Regulation of cellular proliferation and collagen synthesis by relaxin gene therapy in stimulated fibroblasts

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Regulation of cellular proliferation and collagen synthesis by relaxin gene therapy in stimulated fibroblasts

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Jin Soo Park

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Abstract

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Hypertrophied ligamentum flavum(LF), play a major role in the pathogenesis of lumbar spinal stenosis. The previous histological studies showed an increased amount of collagen(scarring or fibrosis), loss of elastic fiber in the thickened LF compared to normal LF. In vitro and in vivo studies, relaxin reduces collagen production, increase procollagenase synthesis and reduce the extent and severity of scarring in a number of experimental models of non-renal and renal fibrosis.

We assessed biologic effect of relaxin gene therapy in fibroblast from LF cells which is stimulated by fibrogenic growth factor. Relaxin gene therapy to fibroblast induce the relaxin receptor expression, does not decrease cell survival, increase the degradation of collagen and decrease the collagen content in cultures stimulated with growth factor.

Relaxin gene therapy suggest another modality of minimally invasive therapy in lumbar spinal stenosis with hypertrophied ligamentum flavum.

Key words : lumbar spinal stenosis, fibroblast, Relaxin, collagen

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I. INTRODUCTION

The posterior spinal structures, including hypertrophied ligamentum flavum(LF), play a major role in the pathogenesis of lumbar spinal stenosis[1,2]. Surgical treatment of symptomatic lumbar spinal stenosis(LSS) provides promising clinical results including increased quality of life and reduced morbidity. Decompressive laminectomy, facetectomy and/or spinal fusion have been a gold standard procedure in LSS. In surgical treatment of lumbar canal stenosis, the hypertrophied LF has to be removed thoroughly for complete decompression of central spinal canal and lateral recess[3,4]. In addition to radical surgery, minimally invasive procedures have been recently introduced i.e., small incision, minimal laminectomy, facetectomy and undercut flavectomy[5,6].

The previous histological studies showed an increased amount of collagen(scarring or fibrosis), loss of elastic fiber, and pseudocystic lesion in

the thickened LF compared to normal LF[7,8,9]. Relaxin, a peptide hormone and member of the insulin-like growth factor family, has traditionally been associated with growth and remodeling of the female reproductive tract during pregnancy[10,11,12]. In vitro studies of relaxin-stimulated dermal, lung and hepatic fibroblasts have demonstrated that relaxin reduces collagen production, increase procollagenase synthesis and reduce tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) expression[13-15]. Furthermore, in vivo, recombinant relaxin has been shown to reduce the extent and severity of scarring in a number of experimental models of non-renal and renal fibrosis[16].

Anti-fibrogenic and anti-scarring effect of relaxin in various tissues suggest an important mechanism of treating lumbar spinal stenosis which was caused, in part, LF hypertrophy. Therefore it can be hypothesized that the antifibrogenic effect of relaxin might reduce collagen synthesis and stimulate collagenolysis in human LF. Hence relaxin gene therapy might provide minimally invasive modality in the treatment of symptomatic LSS without violating bony structures i.e., lamina and facet.

Therefore the objectives of current experimental study were firstly, to assess biologic effect of relaxin gene therapy to fibroblasts from ligamentum flavum(LF cells), secondly, to elucidate the effect of relaxin gene therapy in LF cells stimulated by fibrogenic growth factor which might simulate accelerated scarring condition.

II. MATERIALS AND METHODS

1. Study design

Firstly, human LF cells were transduced by adenovirus relaxin gene construct(Ad/Rel), then the mRNA expression of relaxin receptor(LGR8) was analyzed with reverse transcription polymerase chain reaction(RT-PCR). Secondly, biologic effects of human LF cells to Ad/Rel mediated gene transfer were analyzed. Cellular viability was assessed by crystal violet stain and MTT assay. Apoptosis of human LF was assessed by flow cytometry and western blot analysis. Synthesis of collagens was analyzed by RT-PCR for various collagen mRNAs and total content of soluble collagen. Matrix metalloproteinase (MMP)s was assessed by RT-PCR and western blot analysis. Thirdly, human LF cells were transduced with Ad/Rel and stimulated by transforming growth factor-β1(TGF-β1) and fibroblast growth factor-1(FGF-1) then cellular proliferation, collagen synthesis, and MMP activities were analyzed. Cultures with saline control served as control.

A. *Human LF cell isolation and culture*

Specimens from the interlaminar portion of LF were collected during surgery from 12 patients(age range 25-57 years) with lumbar spinal stenosis. LF cells were then isolated from the ligament as described before[17]. The dissected specimens were minced with a scalpel. LF tissues were then digested for 60 minutes at 37°C under gentle agitation in a medium composed of equal parts of Dulbecco's Modified Eagle Medium and Ham's F-12 medium(DMEM/F12, Gibco-BRL) containing 5% heat-inactivated fetal bovine serum (FBS, Gibco-BRL) with 0.2% pronase(Sigma, St. Louis, MO) and 0.004% deoxyribonuclease II type IV(DNase, Sigma). The tissue was then washed 3 times with DMEM/F12 and digested overnight under the same conditions, with the exception of pronase being replaced with bacterial 0.02%

collagenase type II (Worthington Biochemical Corp., Lakewood, NJ). Cells were filtered through a sterile nylon mesh filter(pore size: 75um) and were then counted in a haemocytometer and plated in 24 well plates (Falcon, Franklin Lakes, NJ) at a density of approximately 5 x 10^5cells/ml. Primary cultures were sustained for 2 to 3 weeks in DMEM/F12 containing 10% FBS, 1% v/v penicillin, streptomycin and nystatin (all antibiotics from Gibco-BRL) in a 5% CO_2 incubator with humidity. Culture medium was changed twice a week.

B. ***Adenoviral vectors***

Three different adenoviral constructs were prepared for this study: Adenovirus luciferase construct(Ad/luciferase) encoding firefly luciferase, adenovirus lacZ construct(Ad/lacZ) expressing lacZ gene as a viral control, and adenovirus relaxin construct(Ad/Rel) expressing human H1 relaxin gene. Each recombinant adenoviral vector originated from replication-deficient type 5 adenovirus lacking the E1 and E3 regions of the genome[18]. Each luciferase, relaxin genes were cloned respectively into the E1 region under the control of the human cytomegalovirus early promoter. Recombinant virus was grown in transformed human embryonic kidney 293 cells and underwent CsC1 density gradient purification. Titers were determined by optical density at 260nm (OD260) and a standard plaque assay[19,20].

C. ***In vitro transduction of human LF cells***

At confluence, the LF cell cultures were rinsed with phosphate buffered saline (PBS) three times and exposed to 50 μl of HBSS containing various

doses of Ad/lacZ and Ad/Rel with various MOI. All cultures were incubated in 5% CO_2 at 37°C under humid conditions for one hour. Care was taken to prevent drying up during the transduction. Then culture medium (950 ul) was then added to each well, and the cells were further incubated in a 5% CO_2 humid environment at 37°C.

D. ***Cell viability test by crystal violet staining***

The LF cells were washed with PBS and stained with 0.5% crystal violet in 50% methanol for 10 minutes at 37℃. Deep purple stained cells were regarded as viable cells in microscopy.

E. ***MTT assay***

After incubation for 48 hour, the viability of LF cells in each well was assessed using the MTT assay[21]. In details, in triplicate: 200 μl MTT reagent(2 mg/mL culture medium) were added to each well and incubated for 3 hours in 5% CO_2 at 37°C with humidity. The supernatant was discarded and replaced with DMSO to dissolve the formazan product, which was measured at 550 nm in a spectrophotometric plate reader.

F. ***Detection of apoptosis***

LF cells were harvested and washed with PBS twice, and resuspended at 2 x 10^6 cells/ml in 1 x Annexin-V Binding Buffer. Subsequently, the cells were stained with propidium idodide buffer for 15 minutes to detect apoptotic cells

and then analyzed by flow cytometry. The expressions of caspase 3, 9 were detected with western blot analysis.

G. *Reverse transcription-polymerase chain reaction*

Total cellular RNA was isolated using RNeasy kit (Qiagen). cDNA was synthesized from 1μg total RNA using Accupower RT premix(Bioneer) in a 50μl reaction mixture. One μl cDNA was amplified in a 20μl reaction of Accupower PCR premix(Bioneer). Amplification reactions were performed for the following genes: β-actin, MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, type I collagen, type II collagen, type III collagen, and type XI collagen. PCR products(3μl) were analyzed by electrophoresis in 2% agarose gels, and detected by staining with ethidium bromide. The intensity of the PCR products was quantified using the BioImage Visage 110 system.

H. *Total collagen content*

Total content of soluble collagen was assessed with Sircol soluble collagen assay kit according to the manufacture's instruction.

I. *Western blot analysis*

The whole cell lysates were separated on 10% SDS-PAGE and transblotted onto Hybond-P membrane. Proteins were immunoblotted with anti-α-tublin(Santa Cruz), anti-MMP-1, caspase 3, and capase 9(Calbiochem). Western blot analysis was performed according to the rapid detection protocol provided by Amersham Pharmacia.

J. *Flurokine assay for MMP-1 and 13 activities*

Enzyme activities of MMP-1 and MMP-13 were measured using Flurokine assay.

K. *Statistics*

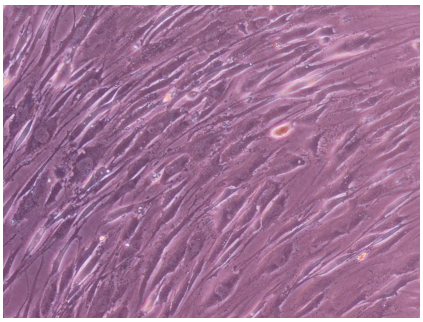
One-way analysis of variance with Fisher's protested LSD postdoc test was performed to test difference in densitometry data. Significance level was set as $p<0.05$.

III. RESULTS

1. *Transduction of LF with Ad/Rel*

Human LF cells transduced with Ad/Rel with an MOI of 150 demonstrated oval shaped morphology compared to control culture with Ad/lacZ at day 3 (Figure 1).

<LF cells>



<LF cells with Ad/Rel>

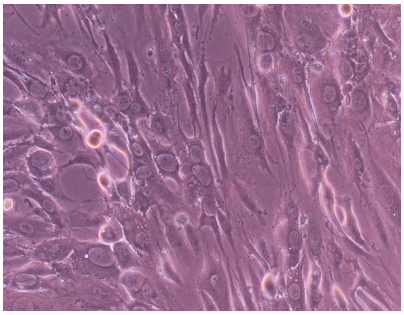


Figure 1. Morphology of Ad/Rel(100MOI)-transduced ligamentum flavum cells (X100). *LF: ligamentum flavum, Ad/Rel: adenovirus relaxin gene construct

Human LF cells with Ad/Rel with an MOI of 150 showed robust expression of relaxin H1 receptor mRNA (LGR8) at day 3 in reverse transcription polymerase chain reaction(Figure 2).

Figure 2. Reverse transcription polymerase chain reaction of human ligamentum flavum cells with Ad/Rel.

*Ad/Rel: adenovirus relaxin gene construct, Con: control

LGR: leucine-rich repeat -containing G protein-coupled receptor

LacZ: adenovirus LacZ gene construct, Rel: adenovirus Relaxin gene construct

2. *Cellular viability after transduction of Ad/Rel*

Crystal violet staining of human LF cells demonstrated no recognizable difference between cultures with Ad/lacZ and Ad/Rel with MOIs of 0, 1, 2.5, 5, 10, and 50 at day 3(Figure 3).

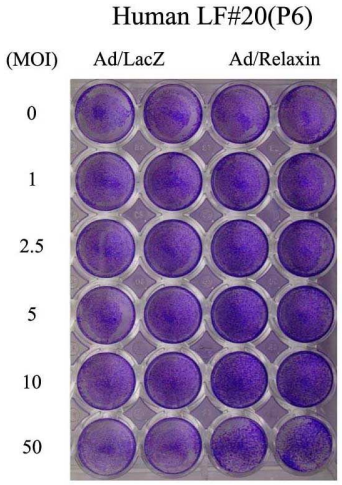


Figure 3. Crystal violet staining of Ad/Relaxin -transduced ligamentum flavum cells.

*Ad/lacZ: adenovirus lacZ gene construct

Ad/Relaxin: adenovirus relaxin gene construct

MTT assay showed that overexpression of relaxin in human LF cell did not render cytotoxic effect in MOIs of 0, 10, 25, 50, 75, and 100 compared to Ad/LacZ at day 3 and 7(Figure 4).

<Day3> <Day7>

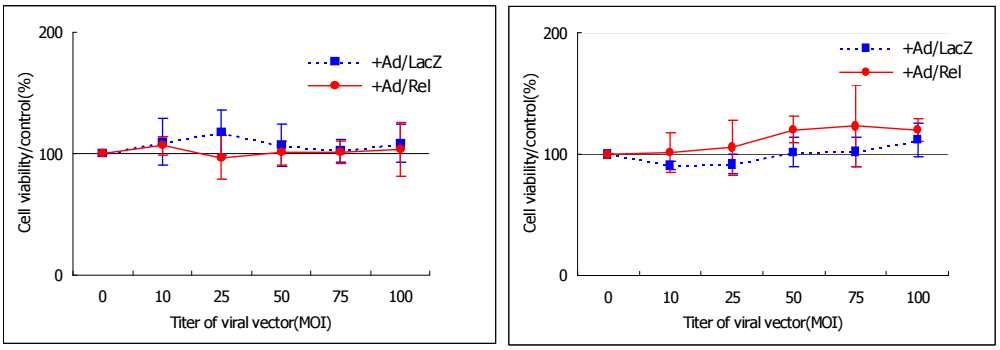


Figure 4. MTT assay of human ligamentum flavum cells with adenovirus relaxin gene construct.

*Ad/lacZ: adenovirus lacZ gene construct,

Ad/Rel: adenovirus relaxin gene construct

3. *Apoptosis of LF cells after transduction of Ad/Rel*

With an apoptosis assay of flow cytometry, human LF cells after transduction of Ad/Rel with MOIs of 0, 50, 75, and 150 demonstrated no difference in apoptotic rate compared to human LF cells with Ad/lacZ with same dosage of virus at day 3(Figure 5). Furthermore, with an apoptotic assay using caspase 3 and 9, human LF cells with Ad/Rel with an MOIs of 150 showed no recognizable difference compared to cells with Ad/lacZ at day 1 (Figure 6).

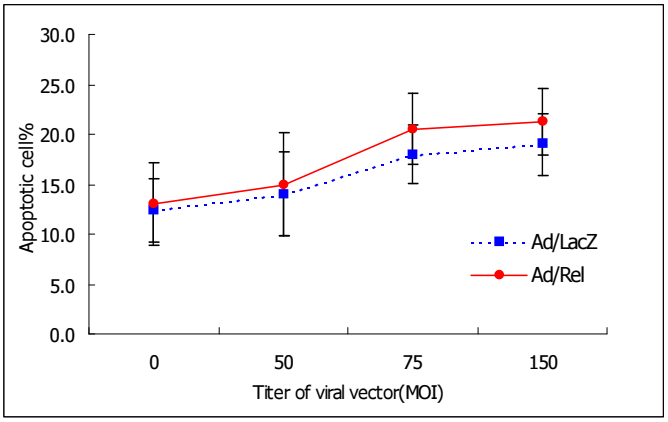


Figure 5. Flow cytometry of human ligamentum flavum cells after transduction of Ad/Rel.

*Ad/lacZ: adenovirus lacZ gene construct

Ad/Rel: adenovirus relaxin gene construct

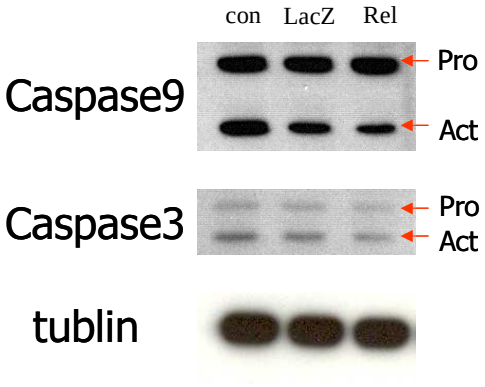


Figure 6. Western blot analysis using caspase 3 and 9 for apoptotic assay of human ligamentum flavum cells with Ad/Rel.

*Con: control, LacZ: adenovirus LacZ gene construct, Rel: adenovirus Relaxin gene construct, Pro: proenzyme, Act: activated enzyme

4. ***Expression of MMPs mRNA***

The expression of various MMPs(MMP-1, 2, 3, 9, and 13) mRNA demonstrated statistically significant increase of MMP-1, MMP-9, and MMP-13 mRNA expression compared to viral control i.e., LF cells with Ad/lacZ and saline control($p<0.05$,Figure 7).

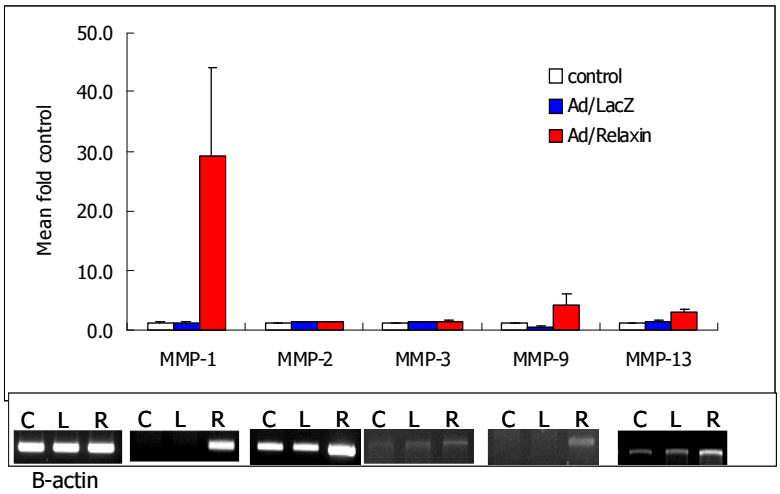


Figure 7. The expression of various matrix metalloproteinase(MMP) mRNAs. *Control(**C**): cultures with saline, Ad/lacZ(**L**): adenovirus lacZ gene construct Ad/Relaxin(**R**): adenovirus relaxin gene construct, MMP: matrix metaloproteinase

5. ***MMP-1 protein expression***

With various concentration of Ad/Rel(i.e., 0, 10, 25, 50, 75, and 100 MOI), human LF cells revealed dose responsive increase in MMP-1 protein expression at day 3 and 7(Figure 8).

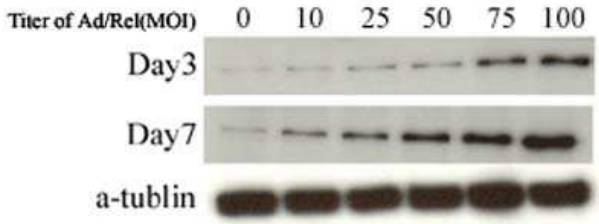


Figure 8. Human ligamentum flavum cells with various concentrations of adenovirus relaxin gene construct.

*Ad/Relaxin: adenovirus relaxin gene construct

6. *Enzyme activities of MMP-1 and MMP-13*

Human LF cells with Ad/Rel with an MOI of 100 showed statistically significant increase in enzyme activities of MMP-1 and MMP-13 compared to LF cells with Ad/lacZ and saline control($p<0.05$, Figure 9).

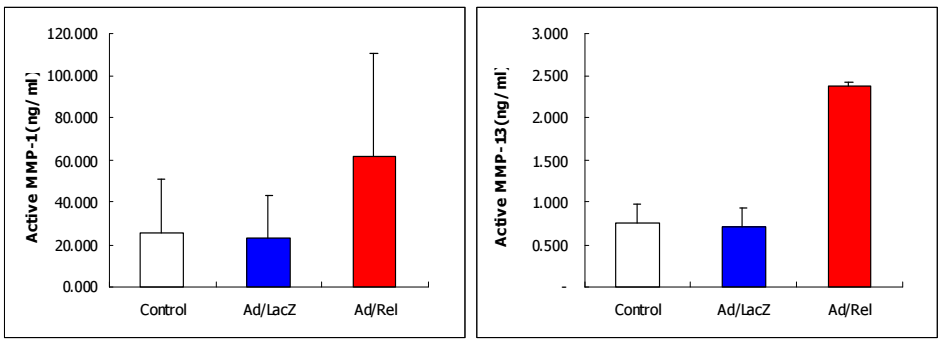


Figure 9. Increased MMP-1,13 activities in human ligamentum flavum cells with adenovirus relaxin gene construct.

*MMP: matrix metaloproteinase, Control: cultures with saline, Ad/lacZ: adenovirus lacZ gene construct, Ad/Relaxin: adenovirus relaxin gene construct

7. *Total content of collagen*

Human LF cells after transduction of Ad/Rel with an MOI of 100 demonstrated statistically significant decrease in total content of collagen compared to LF cells with Ad/lacZ and saline control as analyzed by Sircol method($p<0.05$, Figure 10).

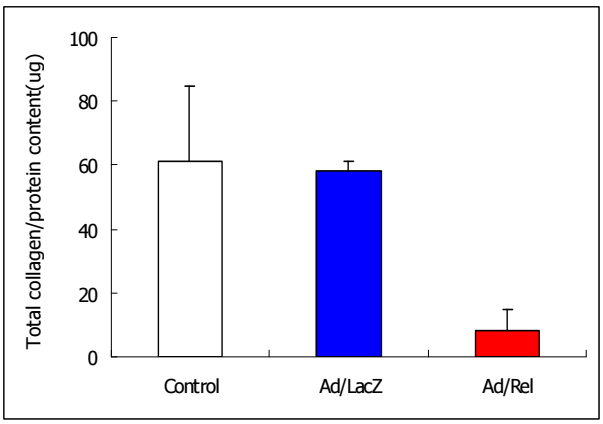


Figure 10. Decrease in total collagen content in human ligamentum flavum cells with adenovirus relaxin gene construct.

*Control: cultures with saline, Ad/lacZ: adenovirus lacZ gene construct Ad/Relaxin:adenovirus relaxin gene construct

8. *Human LF cells with Ad/Rel in response to TGF-β1 and FGF-1*

Human LF cells were allocated to experimental group(cells transduced by Ad/Rel with an MOI of 75) and control group (cells with saline control). Then cultures were stimulated with TGF-β1 and FGF-1. Stimulated human LF cells with TGF-β1 and FGF-1 demonstrated significant increase in total collagen content about 145% compared to control cultures which were LF

culture without growth factor stimulation($p<0.05$). Human LF cells with Ad/Rel without growth factor stimulation showed decrease in total collagen content which was 75% of control cultures($p<0.05$). Human LF cells with Ad/Rel stimulated with TGF-β1 and FGF-1 demonstrated increase in total collagen content about 119% of control ($p<0.05$), however this culture showed decrease in total collagen content compared to that of LF cell cultures stimulated with TGF-β1 and FGF-1alone(without Ad/ Rel) which was 82%($p<0.05$, Figure 11).

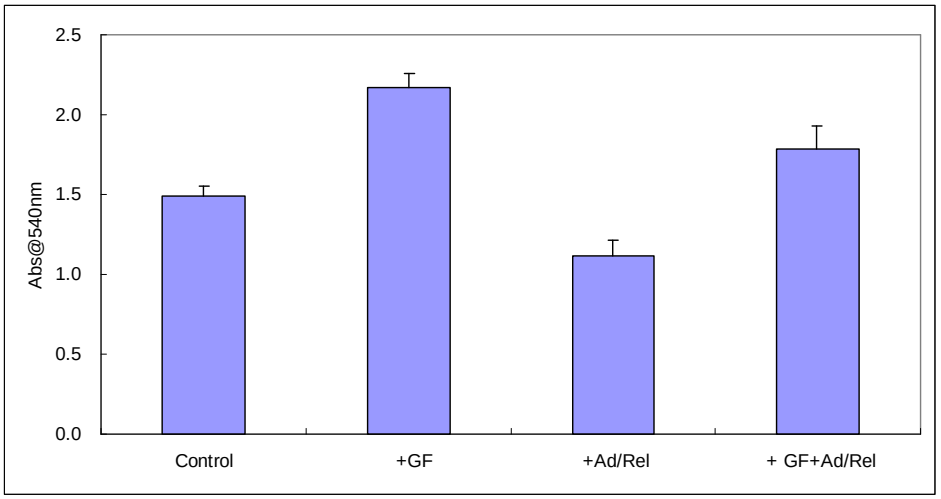


Figure 11. Total collagen content in human ligamentum flavum cells stimulated with growth factor. There was decrease in total collagen content in cells stimulated with growth factor(transforming growth factor-β1 and fibroblast growth factor-1) and transduced with adenovirus relaxin gene construct(+GF+Ad/Rel) compared to cells with only growth factor simulation(+GF).

*Control: cultures with saline, +GF: stimulated with growth hormone

+Ad/Rel: transduced by adenovirus relaxin gene construct

+GF+Ad/Rel: stimulated with growth hormone and transduced with Ad/Rel

IV. DISCUSSION

Fibrosis and scarring render significant morbidity in human organs[10,22]. Various attempts has been introduced to prevent fibrosis and scarring of human organs and tissues[23-26]. Among the attempts relaxin therapy has promising perspectives in the treatment of fibrosis. Numerous studies demonstrated antifibrogenic, anti-scarring effect of relaxin in various conditions involving kidney, liver, and skin[13-15]. Clinical application of relaxin therapy is based upon the mechanism of anti-collagen synthesis and collagenolysis effect mediated by direct inhibition of collagen synthesis, increased activity of procollagenase and inhibition of tissue inhibitor of matrix metaloproteinase (TIMP)[15,27,28].

Lumbar spinal stenosis render significant morbidity including lower back pain, leg pain, and neurogenic intermittent claudication which mandates surgical treatment to relieve symptoms[1,2]. Hypertrophied ligamentum flavum(LF) plays a crucial role in pathogenesis of lumbar spinal stenosis. Therefore, hypertrophied ligamentum flavum should be removed during the surgical intervention[3,4].

Hence it can be hypothesized that antifibrogenic effect of relaxin might provide important mechanism to decompress stenotic spinal canal in lumbar spinal stenosis by reducing thickness of ligamentum flavum. Accordingly, in this experimental study, relaxin gene therapy to human ligamentum flavum was tried to propose feasibility of relaxin as treatment modality in lumbar spinal stenosis.

At first, transduction of human LF cells with adenovirus relaxin gene construct(Ad/Rel) was accomplished to demonstrate feasibility of relaxin gene therapy to human LF cells. Secondly biologic effects of relaxin gene therapy

in terms of cellular proliferation, collagen synthesis, collagenase activity, and apoptosis were assessed. Finally anti-fibrogenic effect of relaxin gene therapy tested using stimulated human LF cells with TGF-β1 and FGF-1.

The results of the current study demonstrated human LF cells were susceptible to adenovirus mediated relaxin gene transfer and transgene expresson was confirmed indirectly by the expression of relaxin receptor in RT-PCR. Human LF cells transduced by Ad/Rel gene demonstrated no significant differences in cellular survival, proliferation, and apoptosis as assessed with crystal violet stain, MTT assay, flow cytometry and western blot analysis for caspase 3 and 9. Furthermore, mRNA expression of collagenolytic enzymes i.e., matrix metaloproteinase(MMP)-1, 9 and 13 were markedly increased in human LF cells transduced with Ad/Rel gene compared to viral and saline control. Enzyme activities of MMP-1 and 13 were also increased in the LF cells with Ad/Rel gene. Relaxin gene therapy to human LF cells which were stimulated with TGF-β1 and FGF-1 prior to gene transfer demonstrated reduced collagen content compared to cells stimulated with TGF-β1 and FGF-1 only. In summary, firstly, relaxin gene therapy did not affect mitogenesis of human LF cells. Secondly, relaxin gene therapy down-regulated total collagen synthesis i.e., type I, II, III, and XI at the level of mRNA, also down-regulated total collagen synthesis. Thirdly, relaxin gene therapy up-regulated the expression of MMP-1, MMP-9, and MMP-13 at the level of mRNA and increased enzyme activities of MMP-1 and MMP-13. Lastly, relaxin gene therapy to human LF cells stimulated with TGF-β1 and FGF-1 demonstrated about 18% decrease in total collagen synthesis compared to cultures stimulated with TGF-β1 and FGF-1. Anti-collagen synthesis and collagenolytic effects which were proved in this study render important clinical significance in the treatment of lumbar spinal stenosis. Hypetrophied

ligamentum flavum might be susceptible to relaxin gene therapy, in consequence, thinning of hypertrophied ligamentum flavum by relaxin gene therapy. As minimally invasive treatment of lumbar spinal stenosis, it would be possible to introduced relaxin gene and obtain thinning of ligamentum flavum by the mechanism of decrease in collagen synthesis and increase in collagenolytic enzyme i.e., MMP-1, MMP-3 and MMP-13.

The clinical and physiological significance of this in vitro results obtained with relaxin gene therapy have to be confirmed in an in vivo system i.e., animal model for lumbar spinal stenosis. Nevertheless, it was interesting to observe that relaxin gene therapy can significantly modify the abundant collagens. These result demonstrated indirectly that relaxin gene therapy appear to be feasible as medical treatment of lumbar spinal stenosis with hypertrophied ligamentum flavum.

V. CONCLUSION

Relaxin gene therapy did not affect mitogenesis of human LF cells. Relaxin gene therapy down-regulated collagen synthesis and up-regulated the expression of matrix metaloproteinases and their enzyme activities. Furtherore, relaxin gene therapy to human fibroblasts from ligamentum flavum stimulated with TGF-β1 and FGF-1 demonstrated about 18% decrease in total collagen synthesis compared to that of control.

Relaxin gene therapy suggest another modality of minimally invasive therapy in lumbar spinal stenosis with hypertrophied ligamentum flavum by inhibiting collagen synthesis and promoting collagen degradation.

REFERENCES

1. Saint-Louis LA. Lumbar spinal stenosis assessment with computed tomography, magnetic resonance imaging, and myelography. Clin Orthop Relat Res 2001:122-36.
2. Szpalski M, Gunzburg R. Lumbar spinal stenosis in the elderly: an overview. Eur Spine J 2003;12 Suppl 2:S170-5.
3. Costa F, Sassi M, Cardia A, Ortolina A, De Santis A, Luccarell G, et al. Degenerative lumbar spinal stenosis: analysis of results in a series of 374 patients treated with unilateral laminotomy for bilateral microdecompression. J Neurosurg Spine 2007;7:579-86.
4. Kornblum MB, Fischgrund JS, Herkowitz HN, Abraham DA, Berkower DL, Ditkoff JS. Degenerative lumbar spondylolisthesis with spinal stenosis: a prospective long-term study comparing fusion and pseudarthrosis. Spine 2004;29:726-33; discussion 33-4.
5. Sasaki M, Abekura M, Morris S, Akiyama C, Kaise K, Yuguchi T, et al. Microscopic bilateral decompression through unilateral laminotomy for lumbar canal stenosis in patients undergoing hemodialysis. J Neurosurg Spine 2006;5:494-9.
6. Fu YS, Zeng BF, Xu JG. Long-term outcomes of two different decompressive techniques for lumbar spinal stenosis. Spine 2008;33:514-8.
7. Sairyo K, Biyani A, Goel VK, Leaman DW, Booth R, Jr., Thomas J, et al. Lumbar ligamentum flavum hypertrophy is due to accumulation of inflammation-related scar tissue. Spine 2007;32:E340-7.
8. Kosaka H, Sairyo K, Biyani A, Leaman D, Yeasting R, Higashino K, et al. Pathomechanism of loss of elasticity and hypertrophy of lumbar ligamentum flavum in elderly patients with lumbar spinal canal stenosis. Spine 2007;32:2805-11.
9. Wildi LM, Kurrer MO, Benini A, Weishaupt D, Michel BA, Bruhlmann P.

Pseudocystic degeneration of the lumbar ligamentum flavum: a little known entity. J Spinal Disord Tech 2004;17:395-400.
10. Samuel CS. Relaxin: antifibrotic properties and effects in models of disease. Clin Med Res 2005;3:241-9.
11. Park JI, Chang CL, Hsu SY. New Insights into biological roles of relaxin and relaxin-related peptides. Rev Endocr Metab Disord 2005;6:291-6.
12. Hayes ES. Biology of primate relaxin: a paracrine signal in early pregnancy. Reprod Biol Endocrinol 2004;2:36.
13. Unemori EN, Amento EP. Relaxin modulates synthesis and secretion of procollagenase and collagen by human dermal fibroblasts. J Biol Chem 1990;265:10681-5.
14. Mookerjee I, Solly NR, Royce SG, Tregear GW, Samuel CS, Tang ML. Endogenous relaxin regulates collagen deposition in an animal model of allergic airway disease. Endocrinology 2006;147:754-61.
15. Bennett RG, Dalton SR, Mahan KJ, Gentry-Nielsen MJ, Hamel FG, Tuma DJ. Relaxin receptors in hepatic stellate cells and cirrhotic liver. Biochem Pharmacol 2007;73:1033-40.
16. Lekgabe ED, Kiriazis H, Zhao C, Xu Q, Moore XL, Su Y, et al. Relaxin reverses cardiac and renal fibrosis in spontaneously hypertensive rats. Hypertension 2005;46:412-8.
17. Specchia N, Pagnotta A, Gigante A, Logroscino G, Toesca A. Characterization of cultured human ligamentum flavum cells in lumbar spine stenosis. J Orthop Res 2001;19:294-300.
18. Robbins PD, Evans CH, Chernajovsky Y. Gene therapy for arthritis. Gene Ther 2003;10:902-11.
19. Graham FL, van der Eb AJ. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 1973;52:456-67.
20. Mittereder N, March KL, Trapnell BC. Evaluation of the concentration and bioactivity of adenovirus vectors for gene therapy. J Virol 1996;70:7498-509.

21. Kostoryz EL, Tong PY, Chappelow CC, Eick JD, Glaros AG, Yourtee DM. In vitro cytotoxicity of solid epoxy-based dental resins and their components. Dent Mater 1999;15:363-73.
22. Franklin TJ. Therapeutic approaches to organ fibrosis. Int J Biochem Cell Biol 1997;29:79-89.
23. Lee JY, Stenzel W, Impekoven P, Theisohn M, Stutzer H, Lohr M, et al. The effect of mitomycin C in reducing epidural fibrosis after lumbar laminectomy in rats. J Neurosurg Spine 2006;5:53-60.
24. Tatsui CE, Martinez G, Li X, Pattany P, Levi AD. Evaluation of DuraGen in preventing peridural fibrosis in rabbits. Invited submission from the Joint Section Meeting on Disorders of the Spine and Peripheral Nerves, March 2005. J Neurosurg Spine 2006;4:51-9.
25. Schimizzi AL, Massie JB, Murphy M, Perry A, Kim CW, Garfin SR, et al. High-molecular-weight hyaluronan inhibits macrophage proliferation and cytokine release in the early wound of a preclinical postlaminectomy rat model. Spine J 2006;6:550-6.
26. Lee BJ, Wang SG, Lee JC, Jung JS, Bae YC, Jeong HJ, et al. The prevention of vocal fold scarring using autologous adipose tissue-derived stromal cells. Cells Tissues Organs 2006;184:198-204.
27. Samuel CS, Hewitson TD, Unemori EN, Tang ML. Drugs of the future: the hormone relaxin. Cell Mol Life Sci 2007;64:1539-57.
28. Van Der Westhuizen ET, Summers RJ, Halls ML, Bathgate RA, Sexton PM. Relaxin receptors--new drug targets for multiple disease states. Curr Drug Targets 2007;8:91-104.

abstract(in Korean)

자극된 섬유세포에서 릴렉신 유전자 전달을 이용한 세포증식 및 교원질 생성 조절

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요추부 척추관 협착증에서 황색인대의 비후는 주요 원인인자중의 하나이다. 이에 대한 지금까지의 연구 결과를 보면 정상적인 황색인대에 비해 척추관 협착증에서의 비후된 황색인대에서는 탄성섬유질이 소실되고 반흔 또는 섬유화에 의해 교원질의 양이 증가한다고 알려져 있다. 신장조직, 혹은 그 외의 조직의 섬유화에 관한 여러 가지 실험적 모델에서도 릴렉신은 교원질의 생성을 감소시키고 교원질 분해효소의 생성을 증가시키며 반흔의 양과 정도를 감소시킨다고 알려져 있다.

본 논문에서는 황색인대로부터 추출한 섬유세포에서 섬유화가 진행되도록 섬유화 유도 성장인자로 자극한 뒤 다시 릴렉신 유전자 치료를 했을때 나타나는 생물학적인 변화를 측정하였다. 섬유세포에서 릴렉신 유전자치료는 릴렉신 수용체를 발현시키고, 세포의 생존에는 영향을 미치지 않으며, 교원질의 분해를 증가시키는 것으로 나타났고, 성장인자의 자극에 의해 섬유화가 촉진되도록 자극된 섬유세포에서도 교원질의 양을 감소시키는 것으로 나타났다.

릴렉신 유전자 치료는 비후되어 있는 황색인대가 원인이 되는 척추관 협착증에서 또 하나의 최소 침습적 치료의 가능성을 제시해줄 수 있을 것으로 기대된다.

핵심되는 말 : 척추관 협착증, 섬유세포, 릴렉신, 교원질