

단백질 전달 영역 융합-Bone Morphogenetic Protein-2가 백서 두개골 결손부에서 골 조직 재생에 미치는 효과

Effect of protein transduction domain fused-bone morphogenetic protein-2 on bone regeneration in rat calvarial defects

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ABSTRACT

Purpose: Recombining bone morphogenetic protein (BMP) is usually acquired from high level animals. Though this method is effective, its high cost limits its use. The purpose of this study was to evaluate the effect of bone morphogenetic protein-2 with protein transduction domain (BMP-2/PTD;TATBMP-2) on bone regeneration. Rat calvarial defect model and osteoblastic differentiation model using MC3T3 cell were used for the purpose of the study.

Materials and Methods: MC3T3 cells were cultured until they reached a confluence stage. The cells were treated with 0, 0.1, 1, 10, 100, 500 ng/ml of BMP-2/PTD for 21 days and at the end of the treatment, osteoblastic differentiation was evaluated using von Kossa staining. An 8mm, calvarial, critical-size osteotomy defect was created in each of 48 male Sprague-Dawley rats (weight 250-300 g). Three groups of 16 animals each received either BMP-2/PTD (0.05mg/ml) in a collagen carrier, collagen only, or negative surgical control. And each group was divided into 2 and 8 weeks healing intervals. The groups were evaluated by histologic analysis (8 animals/group/healing intervals)

Result: In osteoblastic differentiation evaluation test, a stimulatory effect of BMP-2/PTD was observed in 10ng/ml of BMP-2/PTD with no observation of dose-dependent manner. The BMP-2/PTD group showed enhanced local bone formation in the rat calvarial defect at 2 weeks. New bone was observed at the defect margin and central area of the defect. However, new bone formation was observed only in 50% of animals used for 2 weeks. In addition, there was no new bone formation observed at 8 weeks.

Conclusion: The results of the present study indicated that BMP-2/PTD(TATBMP-2) have a positive effect on the bone formation in vitro and in vivo. However, further study should be conducted for the reproducibility of the outcomes.

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KEY WORDS: Bone Regeneration; bone morphogenetic protein.

1965 Urist¹⁾

(Human Recombinant Bone Morphogenetic proteins; BMPs) . 1988

Wozney cloning

가

Human Recombinant Bone Morphogenetic proteins (rhBMPs) TGF- superfamily ,

^{2,3)}, 20

가 BMP가 , rhBMP-2, -4, -5, -6, -7⁴⁻⁸⁾

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DNA (CHO cell)
 rhBMP-2, -7
 가 9,10 가 BMP-2(BMP-2/PTD; TATBMP-2)
 가
 rhBMP-2 rhBMP-7
 Chinese hamster ovary(CHO)
 2)
 rhBMP carrier 1.
 FDA 가가 .
 가 1 1)
 (BMP-2/PTD; TATBMP-2)
 (Protein Transcription
 Domain; PTD) human immunodeficiency virus
 transactivator of transcription(Tat)
 rhBMP 11-13) ,
 가 2 (BMP-2) cDNA RT-PCR
 cloning bacterial expression vector
 BMP-2 5' Tat
 rhBMP 가 E coli (YGRKKRRQRRR)
 rhBMP X-press tag 6
 CHO 3 rhBMP histidine ATG
 rhBMP BL21(E-Coli
 BL21; Invitron Inc) 37 2
 isopropylthio-galactoside 1 mM 가 18
 가 , 8M
 BMP 14,15) 가 BMP 2 3
 가 11,16-20) 가
 가 Ni-Ti bead
 imidazole
 (BMP-2/PTD) 28)
 가 21-25) 2)
 human immunodeficiency virus trans- 250~300 g (Sprague
 activator of transcription 가 Dawley rat) 48
 가 24-27) 가

2.
 1)
 (BMP-2/PTD; TATBMP-2)
 6-well MC3T3 well
 1×10⁴ 10% -MEM
 80% , 5% , 10 mM
 -glycerophosphate, 50 g/ml ascorbic acid PTDBMP-2
 가 , 21
 . 2 3 .
 BMP-2/PTD 0, 0.1, 1, 10, 100, 500 ng/ml
 . 21 von Kossa
 staining 가 .

2)
 (BMP-2/PTD; TATBMP-2) 가
 (1)
 collagen
 , collagen BMP-2/PTD solution(0.05 mg/ml)
 socking .
 2 , 8 ,
 8 48 .
 (2)
 Ketamine hydrochloride(Ketalar,
 Yuhan Co., Seoul, Korea) Xylazine(Rompun, Bayer
 Korea, Seoul, Korea) 4:1 (70



Figure 1. 8 mm circumferential calvarial defect formation.

mg/kg) . povi -
 done iodine 2%
 Lidocaine(2% lidocaine, 1:100,000 epinephrine, Kangmyung
 Pharm., Seoul, Korea)
 8
 mm trephine bur(3i, Palm Beach Gardens, FL, USA)
 8mm .
 ,
 4-0 coated Vicryl®
 (Polyglactin 910, braided absorbable suture, Ethicon,
 Johnson & Johnson Int., Edinburgh, UK) , 1
 . 2 , 8
 가 가 (Fig. 1, 2).

3. 가
 1)
 10% 24
 5% nitric acid 3~7 . 7 μm
 80 μm 4
 ,
 Hematoxylin-Eosin(H-E)
 (Olympus BX50, Olympus Optical Co., Tokyo, Japan)



Figure 2. Collagen matrix used as a carrier.

(dose depend -

ency) (Fig. 3).

1. (BMP-2/PTD; TATBMP-2)

2. (BMP-2/PTD; TATBMP-2) 가

BMP-2/PTD
 MC3T3 BMP-2/PTD 21
 가 von Kossa
 . 500 ng/ml 100 ng/ml 3
 가
 , 500 ng/ml 가
 . 10 ng/ml 21 von
 Kossa
 10 ng/ml von Kossa ,
 가

1)
 2)
 (1) (Sham surgery)
 2

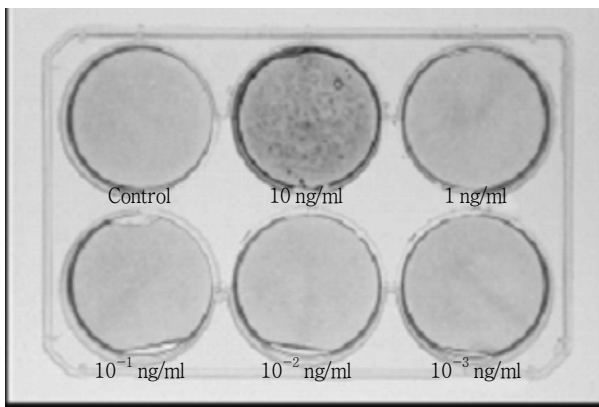


Figure 3. 21 days after treatment with BMP2/PTD (von Kossa staining).

가
 (Fig. 4a).

8

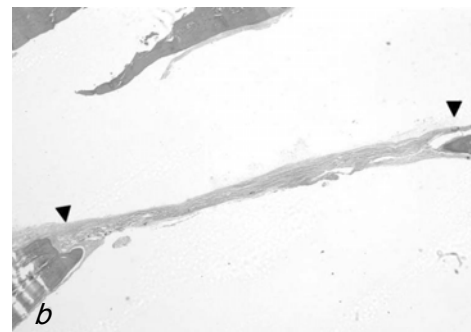
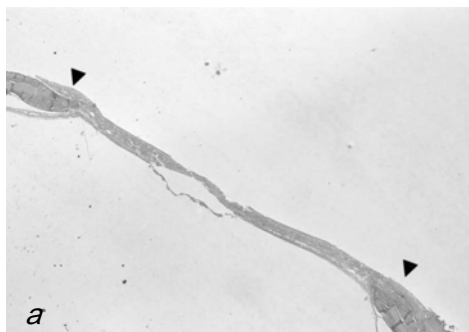


Figure 4. Histological section of Control group. a. Control 2 weeks (HE × 10). b. Control 8 weeks (HE × 10). Arrow head; defect margin.

(Fig. 4b). (3) (BMP2/PTD) 가
 (2) (Collagen only) 2 8
 collagen , collagen collagen
 collagen 가 가 (Fig. 5a). (Fig. 6b).
 , 가 (Fig. 7b, 7c). 가
 8
 Collagen 2
 가 Collagen

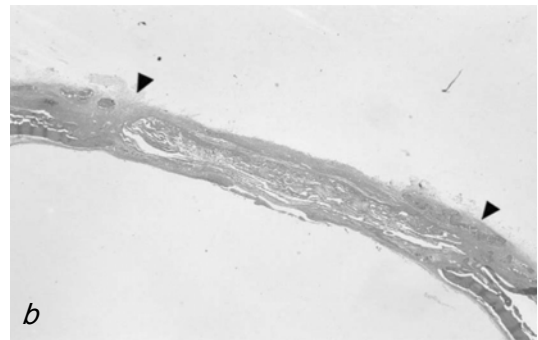
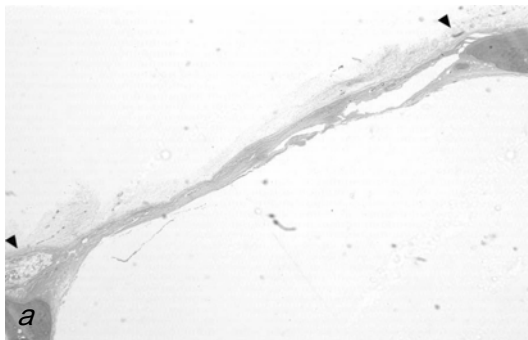


Figure 5. Histological section of Collagen group. a. Collagen 2weeks (HE × 10) b. Collagen 8weeks (HE ×10). Arrow head; defect margin.

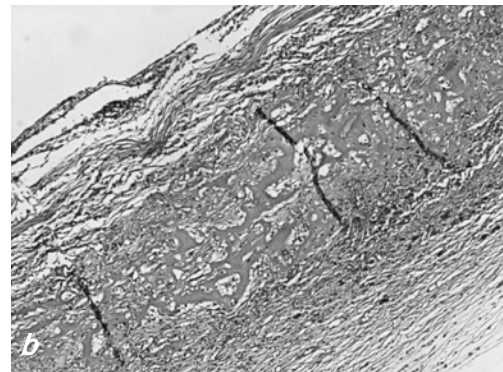


Figure 6. Histological section of BMP-2/PTDweeks with no new bone formation. a. Overall view under low magnification (HE × 10, Arrow head; defect margin). b. Higher magnification(HE × 100).

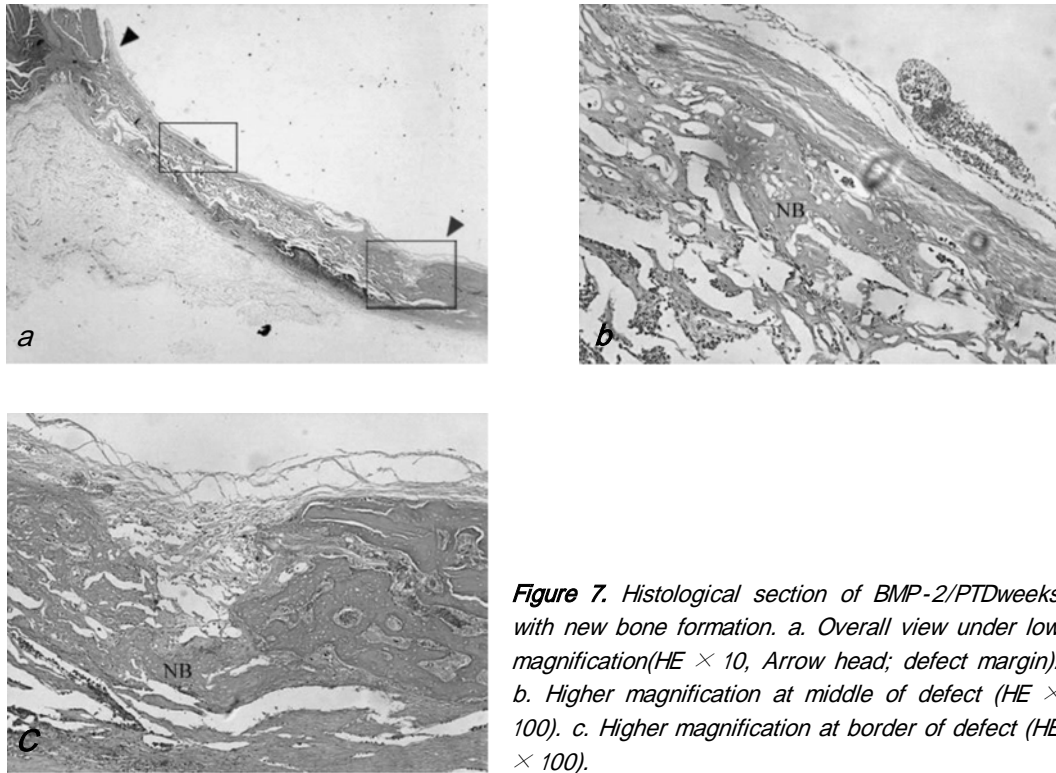


Figure 7. Histological section of BMP-2/PTDweeks with new bone formation. a. Overall view under low magnification(HE × 10, Arrow head; defect margin). b. Higher magnification at middle of defect (HE × 100). c. Higher magnification at border of defect (HE × 100).

defect) , 10%

가

16,17,19,20,29,30)

(BMP-2/PTD; TATBMP-2)

가

rhBMPs

trephine bur 8 mm

가

rhBMPs

collagen , 16,17,19,20,29-31)

BMP-2/PTD collagen carrier , rhBMPs

2 , 8

가 , MC3T3

BMP-2/PTD 가

carrier

BMP가

가

18,32-35)

가

250~300 g

가

22-27,36-39)

8mm (Criticalsize) 가

human immunodeficiency virus
transactivator of transcription
가 가

22-27,36) VP-22, ANTP, Kaposi 21 von Kossa
FGF arginine/lysine-rich peptide 10 ng/ml von Kossa
가
BMP-2/PTD
(dose dependency)

38,39) TAT BMP-2
4 °C 0.05 mg/ml BMP-2/PTD
rhBMP-2 rat calvarial
defect
가 39) rhBMP-2 BMP-2/PTD
BMP-2/PTD heat shock protein
BMP
furin cleavage rhBMP-2
BMP-2/PTD in vitro
43-kd N-terminal fragment가
BMP-2 disulfide
가 dimer 가
40) 114
7 가
disulfide
6 3 disulfide 4,29,41,42) 1999 Wikesjo 5)
3 가 14,15) rhBMP-2
0.05, 0.1,
BMP-2/PTD 0.2 mg/ml , rhBMP-2 8
BMP-2가 , 가
ALP 가 가 Pang 19)
BMP-2/PTD , 0.05 mg/ml 0.025 mg/ml
E coli rhBMP2 가
BMP
가 BMP BMP-2/PTD
가 2
가 8 4
BMP-2/PTD
MC3T3 BMP-2/PTD ALP 가
500 ng/ml 100 ng/ml carrier collagen

4
8
BMP-2/PTD
가
BMP-2/PTD가
BMP-2/PTD 가
, PTD
PTD
endosome
43)
, endosome
BMP-2/PTD가
heat shock
protein
, endosome
chloroquine sucrose PTD
PTD
HA2 peptide TAT
chloroquine
43)
BMP-2/PTD HA2 peptide 가

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