공학석사학위논문

Enhanced bone regeneration with tauroursodeoxycholic acid

타우로우루소디옥시콜릭산을 이용한 뼈 재생 촉진

2013년 2월

서울대학교 대학원
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이 논문을 공학석사학위논문으로 제출함

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마윤지의 석사학위논문을 인준함

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Abstract

Enhanced Bone regeneration with tauroursodeoxycholic acid

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Bone morphogenetic proteins (BMPs) are effective growth factors that widely used in bone tissue engineering. However, since BMPs can induce differentiation of mesenchymal stem cells into both adipocytes and osteoblasts, without meticulous controlling, using BMPs for bone regeneration can cause problems in terms of regenerated bone quality. Thus finding a compound that can minimize the undesirable effects of BMPs in bone regeneration will have its own significance. In the present study, tauroursodeoxycholic acid (TUDCA), an apoptosis inhibitor, was tested and its efficacy was evaluated. To examine the

effect of TUDCA, TUDCA was delivered with or without BMP-2 to calvarial defects of mice using type 1 collagen sponge as a carrier, and its bone regenerating efficacy was evaluated by various analytical methods including micro computed tomography (μCT), histology, and immunohistochemistry. Surprisingly, even without BMP-2, TUDCA delivered by collagen sponge significantly increased bone regeneration area, as well as the quality of regenerated bone tissue, compared to other groups. Furthermore, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and RT-PCR results indicated that TUDCA significantly suppressed apoptosis of the cells migrated into the collagen scaffold, suggesting the possibility of the decreased apoptosis by TUDCA played an important role in the TUDCA-induced bone regeneration. The results of the present study indicate that TUDCA can be an effective bone regenerating agent, and it also can be a potential substitute of BMPs in bone regeneration.

keywords: Tauroursodeoxycholic acid (TUDCA), Bone regeneration, Bone morphogenetic proteins (BMPs), Adipogenesis, Apoptosis

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1. Introduction

For hard-to repair large bone defect, many researchers attempt to induce new bone formation by connecting of filling up the defect site using bone graft of bone graft substitutes, which have adequate mechanical strenth and affinity for patient's biologycal environment [1, 2]. Generally, based bon the source, bone grafts can be categorized into autografts and allografts. Although there are also xenografts, bone grafts from animals, they are rarely used for human because of possible disease transmission and immuneresponse [3]. Autografts are normal bone grafts harvested from patients themselves. Autografts have less side-effect and no immune response, and the results of transplanting autografts are predictable. However, since using, autograft requires harvesting bone fragments from the patients, the patients have to go through additional surgical procedure that can be painful and has potential risks of excessive bleeding. Furthermore, it can be difficult to obtain enough bone fragments to fill up the defect, especially in pediatric and geriatric patients. Reported failure rate of autograft transplantation is 13-30% [3, 4]. Differ from the autografts, allografts are harvested from other person of human cadaver. Although allografts are the common alternatives to the autografts, they are thought to be inferior to autografts because they can cause severe host immune response, transmit serious infectious diesase, and have high failure rates of 20-35% [3, 4]. Furthermore, excessive bone resorption could occur before osteogenesis is completed when the defect site is too large [5].

Although those two common bone grafts were used for many

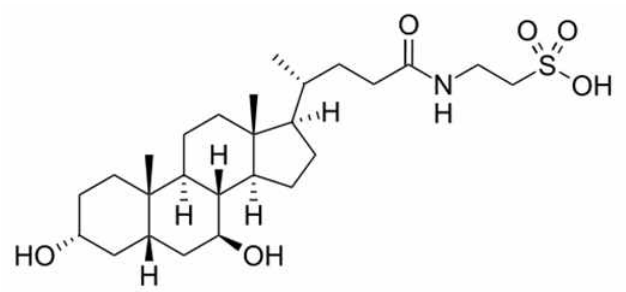
decades in the field of bone regeneration, still they have unsolved problems mentioned above. As a solution to such problems, key factors in bone regeneration such as bone morphogenetic proteins (BMPs) have been identified and are being used along with many different types of scaffolds [6-8]. First, discovered in 1967, BMPs are a group of proteins play important roles in bone [9]. Since then, scientists found additional functions of BMPs on various biological processes such as embryogenesis and postnatal skeleton [10, 11]. Until now 15 types of BMPs have been identified [12]. BMPs have been known to induce osteogenesis of mesenchymal stem cells and ectopic bone formation [13], making them poweful osteogenic agents for bone grafts [11, 14].

However, BMPs are known to stimulate peroxysome proliferator-activated receptor gamma-2 (PPARγ2) which is known to be associated with osteogenesis and adipogenesis of mesenchymal stem cell [15]. Although the exact mechanism is not clear, BMPs can induce both osteogenesis and adipogenesis, and decreased bone mineral density is closely related to increased adipogenesis on bone marrow [16]. There always exists possible undesirable effect of using BMPs for bone regeneration. Since it could compromise the rigidity of regenerated bones, it can be a serious problem in using BMPs for clinical applications intended to induce bone regeneration. Additionally, BMPs have very short half-life and easily lose its bioactivity in solution [17], so it is important to use well-designed drug delivery system which can facilitate sustained release so that BMPs are not released and diffused away rapidly after implantation. Furthermore, uncontrolled BMPs burst could cause over-activated of immune system leading to overgrow of bone tissue, and this also

could be a serious problem. Thus, along with designing an effective drug delivery system, finding a candidate compound or chemical which can minimize the undesired effects on BMPs is also important [18]. In the present study, tauroursodeoxycholic acid (TUDCA) was examined as such compound.

TUDCA is derived from bile acid and produced from cholesterol as a precursor in liver. And FDA already approved TUDCA as a protective medicine for various liver disease including cholestatic liver diseases and cirrhosis because TUDCA inhibits apoptosis by preventing the binding of Bax (Bcl-2-associated X protein) to mitochondria [19-22]. Aside from the anti-apoptotic effect, TUDCA also has an effect on preventing neurodegeneration such as Parkinsons's disease [23, 24], Huntington's disease [25], and acute stroke [26]. Recent studies have reported that TUDCA is highly efficient for reducing retinitis pigmentosa (RP) [27, 28], and myocardial infarction through suppression of apoptosis [28]. It also has been reported that TUDCA, as a chemical chaperone that improves protein folding efficacy, ameliorates obesity by protecting cells from endoplasmic reticulum (ER) stress, which is associated with decreased insulin sensitivity and consequent obesity and diabetes [30]. Consequently, TUDCA can inhibit adipocyte differentiation in dose-dependent manner by decreasing ER stress [31].

Thus, in the present study, the effect of TUDCA, a potent anti-apoptotic agent and inhibitor of adipogenesis, on bone tissue regeneration was investigated using a mouse calvarial defect model.



Molecular formation	$C_{26}H_{45}NO_6S$
Molar mass	499.7g/mol

Fig. 1.1. The structural formula and properties of TUDCA.

2. Materials and Methods

2.1 Preparation of BMP-2 or TUDCA loaded collagen scaffold

Type 1 collagen sponge was purchased from Integra LifeSciences Corporation and was cut as disks (4mm diameter and 1mm thick) for each defect site. BMP-2 (1μg and 3μg; R&D systems, Minneapolis, MN, USA) solution was prepared. TUDCA (500μM; CALBIOCHEM, US and CANADA) solution was prepared by dissolving in PBS (0.01M; Sigma, USA). BMP-2 and TUDCA were loaded in type 1 collagens sponge. Control group was loaded with PBS only.

2.2 Mouse calvarial defect model

Six-week-old Institute of Cancer Research mice (n=8 per each group; KOATECH, KOREA) were anesthetized with xylazine (20mg/kg) and ketamine (100mg/kg). After shaving the scalp hair, a longitudinal incision was made in the middle of the cranium from the nasal bone to the posterior nuchal line, and the periosteum was elevated to the expose the surface of the parietal bones. Using a surgical trephine bur (Ace Surgical Supply Co., Brockton, MA, USA) and a low-speed micrometer (4mm diameter, circular), two transosseouse defects were produced in the skull. This defect size is a critical defect size for the

mouse calvarial defect model. The defect site was irrigated with saline and bleeding points were electrocauterized. Priosteum was removed and each collagen sponge was inserted according to each group. For No treatment group, there was no sponge implanted.

All animal treatments and experiment procedures were approved by Institutional Animal Care and Use Committee in Seoul National University (SNU-120220-3).

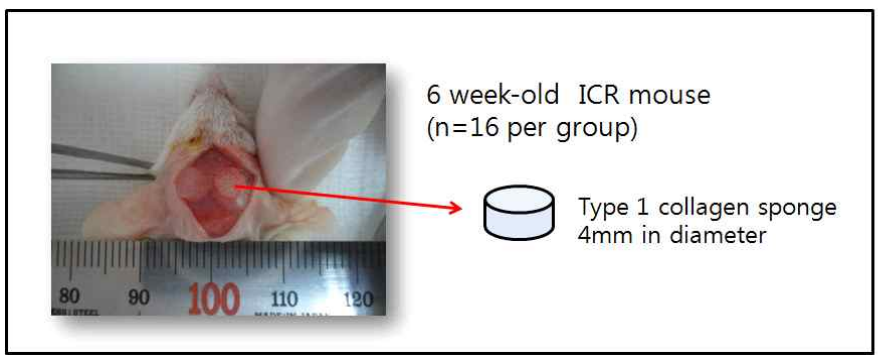


Fig. 2.1. Experimental procedure. Mouse calvarial defect model and implantation of collagen sponge loaded with bone morphogenetic protein-2 (BMP-2) and tauroursodeoxycholic acid (TUDCA).

2.3 Implantation of collagen sponge and injection of TUDCA solution

Calvarial defect sites were filled with type1 collagen sponge loaded with BMP-2 and TUDCA corresponding to each group (Table 1). The dose of BMP-2 is 0, 1 or 3ug and the concentration of TUDCA is 500 μM. In case of group 4, 6 and 8, 25μl of TUDCA was injected every other day for 14days subcutaneously in calverial defect site. And in case of Group3, TUDCA was injected once when the collagen sponge was implanted (single injection at day 0). At the first time of implantation, TUDCA solution was absorbed in collagens sponge. Each mouse has two defects, and eight mice were used for each group (n = 16).

	Groups	
1		No treatment
2	Collagen sponge	PBS only
3		TUDCA (single injection at day0)
4		TUDCA (multiple injection for 14days)
5		BMP-2, 1ug
6		BMP-2, 1ug +TUDCA (multiple injection for 14days)
7		BMP-2, 3ug
8		BMP-2, 3ug +TUDCA (multiple injection for 14days)

Table. 2.1. Groups of in vivo study. In Case of group 4, 6 and 8, TUDCA (500 μM, 25 μl) was injected every other day for 14 days subcutaneously just into the calverial defect site (multiple injection). And in group3, TUDCA was injected just once at the time of implantation of collagens sponge (single injection at day 0).

2.4 Evaluation of in vivo calvarial defect bone formation

Eight weeks after implantation, the mice were sacrificed and the skulls including the defect site were harvested and fixed with 4% paraformaldehyde solution. Three-dimensional images of bone regeneration in critical size defect were visualized with the Micro-computed tomography (μCT, skyscan-1076 in vivo x-ray microtomograph) scan (n = 16 per group).

And bone formation area was evaluated with histological analysis. All samples for histological analysis were decalcified with decalcification solution (Sigma, USA) and passed through dehydration step with alcohol and xylene. After dehydration step, samples were embedded in paraffin and transversely sectioned in a thickness of 4μm. The sectioned samples were stained with Goldner's Trichrome staining and Hematoxilin & Eosin staining (H&E staining) Bone formation area was expressed as the percentage of new bone area in the original bone defect area [(new bone area/original bone defect area) ×100%]. The percentage of bone formation area was calculated by Adobe Photoshop Software (Adobe Systems, Inc., San Jose, CA, USA).

2.5 Immunohistofluorescence analysis

Paraffin embedded mouse calvarial defect sites were cut into 4μm thickness. For qualification of bone regeneration, sample slides were stained with anti-osteocalcin (Abcam, Cambridge, UK). Rhodamin-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) were used to visualize. Sections were counterstained with 4, 6-diamidino-2-phenylindole (DAPI, Vector Laboratories, Burlingame, CA, USA) and examined using a fluorescence microscope (Nikon TE2000, Tokyo, Japan). All samples with X40 magnification were randomly analyzed.

2.6 Apoptosis ananlysis

Calvarial defect sites were filled with collagen sponge and divided into two groups (n = 20). One group was injected TUDCA (500μM, 25μl) and the other group was injected only PBS (0.01M). After 1 week of implantation, collagen sponges were explanted and reverse transcription-polymerase chain reaction (RT-PCR) was performed. Collagen sponge samples were homogenized and lysed in Trizol reagent (Invitrogen, Carlsbad, CA). Reverse transcription was performed using 1μg of pure total RNA and SuperScript^{TM} Ⅱ reverse transcriptase (Invitrogen), followed by PCR using primers listed in Table. Target mRNA expressions were normalized with GAPDH expression.

Collagen sponge from calvarial defect sites were explanted at 1 week and sponges were harvested for use TUNNEL assay. The collagen sponges were fixed in 4% PFA for 24 h and embedded in paraffin blocks. The blocks were sectioned transversely at a thickness of 4 μm. The TUNEL assay was performed to determine the apoptotic activity of the cells in the regenerated tissue using an ApopTag Red *in situ* apoptosis detection kit (Chemicon, Temecula,CA, USA) according to the manufacturer's instructions.

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)
GAPDH	5'-AGG CCG GTG CTG AGT ATG TCT-3'	5'-TGC CTG CTT CAC CAC CTT CT-3'
Bcl-2	5'-ACCGTCGTGACTTCGCAGAG-3'	5'-GGTGTGCAGATGCCGGTTCA-3'
BAX	5'-CGGCGAATTGGAGATGAACTG-3'	5'-GCAAAGTAGAAGAGGGCAACC-3'
Fas	5'-GCTGCAGACATGCTGTGGATC-3'	5'-TCACAGCCAGGAGAATCGCAG-3'
FasL	5'-TCCAGGGTGGGTCTACTTACTAC-3'	5'-CCCTCTTACTTCTCCGTTAGGA-3'

Table. 2.2. Primers used for RT-PCR (mouse genes).

2.7 Statistical analysis

All data were expressed as the mean±standard deviation. And all the results were statistically analyzed with oneway analysis of variance (ANOVA) with Tukey honestly significant difference post hoc test using SPSS software (SPSS Inc., Chicago, IL, USA). $p<0.005$ was considered to be statistically significant.

3. Results

3.1 Bone formation study - Microcomputed tomography image (μCT)

Type 1 collagen sponge loaded with bmp-2 and TUDCA, mouse skulls including the calvarial defect site were explanted. For analyzing the mineralization of the defect site and comparing the effect of TUDCA with BMP-2, microcomputed tomography image (μCT) was used. Fig. 3.1 represents the results. This data shows an obvious difference between collagen sponge only group and collagen sponge + TUDCA group. Also, collagen sponge + TUDCA group was more regenerated than the other groups that using BMP-2 1μg and 3μg. However, groups using 1μg and 3 μg BMP-2 only and groups using 1 and 3 μg BMP-2 with TUDCA have no significant difference between them. Meanwhile, very low mineralization was observed in no treated group (14.51 ± 8.89%). Furthermore, sustained release effect was also important for bone regeneration using TUDCA. The Collagen sponge + TUDCA group regenerated significantly higher than collagen sponge + TUDCA (single injectio at day 0) group.

Bone regeneration area (%) from examination of micro-CT image shows more distinct results (Fig. 3.2). Collagen sponge + TUDCA group represents significant regeneration rate (85.53 ± 13.79%) compare to the other group. Especially bone regeneration area of collagen sponge + TUDCA group has almost the same or higher level with the areas of collagen sponge + BMP-2 3μg (with TUDCA; and w/o TUDCA group). Moreover, collagen sponge + TUDCA group has

2.6-fold higher bone formation area than collagen sponge + TUDCA (single injection at day 0) group (85.53 ± 13.79% versus 32.42 ± 14.79%).

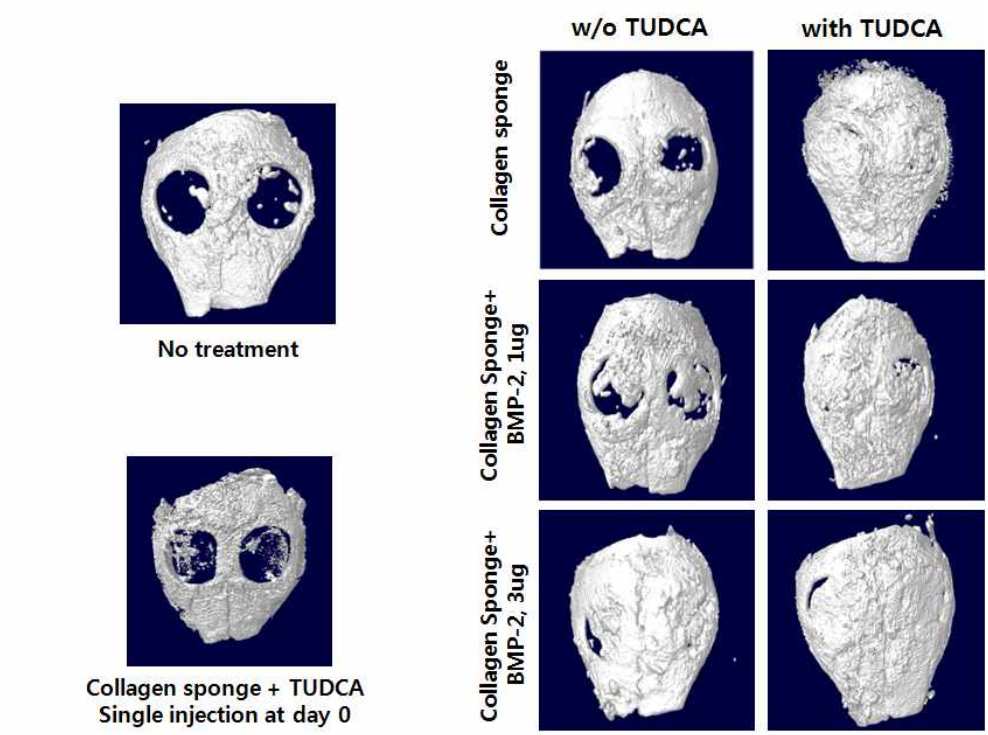


Fig. 3.1. Micro computed tomography (μCT) image.

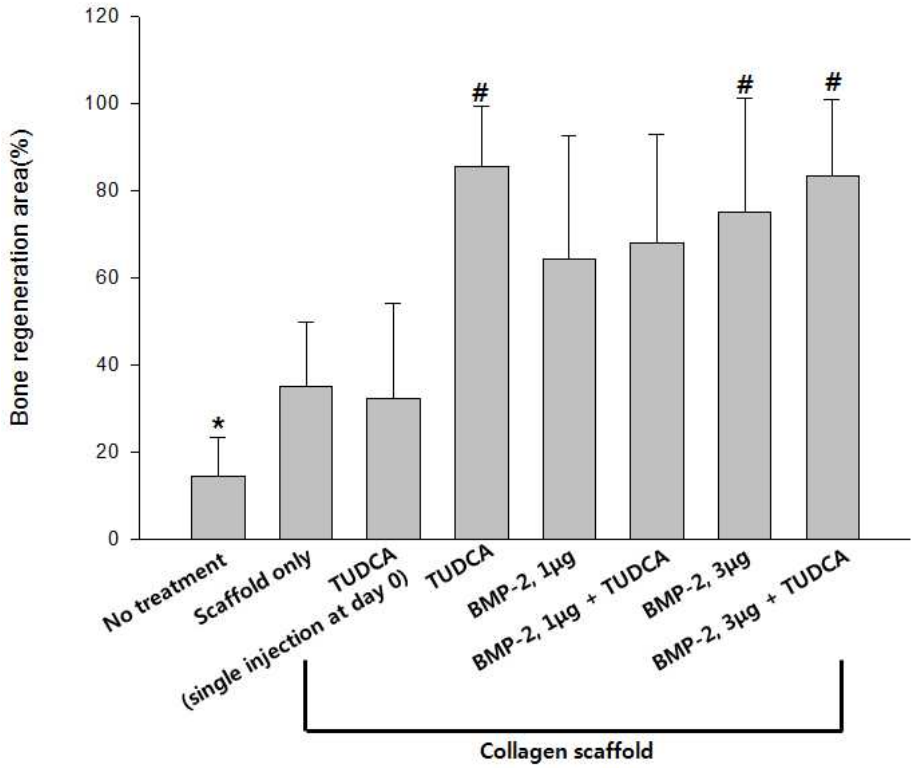


Fig. 3.2. Bone regeneration area (%) evaluated by microcomputed tomopgraphy examination. *p < 0.05 compared with any other group #p < 0.05 compared with scaffold only group and collagen sponge + TUDCA (single injection at day 0) group.

3.2 Histology analysis

Goldner's trichrome staining and H & E staining were used. Goldner's Trichrome staining is the most general staining method to confirm of bone regeneration. Density, strength, mineralization and thickness of bone as well as bone regeneration area estimated from μ CT images are important factors for evaluating the quality of regenerated bone. Goldner's Trichrome staining (Fig. 3.4) shows these factors very efficiently. Collagen scaffolds in the defect site were well degraded after 8 weeks and inflammation was not observed. Green color indicates well mineralized bone matrix. And large red structures are bone marrow. Collagens sponge only group shows very thin fibrous-like tissue and this indicates that there was no bone regeneration. However, collagens sponge + TUDCA group has much more mineralized bone matrix and bone marrow. The thickness of this group showed the most compared to the other groups.

Hematoxilin & Eosin staining results (Fig. 3.5) indicated the same result as the Goldner's trichrome staining. Collagen sponge + TUDCA group has many lacuna structure including osteocyte (red spots) and this group has much more spots than the other groups that contained BMP-2.

Bone regeneration area (%) evaluation (Fig.3.6) also shows the collagen sponge + TUDCA group have significant higher level (89.19 ± 41.89%). In comparison with collagen sponge + TUDCA (single injection at day 0), collagen sponge + TUDCA group has significant higher level of bone formation area (30.32 ± 9.68% versus 89.19 ± 41.89%).

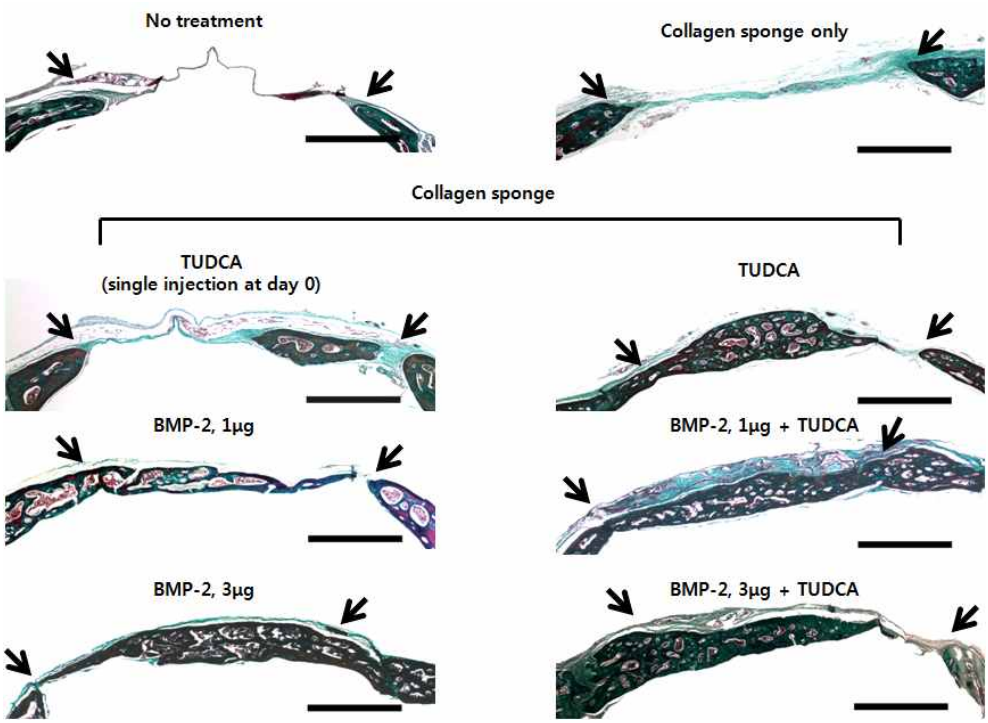


Fig. 3.3. Goldner's trichrome staining (scale bars = 1mm). Black arrows indicate the calvarial defect margins.

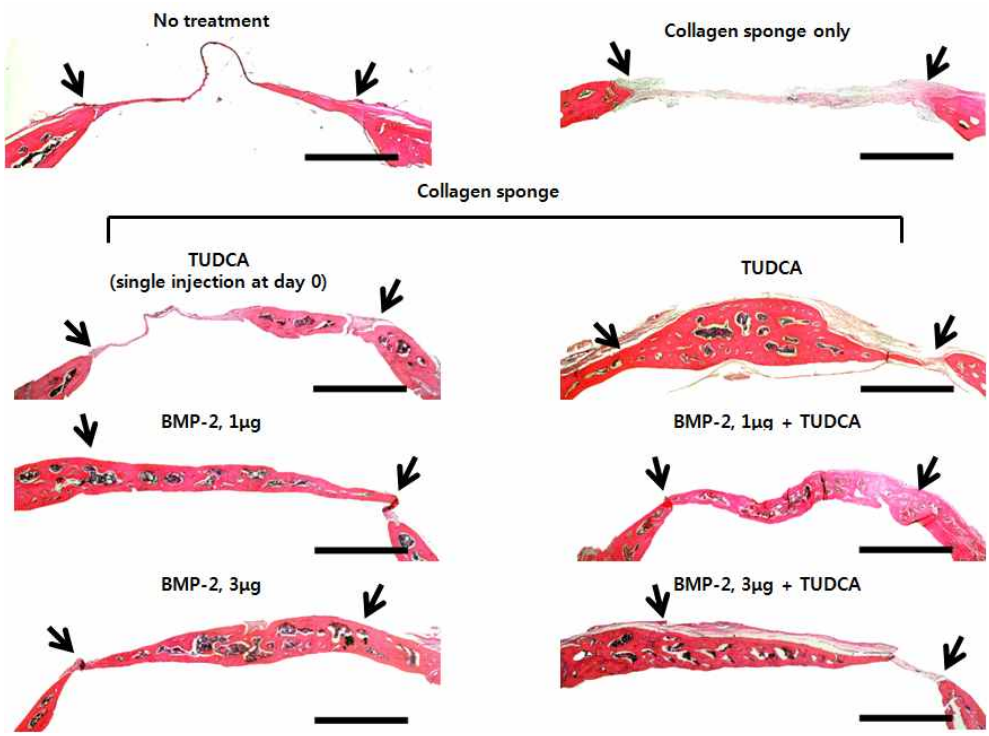


Fig. 3.4. H & E staining (scale bars = 1mm). Black arrows indicate the calvarial defect margins.

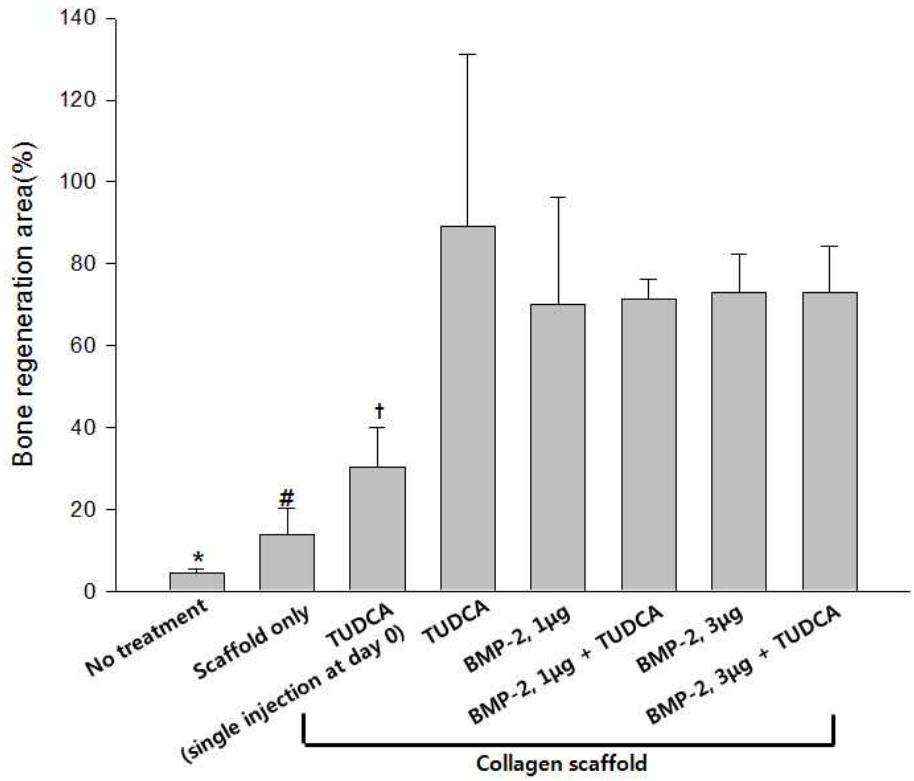


Fig. 3.5. Bone regeneration area (%) evaluated by histomorphography. *p < 0.05 compared with any other group, #p < 0.05 compared with collagen sponge group, † p < 0.05 compared TUDCA (single injection at day 0) group.

3.3 Immunohistochemistry

Fig.9 shows the immunohistochemistry staining images of osteocalcine (OC, red) at the regenerated site. No treatment and collagen sponge only groups show weak expression of osteocalcin. On the other hands, collagen sponge + TUDCA group shows that the regenerated matrix is highly positive for osteocalcin protein. Comparing with BMP-2 groups or BMP-2 + TUDCA groups, osteocalcin expression levels depend on the thickness of regenerated bone tissue.

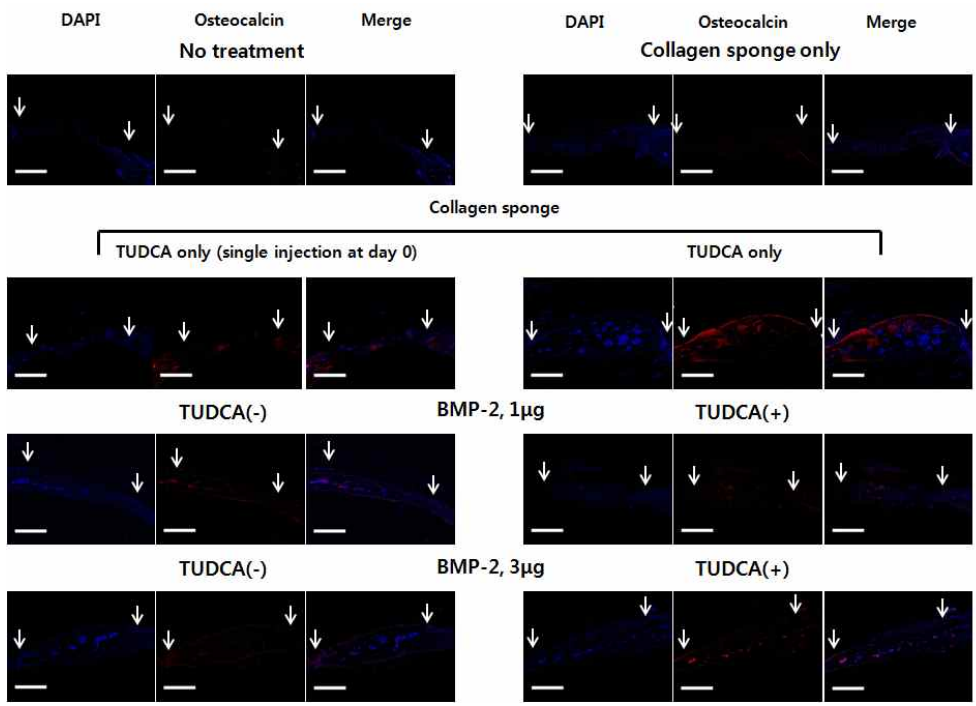


Fig. 3.6. Immunohistochemistry. Wihte arrows indicate the defect margins (scale bars = 500μm).

3.4 Apoptosis analysis

After 1 week of implanted collagen sponge, TUNERL assay was performed for measuring the apoptosis level in migrated cells into the implanted collagen sponges (Fig. 3.7A). DAPI (blue) staining indicates the healthy cells in the collagen sponges and TUNEL-positive staining (green) indicates the apoptosis-induced cell death. TUDCA (-) groups show higher TUNEL-positive level than TUDCA (+) group. This result reveals that TUDCA suppressed apoptosis and powerfully reduced apoptotic cell number.

At the same time, anti-apoptosis and pro-apoptosis signals were significantly expressed between TUDCA (+) group and TUDCA (-) group. RT-PCR analysis (Fig. 3.7B) revealed that the expression levels of BAX (pro-apoptosis), Fas (pro-apoptosis), Fas L (pro-apoptosis) and Bcl-2(anti-apoptosis). In case of TUDCA (+), Bcl-2 (anti-apoptosis) signal was highly expressed. On the other hands, pro-apoptosis signals were highly expressed in TUDCA (-) group. And TUDCA (+) group, BAX/Bcl-2 ratio was 1.5 times lower than TUDCA (-) group (Fig 10B). This indicated that TUDCA is highly suppressing the apoptosis when the bone tissue is regenerated.

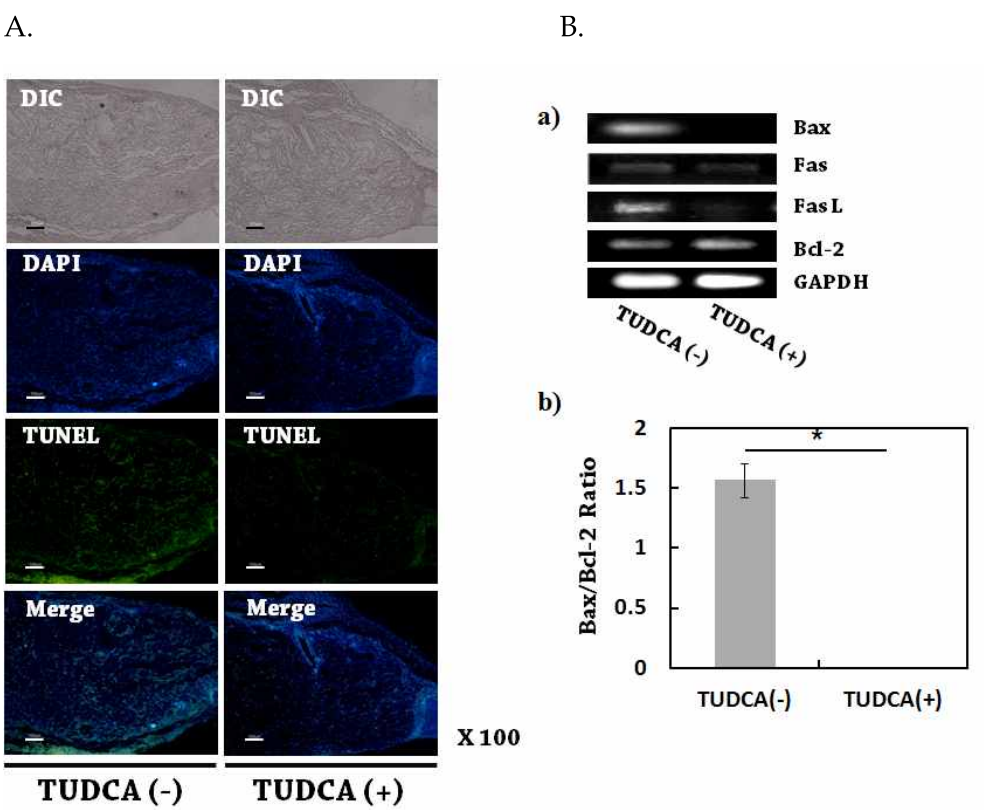


Fig 3.7. Apoptosis ananlysis. (A) TUNEL assay (scale bars = 100μm). (B) Apoptosis marker. a) RT-PCR, b) Bax/Bcl-2 Ratio (*$p < 0.05$).

4. Discussion

In the present study, multiple injections of TUDCA significantly increased new bone formation compared to control group in a mouse calvarial model. This study was set out to investigate whether addition of TUDCA could increase the bone regeneration potency of BMP-2 by inhibiting possible BMP-2-induced adipogenesis. Differ from the hypothesis, the data indicate that there is no synergistic effect of using BMP-2 in combination with TUDCA. However, surprisingly, multiple injection of TUDCA with collagen sponge scaffold implantation was as potent as other treatments using BMP-2. In addition to the regenerated area, quality of the bone is also important and it was evaluated by histology and immunohistochemistry. The results of goldner's trichrome and H&E staining to measure the bone thickness and bone matrix mineralization indicated that the collagen sponge + TUDCA group showed thicker bones and well mineralized matrix (Fig. 3.3 - 3.5). Immunohistochemistry for osteocalcin, general bone formation marker and related to pro-osteoblastic and bone-building [32], showed similar results, suggesting TUDCA by itself has bone formation ability comparable to BMP-2.

The molecular mechanism of how TUDCA increases bone formation is not yet clear, however, there are possible explanations. TUDCA, as a chemical chaperone, can reduce ER stress by inactivating the unfolded protein response, which is highly related the inhibition of adipocyte differentiation. Besides, adipocyte-derived hormone, leptin which is negatively affected bone formation is also suppressed due to adipogenesis inhibition. Generally, bone regeneration is involved in

two different linage, pre-osteoblast and pre-adipocyte progenitor cells, and the cell signaling pathway of osteoblast and adipocyte differentiation is overlapped mutually exclusive [30]. Thus, suppression of adipogenesis during bone formation can enhance the extent of bone regeneration and quality.

In addition, TUDCA acts on BAX pathway of mitochondria and Fas pathway suppress the apoptosis of cells [33-36]. So, in the same mouse model (calvarial defect model), collagen sponges were explanted after 1 week to evaluated the anti-apoptosis effect of TUDCA using TUNEL assay and RT-PCR. TUDCA (+) group shows high anti-apoptosis signal, and TUDCA (-) group shows high pro-apoptosis signals. This result indicates that TUDCA is suppressing apoptosis when bone was regenerated at the defect site (Fig. 3.7B). TUNEL assay and RT-PCR results also supports the suppression of apoptosis of TUDCA on defect sites (Fig. 3.7A). In previous studies revealed that apoptosis is critical factor for bone turn-over, fracture healing and bone regeneration [33], especially, the relation between apoptosis and ostoblast is worthy of notice. In mouse calvarial model, apoptosis of osteoblast was occurred in fractured cellus [34], and controlling the apoptosis of osteoblast during bone healing played a crucial role in regulating bone regeneration, especially early stage of differentiation of osteoblast. So it is highly possible that TUDCA suppress the apoptosis of cells during bone regeneration enhancing bone regeneration.

This study demonstrates that TUDCA with collagen sponge as scaffold enhanced bone regeneration and the quality of regenerated bone was significantly high due to its inhibition capacity of adipogenesis and apoptosis.

5. Conclusion

Tauroursodeoxycholic acid (TUDCA) enhanced bone regeneration at the critical sized defect in mice. The regenerated bone in collagen sponge + TUDCA (multiple injections) group had high bone formation area (%) and quality *in vivo* compared to the groups using BMP-2, sponge only and collagen sponge + TUDCA (single injection at day 0).

TUDCA suppressed apoptosis of cells migrated into the collagen sponges and, as a chemical chaperone, may inhibit the adipogenesis during bone formation *in vivo*. This synergic effect of inhibition adipogenesis and apoptosis would be associated with the enhanced bone regeneration. Tauroursodeoxycholicacid (TUDCA) can be used as a chemical substitute for osteogenic growth factor such as BMP-2 in bone tissue engineering.

6. References

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요약 (국문초록)

타우로우루소디옥시콜릭산을 이용한 골 재생 촉진

골형성단백질(BMPs) 은 효과적인 성장인자로 골 조직재생분야에서 광범위하게 사용되고 있다. 하지만 중간엽줄기세포 (MSCs) 를 지방세포 (Adipocyte)와 골세포 (Osteoblast)로 섬세한 제어과정 없이 동시에 분화시키기 때문에 BMPs를 사용한 골 조직 재생에서는 양질의 골 재생이 중요한 문제가 될 수 있다. 따라서, 조직재생에서 바람직하지 않은 BMPs의 이러한 효과를 최소화 하는 화합물을 찾는 것이 굉장히 중요한 의미를 가질 수 있다. 이번 연구에서는, 강력하게 세포사멸 (Apoptosis)을 억제하고, 화학적 샤페론 (Chaperone)으로써 지방분화를 억제하는 약물인 타우로우루소디옥시콜릭산 (TUDCA) 을 이용해 골 재생 효과와 그 정도를 확인하였다. TUDCA의 효과를 입증하기 위해 타입 1 콜라겐 스폰지를 지지체로 사용하여 TUDCA 단독, 혹은 TUDCA와 BMP-2을 함께 마우스 골 결손 모델에 전달하고, 그 정도를 micro computed tomography (μCT) 와 조직학 그리고 면적조직화학을 이용해 평가했다. 콜라겐 스폰지를 통해 전달된 TUDCA는 다른 군들과 비교했을 때 양질의 골을 효과적으로 재생한 것을 확인할 수 있었다. 또한 Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay와 RT-PCR 결과를 통해 콜라겐 지지체로 이동 한 세포의 사멸을 감소시키는 것을 확인했고, 이는 TUDCA로 유도된 골의 재생에 세포사멸이 중요한 요소가 될 수 있다는 가능성을 제시한다. 이번 실험 결과를 통해, TUDCA는 세포사멸과 지방 분화 억제를 통해 효과적인 골 재생 약물로서 사용될 수 있다는 가능성을 보여주었고, 나아가 BMPs의 대체제로서의 가능성도 엿볼 수 있었다.

주요어: Tauroursodeoxycholic acid (TUDCA), Bone regeneration, Bone morphogenetic proteins (BMPs), Adipogenesis, Apoptosis

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