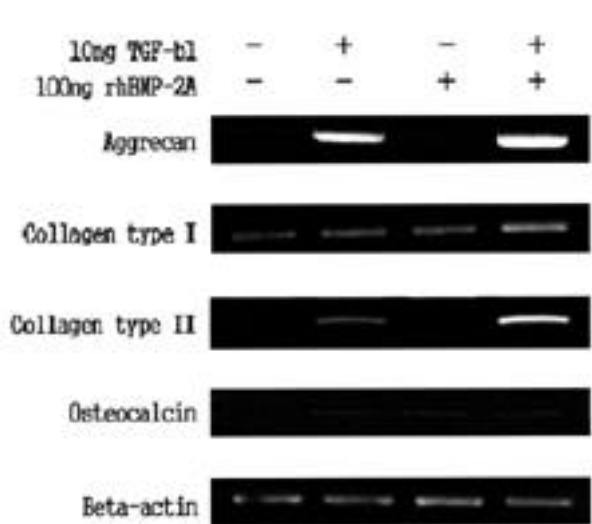
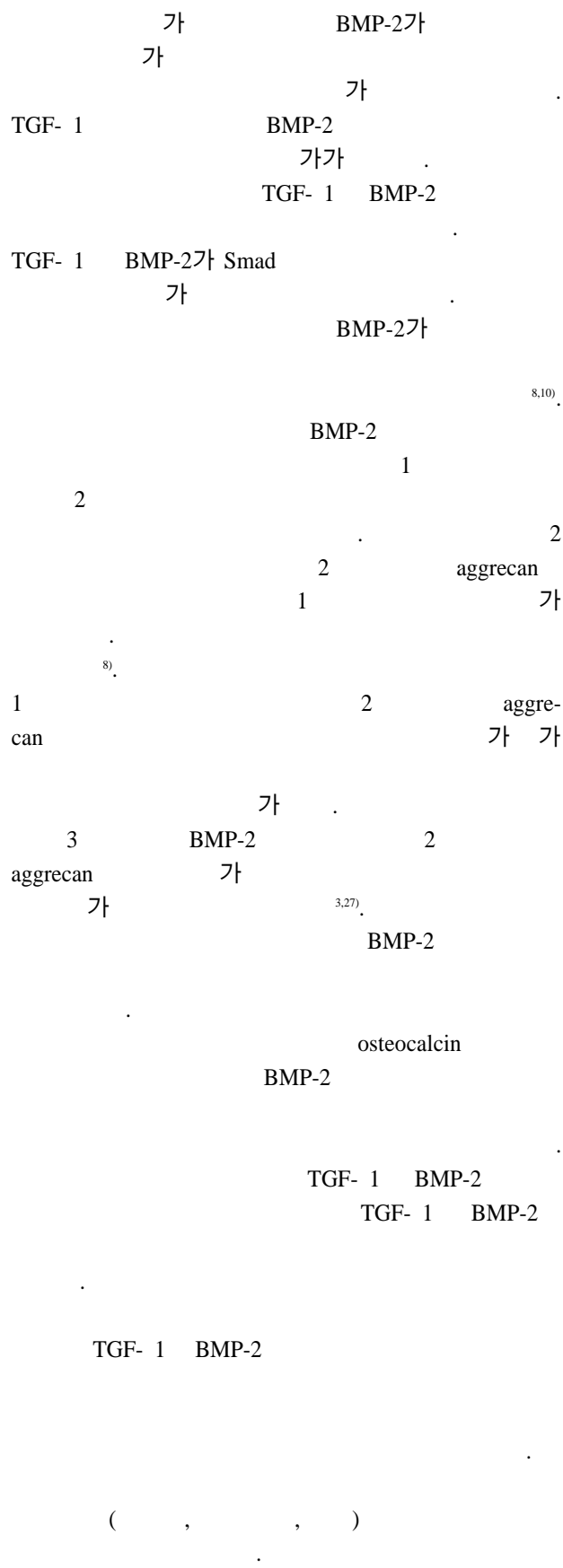


Fig. 3. Effect of BMP-2 and TGF- β 1 in mRNA expression of collagen type I, collagen type II, aggrecan, and osteocalcin as measured by reverse transcriptase-polymerase chain reaction. Even with combination of two growth factors, there was no synergistic effect in matrix mRNA expression. In given doses, there was no expression of osteocalcin mRNA.



TGF- 1 BMP-2
가

IGF-1

TGF- 1 BMP-2
TGF- 1 BMP-2
가 (aggre-
can, 1 , 2) 가
BMP-2
(osteocalcin)
TGF- 1 BMP-2가

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: TGF- 1 BMP-2
 : , TGF- 1 R&D
 BMP-2 pcDNA3.1/Hygro/BMP-2 Lipofectamine 2000 CHO cell BMP-2
 Heparin-Sepharose A chromatography . TGF-b1 BMP-2 3
 35S incorporation , aggrecan, collagen type I, collagen type II, osteocalcin mRNA
 RT-PCR
 : TGF- 1 BMP-2 가 가 aggrecan, collagen type
 I, II mRNA 가 . TGF- 1 BMP-2 . TGF- 1 BMP-2
 osteocalcin mRNA
 : TGF- 1 BMP-2
 : TGF- 1, BMP-2, aggrecan, collagen I, collagen II, osteocalcin