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Direct chitosan-mediated gene delivery to the rabbit knee joints in vitro and in vivo ☆

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Abstract

Chitosan vector system is expected to be useful for direct gene therapy for joint disease. This study first sought to confirm that foreign genes can be transferred to articular chondrocytes in primary culture. Next, chitosan–DNA nanoparticles containing IL-1Ra or IL-10 gene were injected directly into the knee joint cavities of osteoarthritis rabbits to clarify the in vivo transfer availability of the chitosan vectors. Clear expression of IL-1Ra was detected in the knee joint synovial fluid of the chitosan IL-1Ra-injected group. While no expression was detected in the chitosan IL-10-injected group, this demonstrates that the transfection efficiency of chitosan–DNA nanoparticles was closely related to the type of the gene product. A significant reduction was also noted in the severity of histologic cartilage lesions in the group that received the chitosan IL-1Ra injection. This avenue may therefore represent a promising future treatment for osteoarthritis.

Keywords: Gene therapy; Citosan; Chondrocyte; Osteoarthritis; Knee joint

Gene therapy is a powerful treatment for curing inborn and acquired diseases. There are primarily two types of gene carriers: viral and non-viral vectors, such as retrovirus, adenovirus, etc., which have been utilized for approximately 80% of approved phase I clinical trials due to their ability to transfect [1]. However, they have limitations in vivo such as wild-type reversion and immunogenicity [2]. Non-viral delivery systems for gene therapy have been increasingly proposed as safer alternatives to viral vectors [3,4]. They have the potential to be administered repeatedly with minimal host immune response, are targetable, stable in storage, and easy to produce in large quantities. These advantages have provided the impetus to continue their development. Cationic phospholipids and cationic polymers are the two major types of non-viral gene delivery vectors currently investigated [5]. Because of their permanent cationic charge, both types interact electrostatically with negatively charged DNA and form complexes. Although liposomes formed from cationic phospholipids offer several advantages over viral gene transfer, e.g., low immunogenicity and ease of preparation [6], the success of the liposomal approach is limited. Toxicity of the cationic lipids and the relatively low transfection efficiency compared to those of viral gene delivery vectors [7] are the main disadvantages.

In recent years, the potential of chitosan as a polycationic gene carrier has been explored in several research groups.

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Chitosan is a biodegradable polysaccharide [8] extracted from crustacean shells. It has been shown to be non-toxic in a range of toxicity tests, both in experimental animals [9] and humans [10]. Its soft tissue compatibility has also been demonstrated by Tomihata and Ikada [11]. Chitosan has been shown to effectively bind DNA in saline or acetic acid solution and partially protect DNA from nuclease degradation [12,13]. Several transfection studies involving chitosan derivatives have been reported in the last few years. Chitosan polymers and chitosan–DNA complexes have been investigated for their ability to condense and deliver plasmid DNA in Cos-1 cells, HeLa cells, Hep-G2 cells, and 293 human embryonal kidney cells, so on [12,14,15]. Chitosan–DNA nanospheres were prepared by self-induced complex coacervation method to yield particles in the size range of 200–750 nm these nanospheres being efficient vectors in the Luciferase-293 cell system. However, their transfection efficiency varied when tested in different cell lines [7]. In vivo gene expression is observed in the upper small intestine and colon of rabbits by direct administration of these complexes including a pH-sensitive endosomolytic peptide [12]. The chitosan–DNA nanoparticles formed as a result of complex coacervation between chitosan and DNA. The cationic characteristic of chitosan is a crucial parameter for the complex formation. Zetasizer measurement of these particles showed a fairly unimodal distribution from 150 to 300 nm[16]. In another study, oral application of chitosan nanoparticles bearing a plasmid encoding for blood coagulation factor (FIX) resulted in detectable FIX plasma levels, which gradually declined over a time period of 14 days [5]. All these studies point to the versatility and promise of chitosan as a gene carrier, although only limited in vivo efficacy has been demonstrated.

Due to the complex pathology of osteoarthritis (OA), gene transfer strategies have become of major interest for interfering with key processes of disease. Specifically, gene therapy has been associated with a highly specific targeting of disease-relevant mechanisms and thus with treating the causes of OA rather than the symptoms. In recent years, several strategies have been developed to modulate OA using gene transfer. Among them, gene transfer with the interleukin-1 receptor antagonist (IL-1Ra) has been of particular importance. This is based on several data suggesting that interleukin-1 beta (IL-1β) plays a pivotal role both in early and late stages of OA, and is produced by synoviocytes and chondrocytes [17]. Previous studies demonstrated that the local production of the IL-1Ra significantly reduces the OA cartilage breakdown [18]. Another cytokine of particular interest as a therapeutic agent for OA is interleukin-10 (IL-10). IL-10 is a 35-kDa homodimeric cytokine product of Th2 cells, B cells, and macrophages. Primarily, IL-10 can act as an anti-inflammatory by inhibiting synthesis of macrophage-derived pro-inflammatory cytokines, such as TNF-α, IL-1α, IL-1β, IL-6, and IL-8 [19–22]. IL-10 is elevated in the serum of OA patients [23].

Here, we first sought to confirm whether foreign genes can be transferred to articular chondrocytes in primary culture by chitosan–EGFP nanoparticles. Then, we injected chitosan–DNA nanoparticles containing IL-1Ra gene or IL-10 gene into the OA rabbit knee joints and examined: (1) the site of the transgene expression and the efficiency of gene transduction and (2) the efficiency of in vivo gene therapy through the direct delivery of chitosan–DNA nanoparticles in a rabbit model of OA, as a new therapeutic approach to control the progression of the disease.

Materials and methods

Plasmid constructs and cell culture. During this study, three plasmids were used. pEGFP-C_3 (4.7 kb) was an enhanced green fluorescence protein (EGFP) gene construct with a cytomegalovirus (CMV) promoter (Clontech). Another plasmid PcDNA3.1-IL-1Ra was a 6.1 kb cDNA encoding human IL-1Ra driven by a CMV promoter inserted into an Invitrogen PcDNA3.1 vector, while PcDNA3.1-IL-10 encoding the human IL-10 with a size of 6.5 kb driven by the CMV promoter inserted into an Invitrogen PcDNA3.1 vector. These plasmids were amplified and purified by the differential precipitation method [24].

Chondrocytes were isolated from the articular cartilage of the knees of two young adult New Zealand rabbits, as previously described [25]. The chondrocytes used in this study were maintained as monolayer cultures for no more than two passages, to maintain the differentiated chondrocyte phenotype.

Primary synoviocytes were obtained by enzymatic dispersal of synovia from the knee joints of euthanized, young adult New Zealand white rabbits as previously described [26].

Preparation of chitosan–DNA nanoparticles. Chitosan (minimum 85% deacetylated) was purchased from Sigma–Aldrich (St. Louis, MO, USA). A solution of 0.02% chitosan was prepared by dissolving the chitosan in 25 mM of sodium acetate buffer. The pH of the chitosan solution was adjusted to 5.7 with sodium hydroxide and sterile-filtered through a 0.22-μm filter.

A chitosan solution (0.02% in 25 mM sodium acetate buffer, pH 5.7) and a DNA solution of 100 μg/ml in 50 mM of sodium sulfate solution were preheated to 55 °C separately. An equal volume of both solutions was quickly mixed together and vortexed (2500 rpm) for 30 s. The final volume of the mixture in each preparation was limited to below 500 μl in order to yield uniform nanoparticles. The nanoparticles were used for transfection study without further purification.

In vitro transfection of chondrocytes and synoviocytes with chitosan–DNA nanoparticles. Cells were seeded 24 h prior to transfection into a 12-well plate at a density of 8×10^4 cells/well in 1 ml of complete medium (MEM containing 10% FBS, supplemented with 2 mM L-glutamate, 50 U/ml penicillin, and 50 μg/ml streptomycin). At the time of transfection, the medium in each well was replaced with 1 ml of serum-free media. The amount of chitosan–DNA nanoparticles (chitosan–EGFP) equivalent to 2–4 μg DNA of pEGFP-C_3 was added to each well and incubated with the cells for 8 h after further incubation for 24 h by adding 1 ml of fresh complete medium, the medium in each well was replaced with fresh complete medium. In control experiments, cells received the same amount of DNA (in sodium sulfate buffer). All transfection experiments were performed in triplicate. After 48- to 72-h incubation, cells were directly viewed under a fluorescence microscope (Olympus America, Melville, NY).

Introduction of plasmid DNA into OA rabbit knee joints. Fifteen white New Zealand rabbits weighing 2.5–3 kg each were used in this study. Each rabbit had undergone operations on both knees. The operation model was excision of the medial collateral ligament plus medial meniscectomy [27]. After each treatment, animals were allowed to move freely in their cages.

The rabbits were then divided into three experimental groups. Rabbits in Group 1 ($n = 3$) received three consecutive intra-articular injections at 48-h

intervals of chitosan–DNA nanoparticles (0.4 ml) containing PcDNA3.1, which does not have the expression unit (placebo). Rabbits in Group 2 ($n = 6$), the right knee received three injections at 48-h intervals of 0.4 ml chitosan–DNA nanoparticles containing 20 μg of PcDNA3.1-IL-1Ra plasmid (chitosan-IL-1Ra), the left knee received an injection of the same volume of sodium sulfate buffer containing 20 μg of PcDNA3.1-IL-1Ra plasmid (control). Rabbits in Group 3 ($n = 6$), the right knee received three injections at 48-h intervals of 0.4 ml chitosan–DNA nanoparticles containing 20 μg of PcDNA3.1-IL-10 (chitosan-IL-10), the left knee received an injection of the same volume of sodium sulfate buffer containing 20 μg of PcDNA3.1-IL-10 plasmid (control). All injections were given beginning 5 days postsurgery.

Enzyme-linked immunosorbent assay (ELISA). Seven days after the first injection, both knees of each rabbit were lavaged with saline, at 14 days post first injection, the rabbits were sacrificed, the knees lavaged, dissected, and analyzed for effects of transgene expression. Levels of human IL-1Ra and human IL-10 expression in recovered lavage fluids were measured using a cytokine ELISA kit (human IL-1Ra kit, R&D Systems, USA; human IL-10 kit, Diaclone, France) according to the manufacturer's instructions. The assay used in this study did not recognize the rabbit IL-1Ra and rabbit IL-10.

Immunohistochemical analysis. Slides of synovial membrane were pre-incubated with 0.3% hydrogen peroxide and blocking solution. The first antibodies were mouse monoclonal antibodies against human IL-1Ra (specificity for human origin, Human Disease Genomics Research Center, Peking University, Beijing) or human IL-10 (specificity for human origin, Santa Cruz Biotechnology, USA), The second antibody was biotinylated anti-mouse immunoglobulin. After several washes with phosphate-buffered saline (pH 7.4), the slides were incubated with peroxidase-labeled streptavidin solution. The color was revealed by aminoethylcarbazole and counterstaining with hematoxylin.

Histological examination. Histologic evaluation was performed on sagittal sections of cartilage from the lesion areas on medial femoral condyle. After dissection, specimens were fixed in 10% buffered formalin and embedded in paraffin. Serial sections (5 μm) were prepared and stained with hematoxylin and eosin (H&E) and toluidine blue.

Statistical analysis. All values, expressed as means ± SE, were subjected to t test and one-way ANOVA employing the computer SPSS 10.0 statistic package.

Results

Expression of delivered EGFP in the cultured articular chondrocytes and synoviocytes

Fig. 1 shows the results of the expression of EGFP of the chitosan–EGFP transfected chondrocytes. Chitosan–EGFP transfected chondrocytes expressed EGFP clearly. According to the increase in DNA from 1 to 5 μg, the percentage of cells transfected also clearly increased. While DNA transfected (control) chondrocytes did not show

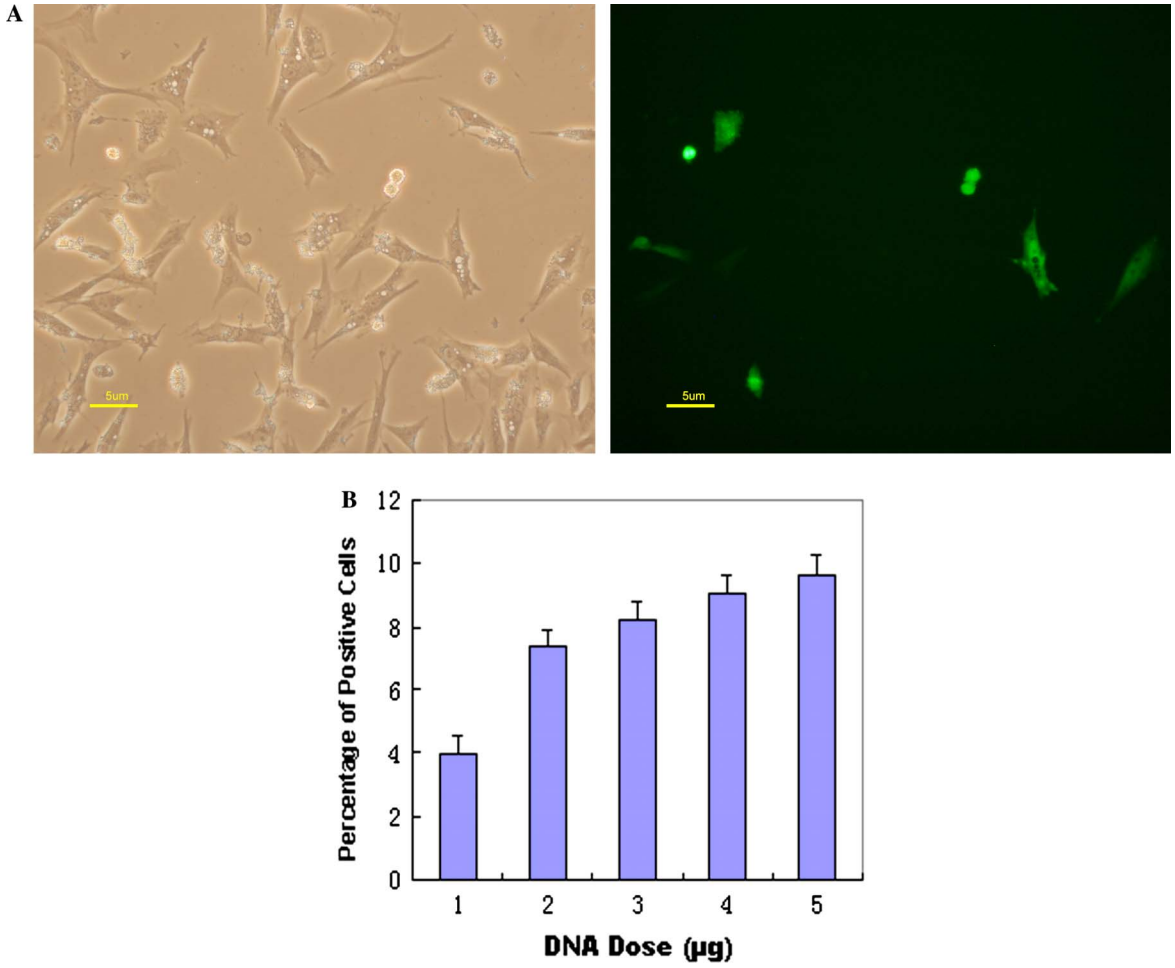

Fig. 1. The expression of EGFP of chitosan–EGFP transfected chondrocytes. (A) Phase contrast microscopic images of chitosan–EGFP transfected chondrocytes: light microscopy imaging (a-1) and fluorescence microscopy imaging (a-2) (200× magnification). (B) Percentage of EGFP-positive cells at different concentrations of DNA.

any expression at all. The results of the expression of EGFP of the chitosan–EGFP transfected synoviocytes showed that this vector is poorly efficient in transfecting articular synoviocytes (data not shown).

Expression of delivered genes in the synovial fluid

A detectable level of human IL-1Ra was found in the rabbit knees treated with the chitosan-IL-1Ra nanoparticles (Fig. 2). Human IL-10 was not detectable in the rabbit knees treated with the chitosan-IL-10 nanoparticles.

Immunohistochemistry

Immunohistochemistry demonstrated human IL-Ra could not be detected in the specimens of control or placebo rabbit knees. Positive staining was noted in the knees injected with chitosan-IL-1Ra nanoparticles, in these specimens, a small group of chondrocytes in a few areas of the superficial and middle zones were found to stain positive for human IL-1Ra (Fig. 3). The immunohistochemistry of synovial membrane specimens of the rabbit knees given chitosan-IL-1Ra nanoparticle injection was negative for human IL-1Ra.

Histological findings of articular cartilage

Specimens from the placebo and control rabbit knees showed a similar degree of morphologic characteristic change of early OA. Transverse clefts occurred which resulted in loss of articular cartilage mass, several chondrocytes in one lacuna forming a cluster in the superficial and intermediate layers. H&E staining showed chondrocyte death (Fig. 4A), and toluidine blue staining for proteoglycan was distributed unevenly, the areas in the superficial and intermediate layers mostly destained and only the areas around cell clusters still darkly stained (Fig. 4B).

Treatment of joints with chitosan-IL-1Ra nanoparticles had less severe lesions compared with the control. Surface irregularities developed which appeared to link up with disarrangement of chondrocytes (Fig. 4C). Toluidine blue staining showed partially destaining (Fig. 4D).

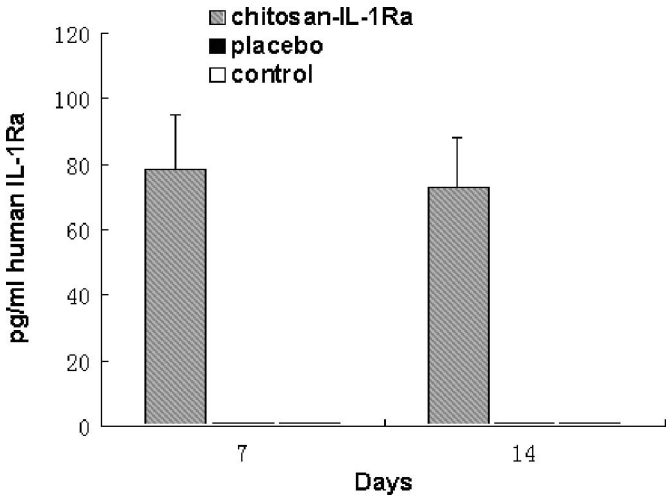

Fig. 2. In vivo expression of human IL-1Ra after direct chitosan-IL-1Ra nanoparticles, delivery to rabbit knees with OA. Five days after induction of OA, a group of three rabbits received three consecutive intra-articular injections at 48-h intervals of chitosan–DNA nanoparticles (0.4 ml) containing PcDNA3.1 in both knees (placebo), a group of six rabbits was injected in the right knee with 0.4 ml chitosan-IL-1Ra nanoparticles and in the left knee with the same volume of sodium sulfate buffer containing PcDNA3.1-IL-1Ra plasmid (control). At days 7 and 14 after first injection, the knees were lavaged and the recovered fluids were analyzed by ELISA for levels of human IL-1Ra. Values shown represent means ± SE.

Discussion

The articular surface is the primary site of cartilage degeneration in a joint disease such as osteoarthritis. Currently available therapies assist with pain and control inflammation but do little to regain this surface. This may be because (1) there are no blood vessels in the articular cartilage, (2) the self-repair capability of the cartilage tissues is low because highly differentiated chondrocytes are surrounded with rich extracellular matrix, or (3) it is difficult to maintain the effective dose of medicines that are locally injected into the joint cavity. Gene therapy might prove applicable to osteoarthritic disease, offering the potential for greater and more prolonged clinical efficacy [28]. Recently, there have been several clinical trials of gene therapy for this intractable disease. With in vivo methods, vector or virus suspensions are directly injected into the OA joints. These studies include both viral and non-viral constructs [29–33]. Viral vectors are very effective in terms of transfection efficiency, but they have limitations in vivo including wide-type reversion and immunogenicity [2]. Non-viral delivery systems include cationic lipids and cationic polymers. Cationic lipids provide effective synthetic transfection systems, but their use in vivo is limited by general toxicity, complement activation, and liver, and lung tropism [13].

Since the studies with poly (L-lysine) in the late 1980s, many cationic polymers have been explored as non-viral vectors. Chitosan was first described as a delivery system for plasmids by Mumper et al. [34]. Chitosan is a biodegradable polysaccharide composed of two subunits, D-glucosamine and *N*-acetyl-D-glucosamine, linked together by β(1,4) glycosidic bonds. It is derived from the second most universally abundant biopolymer, chitin. Many chitosans of different molecular weights and degrees of deacetylation are commercially available. A number of properties of chitosan render it a potentially useful system for the delivery of plasmid. The general consensus is that chitosan is non-toxic and biocompatible [35,10], chitosan has been shown to bind mammalian and microbial cells (by interacting with surface glycoproteins), and some studies have indicated that chitosan may actually be endocytosed into the cell [12]. In our study, the in vitro transfection ability of

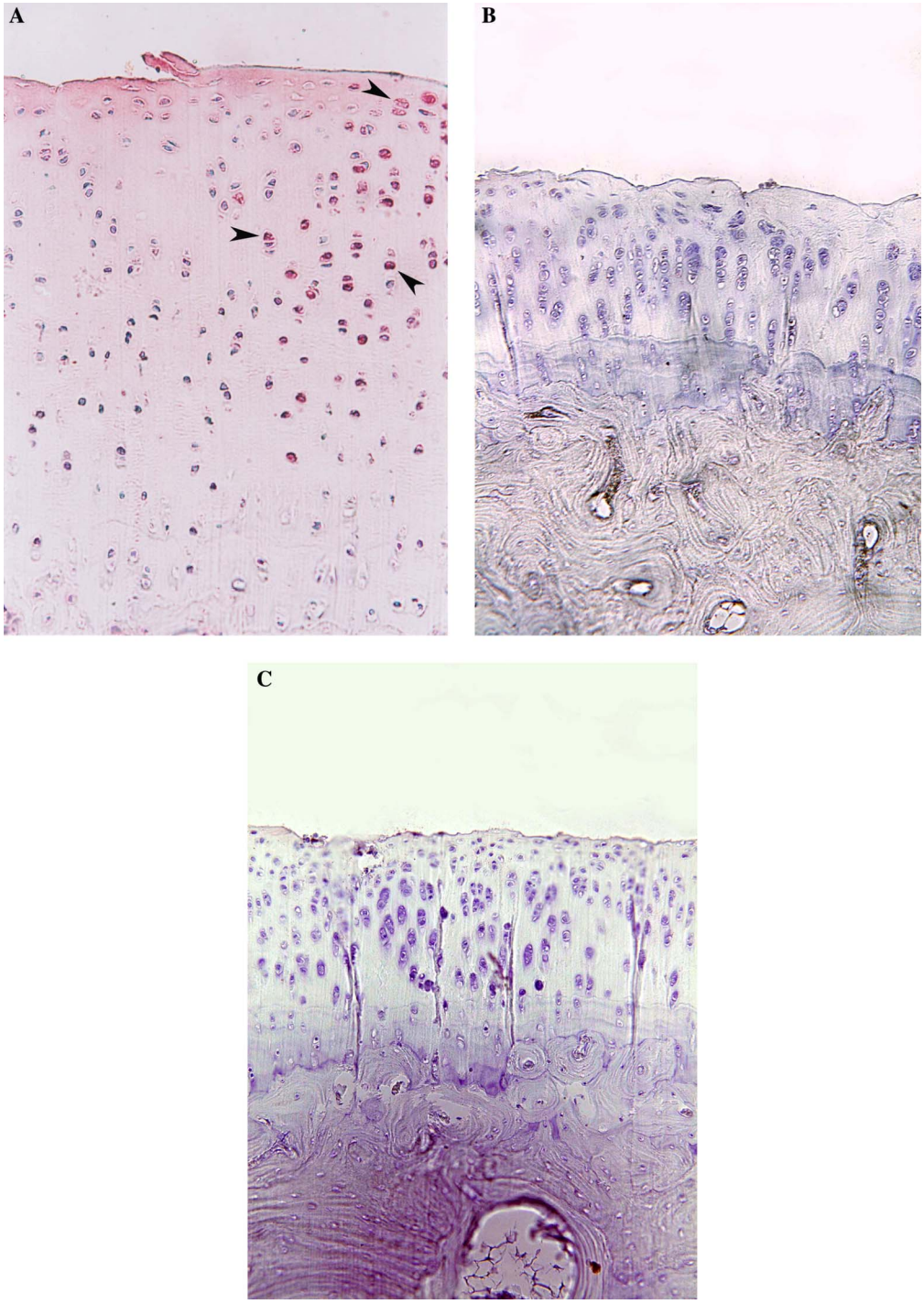

Fig. 3. Immunohistochemical detection of human IL-1Ra. (A) Positive staining was noted in cartilage of OA rabbit injected with chitosan-IL-1Ra nanoparticles. AEC was used as chromogen, red in color represents positive staining (arrows). Human IL-1Ra could not be detected in the specimens of placebo (B) and control (C) rabbit knees. 200×. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

chitosan–DNA nanoparticles was first evaluated in articular chondrocytes and synoviocytes using the pEGFP-C_3 plasmid. The result confirmed that higher gene expression levels were found in articular chondrocytes, compared with that in synoviocytes. There were several indications that the transfection efficiency of chitosan–DNA complexes was cell-type dependent [14].

So far, the results for in vivo gene transfer into OA articular cartilage have been obtained ([29, adenovirus]; [33, lipid]). In this study, we applied an in vivo method using chitosan vector because it is simple and has no report on the delivered gene in the joint cavity by it. The present study shows that the IL-1Ra gene can be transduced directly in vivo using a non-viral chitosan technology and

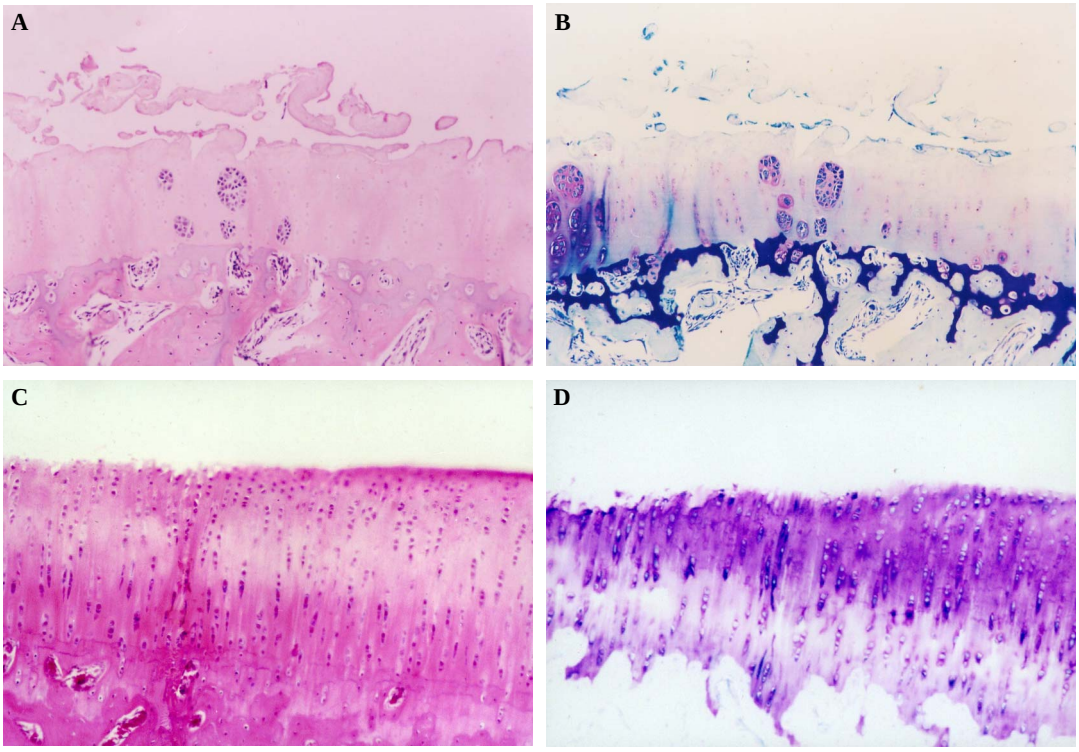

Fig. 4. Representative sections of articular cartilage from the femoral condyle were stained with H&E (left panels) and with toluidine blue (right panels) of (A,B) rabbit knee received an intraarticular injection of sodium sulfate buffer containing PcDNA3.1-IL-1Ra plasmid (control), (C,D) rabbit knee received an intraarticular injection of chitosan-IL-1Ra nanoparticles. 100×.

incorporated into the chondrocytes to produce the IL-1Ra protein. Immunohistochemistry demonstrated human IL-Ra could be detected in the rabbit knees injected with chitosan-IL-1Ra nanoparticles. In these specimens, a small group of chondrocytes in a few areas of the superficial and middle zones were found to stain positive for human IL-1Ra. However, the immunohistochemistry of synovial membrane specimens of the rabbit knees given chitosan-IL-1Ra injection was negative for human IL-1Ra. This could be explained by in vitro experiments in cell culture, which have demonstrated that the transfection of the pEGFP-C_3 gene in synovial cells using chitosan–DNA nanoparticles was less efficient than in chondrocytes. In this study, human IL-10 was not detectable in lavaged fluid recovered from rabbit knees treated with the chitosan-IL-10 nanoparticles. Perhaps, the level of production of IL-10 is too little to be detected, It is worth noting that this pattern of gene expression is also closely related to the type of the gene product.

This study demonstrates that, using a direct in vivo technology for gene transfer, it is possible to ensure a detectable level of expression of the IL-1Ra with persistence of the plasmid for at least 2 weeks following the chitosan-IL-1Ra nanoparticle injections. These results are most encouraging and further studies are needed to explore the length of time for gene expression to last. Direct transfection of the IL-1Ra gene and its in vivo expression were also found to effectively reduce the progression of cartilage lesions and the severity of histologic lesions was reduced. The pathophysiology of OA lesions, both in the model and in the natural disease, has been shown to be induced by proteolytic enzymes, the synthesis of which is up-regulated by pro-inflammatory cytokines such as IL-1 [36–38]. Therefore, the actual findings with regard to cartilage lesions are most likely explained by the inhibitory action of increased local production of IL-1Ra on IL-1 action.

In summary, this study demonstrates that the use of chitosan–DNA plasmid represents a promising method for the direct transfer of therapeutic gene(s) in vivo. Though there were several indications that the level of transfection of chitosan–DNA complexes is lower than that observed with complexes made from commercially available carriers [39]. This technology still presents several obvious advantages for in vivo use, including biocompatible (non-toxic and non-immunogenic, and preferably biodegradable); able to complex DNA to form small particles which can be formulated reproducibly; able to protect the complexed DNA from degradation. Based on the results of this study, the use of the chitosan-IL-1Ra nanoparticles could reduce cartilage degradation and retard the progression of structural changes in OA. It seems to hold interesting future prospects for the treatment of OA.

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References

[1] N.A. Wivel, J.M. Wilson, Methods of gene delivery, Hematol. Oncol. Clin. North. Am. 12 (1998) 483–501.
[2] I.M. Verma, N. Somia, Gene therapy—promises, problems and prospects, Nature 389 (1997) 239–242.
[3] R.G. Crystal, The gene as the drug, Nat. Med. 1 (1995) 15–17.
[4] C.W. Pouton, L.W. Seymour, Key issues in non-viral gene delivery, Adv. Drug. Deliv. Rev. 34 (1998) 3–19.
[5] M.N. Kumar, S.S. Mohapatra, X. Kong, P.K. Jena, U. Bakowsky, C.M. Lehr, Cationic poly (lactide-co-glycolide) nanoparticles as efficient in vivo gene transfection agent, J. Nanosci. Nanotechnol. 4 (2004) 990–994.
[6] D. Deshpande, P. Blezinger, R. Pillai, J. Duguid, B. Freimark, A. Rolland, Target specific optimization of cationic lipid-based systems for pulmonary gene therapy, Pharm. Res. 15 (1998) 1340–1347.
[7] K.W. Leong, H.Q. Mao, V.L. Truong-Le, K. Roy, S.M. Walsh, J.T. August, DNA-polycation nanospheres as non-viral gene delivery vehicles, J. Control Release 53 (1998) 183–193.
[8] H. Onishi, Y. Machida, Biodegradation and distribution of water-soluble chitosan in mice, Biomaterials 20 (1999) 175–182.
[9] S.B. Rao, C.P. Sharma, Use of chitosan as a biomaterial: studies on its safety and hemostatic potential, J. Biomed. Mater. Res. 34 (1997) 21–28.
[10] T.J. Aspden, J.D. Mason, N.S. Jones, J. Lowe, O. Skaugrud, L. Illum, Chitosan as a nasal delivery system: the effect of chitosan solutions on in vitro and in vivo mucociliary transport rates in human turbinates and volunteers, J. Pharm. Sci. 86 (1997) 509–513.
[11] K. Tomihata, Y. Ikada, In vitro and in vivo degradation of films of chitin and its deacetylated derivatives, Biomaterials 18 (1997) 567–575.
[12] F.C. MacLaughlin, R.J. Mumper, J. Wang, J.M. Tagliaferri, I. Gill, M. Hinchcliffe, A.P. Rolland, Chitosan and depolymerized chitosan oligomers as condensing carriers for in vivo plasmid delivery, J. Control Release 56 (1998) 259–272.
[13] S.C. Richardson, H.V. Kolbe, R. Duncan, Potential of low molecular mass chitosan as a DNA delivery system: biocompatibility, body distribution and ability to complex and protect DNA, Int. J. Pharm. 178 (1999) 231–243.
[14] P. Erbacher, S. Zou, T. Bettinger, A.M. Steffan, J.S. Remy, Chitosan-based vector/DNA complexes for gene delivery: biophysical characteristics and transfection ability, Pharm. Res. 15 (1998) 1332–1339.
[15] X.W. Li, D.K. Lee, A.S. Chan, H.O. Alpar, Sustained expression in mammalian cells with DNA complexed with chitosan nanoparticles, Biochim. Biophys. Acta 1630 (2003) 7–18.
[16] H.Q. Mao, K. Roy, V.L. Troung-Le, K.A. Janes, K.Y. Lin, Y. Wang, J.T. August, K.W. Leong, Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency, J. Control Release 70 (2001) 399–421.
[17] C. Guincamp, T. Pap, J. Schedel, G. Pap, U. Moller-Ladner, R.E. Gay, S. Gay, Gene therapy in osteoarthritis, Joint Bone Spine 67 (2000) 570–571.
[18] T.A. Holland, A.G. Mikos, Advances in drug delivery for articular cartilage, J. Control Release 86 (2003) 1–14.
[19] K.W. Moore, A. O'Garra, R. de Waal Malafyt, P. Vieira, T.R. Mosmann, Interleukin-10, Annu. Rev. Immunol. 11 (1993) 165–190.
[20] R. de Waal Malefyt, J. Abrams, B. Bennett, C.G. Figdor, J.E. de Vries, Interleukin10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes, J. Exp. Med. 174 (1991) 1209–1220.
[21] C. Bogdan, Y. Vodovotz, C. Nathan, Macrophage deactivation by interleukin10, J. Exp. Med. 174 (1991) 1549–1555.
[22] P. Wang, P. Wu, M.I. Siegel, R.W. Egan, M.M. Billah, IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells, J. Immunol. 153 (1994) 811–816.
[23] J. Martel-Pelletier, N. Alaaeddine, J.P. Pelletier, Cytokines and their role in the pathophysiology of osteoarthritis, Front. Biosci. 4 (1999) D694–D703.
[24] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.
[25] V.M. Baragi, R.R. Renkiewicz, H. Jordan, J. Bonadio, J.W. Hartman, B.J. Roessler, Transplantation of transduced chondrocytes protects articular cartilage from interleukin 1-induced extracellular matrix degradation, J. Clin. Invest. 96 (1995) 2454–2460.
[26] S.S. Makarov, J.C. Olisen, W.N. Johnston, J.H. Schwab, S.K. Anderle, R.R. Brown, J.S. Haskill, Retrovirus mediated in vivo gene transfer to synovium in bacterial cell wall-induced arthritis in rats, Gene Ther. 2 (1995) 424–428.
[27] C.L. Yu, M.Y. Qu, D.X. Tian, Y.F. Ao, J.Y. Zhao, Early articular cartilage changes in traumatic osteoarthritis, J. Orthop. Surg. 6 (1998) 41–47.
[28] T. Kuboki, T. Nakanishi, M. Kanyama, W. Sonoyama, T. Fujisawa, K. Kobayashi, T. Ikeda, T. Kubo, A. Yamashita, M. Takigawa, Direct adenovirus-mediated gene delivery to the temporomandibular joint in guinea-pigs, Arch. Oral Biol. 44 (1999) 701–709.
[29] T. Ikeda, T. Kubo, Y. Arai, T. Nakanishi, K. Kobayashi, K. Takahashi, J. Imanishi, M. Takigawa, Y. Hirasawa, Adenovirus mediated gene delivery to the joints of guinea pigs, J. Rheumatol. 25 (1998) 1666–1673.
[30] D.D. Frisbie, S.C. Ghivizzani, P.D. Robbins, C.H. Evans, C.W. McI1wraith, Treatment of experimental equine osteoarthritis by in vivo delivery of the equine interleukin-1 receptor antagonist gene, Gene Ther. 9 (2002) 12–20.
[31] J.P. Pelletier, J.P. Caron, C. Evans, P.D. Robbins, H.I. Georgescu, D. Jovanovic, J.C. Femandes, J. Martel-Pelletier, In vivo suppression of early experimental osteoarthritis by interleukin-1 receptor antagonist using gene therapy, Arthritis Rheum. 40 (1997) 1012–1019.
[32] H. Madry, M. Cucchiarini, E.F. Terwilliger, S.B. Trippel, Recombinant adeno-associated virus vectors efficiently and persistently transduce chondrocytes in normal and osteoarthritic human articular cartilage, Hum. Gene Ther. 14 (2003) 393–402.
[33] J. Fernandes, G. Tardif, J. Martel-Pelletier, V. Lascau-Coman, M. Dupuis, F. Moldovan, M. Sheppard, B.R. Krishnan, J.P. Pelletier, In vivo transfer of interleukin-1 receptor antagonist gene in osteoarthritic rabbit knee joints: prevention of osteoarthritis progression, Am. J. Pathol. 154 (1999) 1159–1169.
[34] R.J. Mumper, J.J. Wang, J.M. Claspell, A.P. Rolland, Novel polymeric condensing carriers for gene delivery, in: Proceedings of the International Symposium on Controlled Release Bioactive Materials 22 (1995) 178–179.
[35] L. Illum, N.F. Farraj, S.S. Davis, Chitosan as a novel nasal delivery system for peptide drugs, Pharm. Res. 11 (1994) 1186–1189.
[36] J. Saklatvala, Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage, Nature 322 (1986) 547–549.
[37] C.A. Towel, H.H. Hung, L.J. Bonassar, B.V. Treadwell, D.C. Mangham, Detection of interleukin-1 in the cartilage of patients with osteoarthritis: a possible autocrine/paracrine role in pathogenesis, Osteoarthritis Cartilage 5 (1997) 293–300.
[38] M. Kobayashi, G.R. Squires, A. Mousa, M. Tanzer, D.J. Zukor, J. Antonious, U. Feige, A.R. Poole, Role of interleukin-1 and tumor necrosis factor alpha in matrix degradation of human osteoarthritic cartilage, Arthritis Rheum. 52 (2005) 128–135.
[39] T. Ishii, Y. Okahata, T. Sato, Mechanism of cell transfection with plasmid/chitosan complexes, Biochim. Biophys. Acta 1514 (2001) 51–64.