Tissue and Cell 41 (2009) 13–22

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Degeneration of normal articular cartilage induced by late phase osteoarthritic synovial fluid in beagle dogs

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ARTICLE INFO

Article history:
Received 28 November 2007
Received in revised form 21 June 2008
Accepted 28 June 2008
Available online 9 August 2008

Keywords:
Osteoarthritis
Degeneration
Cartilage
Pathogenesis
Synovial fluid

ABSTRACT

Objective: To investigate the pathogenesis of late phase osteoarthritic (OA) synovial fluid (SF) on normal articular cartilage in vivo and provide an understanding of degenerative cartilage extending in OA joint.
Methods: A random knee, each of 8 beagle dogs, received anterior cruciate ligament transection (ACLT) and was confirmed to have late phase OA degenerative changes at 24 weeks after operation. Thereafter, one random elbow of each canine was injected with autologous late phase OA knee SF. The contralateral elbow was injected with normal saline (NS) of the same volume as SF aspirated from ACLT knee. These two groups of elbows were labeled "SF" and "NS". 8 other beagle dogs were left intact and placed in Group Control. After aseptic arthrocentesis was performed weekly on both elbows for 24 weeks, morphological changes were observed in the cartilage of the elbows, and expressions of 7 biological etiological factors of chondrocytes of the elbows were determined in Group SF, Group NS and Group Control, respectively.
Results: Morphological changes were observed in articular cartilage of the elbows in Group SF. Levels of unit area of collagen type I in the noncalcified, calcified and full zones of articular cartilage of the elbows in Group SF increased significantly. Level of unit area of collagen type III in the calcified zone of articular cartilage of the elbows in Group SF remained unchanged. Meanwhile, expressions of MMP-1 and MMP-3 of chondrocytes of the elbows in Group SF increased significantly. There was almost no difference between articular cartilage in Group NS and Group Control.
Conclusion: Based on these results, we conclude that OA degeneration of normal articular cartilage can be independently induced by late phase OA SF. Endogenous OA biological etiological factor may be one of the reasons causing degenerative cartilage extending in OA joint.

The current opinion is that OA is an independent degenerative joint disease related to mechanical and biological factors (Creamer and Hochberg, 1997). A number of OA animal models have considerably advanced the understanding of how mechanical factors contribute to the onset of OA (Kääb et al., 2000; Haapala et al., 2001; Patwari et al., 2001). However, the biological etiopathogenesis of OA is still unclear. In clinical settings, the mechanical and biological factors (Okazaki et al., 2001; Fahlgren et al., 2001; Xu et al., 2007; Panula et al., 1998; Aigner et al., 2001; Bank et al., 2000; Uesaka et al., 2001; Väätäinen et al., 1998; Kobayashi et al., 1997; Grabowski et al., 1996; Blanco et al., 1998) related to the degeneration of OA are difficult to discriminate. The katogene of some kind of biological etiological factors have been demonstrated directly by injecting them into normal joints (Yeh et al., 2008; Blaney Davidson et al., 2007; Murat et al., 2007; Barve et al., 2007; Céleste et al., 2005). However, most of them were ectogenic with significantly higher doses than the physiological or pathologic endogenous doses. Thus, the true enduring capacity of normal articular cartilage to biological etiological factors; the destructive degree of pathologic doses of biological etiological factors to normal articular cartilage in vivo; and the reactive compensation and renovation of articular cartilage following disequilibrium of biological dynamic balance could not be effectively and truly reflected.

The aim of the present study was to investigate the pathogenesis of endogenous OA biological etiological factors in OA SF on normal articular cartilage in vivo. We tried to rely on a different method to understand degenerative cartilage extending in OA joint, and thus to gain more insight into the beginning of biologically induced cartilage degeneration. The major objective was to focus on the histological appearance and gene expression of cartilage degeneration in elbow induced by late phase OA SF of autologous knee joint.

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[1] Dr. Dong's work was supported by a grant from the research fund of the Science and Technology Committee of Shanghai Metropolis.

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doi:10.1016/j.tice.2008.06.004

1. Materials and methods

1.1. Animals

1.1.1. 1st stage

Eighteen mature beagle dogs of pure breed were divided randomly into 2 groups: Group I ($n = 9$) and Group II ($n = 9$). Their ages ranged from 3 to 5 years (average, 47 months) whereas their body mass ranged from 12.5–17.5 kg (average, 15.6 kg). The canines were cared for as per the guidelines of the Local Council for Animal Care. On a random knee of each canine in Group I, ACLT through arthrotomy was performed under anaesthesia (Ketamine 1.0 mg/kg, intravenous). Positive Lachmann test was confirmed before incision closure. After 3 days rest in individual indoor kennels, the canines were allowed to move about freely in outdoor pens. The canines showed no abnormalities in posture and movement before treatment and at euthanasia. Canines in Group II served as non-operative Controls. One canine in each group was euthanized at the 24th week after ACLT, and late phase OA of ACLT knee in Group I was verified by its gross appearance.

1.1.2. 2nd stage

Under anaesthesia, one random elbow of each canine in Group I was injected with SF through a posteromedial approach just after it was aspirated from the autologous ACLT knee by OT injector. This was done weekly from the 25th week after ACLT for a total of 24 times. The SF-treated elbows were placed in Group SF ($n = 8$). Before SF was injected into elbow, it was confirmed that the needle of injector was properly positioned into the space of elbow joint by obtaining elbow SF. The SF volume of ACLT knee ranged from 0.1 ml to 1.1 ml (average, 0.5625 ml). Meanwhile, the contralateral elbow of each canine in Group I was injected with NS of the same volume as SF aspirated from ACLT knee weekly. The NS-treated elbows were placed in Group NS ($n = 8$). One random elbow of each canine in Group II was placed in Group Control ($n = 8$) which served as non-treated Controls. Canines in all groups were euthanized at the 48th week after ACLT.

1.1.3. SF samples

SF specimens were aspirated from ACLT knee of each canine in Group I and also from one random knee of each canine in Group II at the 24th week after ACLT. Supernatant collected from centrifuged (3000 rpm) SF was stored frozen at −70 °C pending analysis.

1.1.4. Serum samples

Superficial venous blood specimens were collected from anterior limbs of canines in Group I and II at the 24th week after ACLT. Serum sample collected from centrifuged (3000 rpm) venous blood was stored frozen at −70 °C pending analysis.

1.1.5. Assays of tumor necrosis factor α (TNF-α), transforming growth factor β1 (TGF-β1), matrix metalloproteinase 1 (MMP-1, collagenase-1), MMP-3 (stromelysin-1), MMP-13 (collagenase-3), tissue inhibitor of metalloproteinase 1 (TIMP-1) and NO in SF supernatant and serum

Concentrations of TNF-α, TGF-β1, MMP-1, MMP-3, MMP-13 and TIMP-1 in SF supernatant and serum were determined by sandwich ELISAs using the quantitation kits as per the instructions of the manufacturer (TPI Inc., Washington, USA). All assays used a monoclonal antibody against the enzyme or factor as a trapping reagent. Polyclonal antisera against the specific proteins generated in rabbits were used as secondary reagents. The assay for TIMP-1, as used in this study, detected only free TIMP-1 (i.e., not the inhibitor complexed with metalloproteinases). NO was determined by estimating the nitrite concentration in SF supernatant and serum using NO quantitation kits as per the instructions of the manufacturer (TPI Inc., Washington, USA). For each factor, concentrations were quantified in the samples using a standard curve prepared with known concentrations of factors.

1.1.6. Cartilage samples

Cartilage specimens were collected immediately following euthanasia of the canines at the 48th week after ACLT. The elbow joints in each group were isolated through 2 horizontal cuts, one up the humeral epicondyle and the other below the radial neck, using a band saw. Two full depth articular cartilages from the center of medial (ulnar) articular surface of humeral trochlea, measuring $1\,mm^2 \times 1\,mm^2$ and $2\,mm^2 \times 2\,mm^2$, were sampled from each elbow in each group. Cartilage samples were fixed in 2% (v/v) glutaraldehyde phosphate buffered solution (PBS) at 4 °C pending analysis by electron microscope. Full depth articular cartilages from the medial articular surface of ulnar trochlear notch and the remaining medial articular surface of humeral trochlea were harvested and snap frozen in liquid nitrogen before storage at −70 °C for subsequent use. Similarly, full thickness sagittal osteochondral slabs (5 mm), near lateral (radial) border of articular surface of humeral trochlea (about 2 mm), were then prepared through the lateral articular surface of humeral trochlea and ulnar trochlear notch.

1.1.7. Histology

The sagittal humeral and ulnar slices were fixed in 10% (v/v) neutral buffered formalin for 48 h and then decalcified in 10% ethylenediamine tetraacetic acid (EDTA) (v/v) which was replaced every 3 days for the next 45 days. The specimens were then dehydrated in graded alcohols and double-embedded in celloidin–paraffin blocks. Tissue sections (4 μm) were cut using a microtome (RM2135, leica, Germany) and attached to microscope slides. The 11th, 21st and 31st sections of each specimen were then deparaffinized in xylene and washed in graded alcohols to 70% (v/v) ethanol and then stained with Toluidine Blue (Bancroft and Stevens, 1990), Safranin-O (Brophet et al., 1992) and Picro Sirius Red (PSR) (Junqueira et al., 1979) in that order. Articular cartilage zone thickness; matrix abundance and content; and arrangement of collagen type I and III were evaluated through Toluidine Blue staining, Safranin-O staining and PSR staining (under polarized light microscopy), respectively. The slides were photographed and analyzed with a microscope image analysis system (Axioplan 2 imaging, Zeiss, Germany).

Four zones of articular cartilage are defined: superficial, transitional, radial and calcified (Hunziker, 1992). Superficial, transitional and radial zones are called noncalcified zone. The borderline between hyaline articular cartilage and the zone of calcified cartilage is called the tidemark.

1.1.8. Electron microscope

$1\,mm^2 \times 1\,mm^2$ full depth articular cartilages were fixed in 2% glutaraldehyde for 2 h. After being washed in PBS, the specimens were postfixed in 1% (v/v) osmium tetroxide for 1 h, rewashed, dehydrated in ethanol, and placed in propylene oxide followed by a 50:50 propylene oxide/Araldite mix before final embedding in Araldite. Semithin sections (1 μm) were cut, stained with Toluidine Blue, and examined under light microscopy. Ultrathin sections (60 nm) were cut and stained with lead citrate before examination on a transmission electron microscope (H–500, Hitachi, Japan).

$2\,mm^2 \times 2\,mm^2$ full depth articular cartilages were fixed in 2% glutaraldehyde for 2 h. After being washed in PBS, the specimens were dehydrated in ethanol, stuck to object stage orientally, and sputter coated with BAL-TEC ion before examination on a scanning electron microscope (QUANTA–200, Philips, Holland).