Osteoarthritis and Cartilage 22 (2014) 519–526

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Association between adiponectin and cartilage degradation in human osteoarthritis

P.-J. Francin, A. Abot, C. Guillaume, D. Moulin, A. Bianchi, P. Gegout-Pottie, J.-Y. Jouzeau, D. Mainard, N. Presle*

UMR 7365 CNRS-Universite de Lorraine, Biopôle de l'Universite de Lorraine, campus Biologie-Sante, Avenue de la forêt de Haye, BP 184, 54505 Vandoeuvre-les-Nancy, France

ARTICLE INFO

Article history:
Received 20 June 2013
Accepted 15 January 2014

Keywords:
Adipokines
Osteoarthritis
Obesity
Cartilage

SUMMARY

Objective: Conflicting findings raise questions about the role of adiponectin in osteoarthritis (OA). The current study aimed to investigate in OA patients the association between the production of adiponectin and the grade of cartilage destruction, and to provide functional evidence for a potential role of adiponectin in OA.

Design: The expression of adiponectin was examined by immunohistochemistry in cartilage obtained from healthy individuals ($n = 2$; ages 56 and 41 years; 1 male and 1 female) and OA patients ($n = 11$; ages 64–79 years; 2 male and 9 female). The association between its production in chondrocytes and the grade of cartilage destruction was established on full-depth cartilage biopsies. The functional activity of adiponectin in OA cartilage was determined from the relation between the expression of adiponectin, its receptor, cartilage-specific components and factors involved in matrix degradation, and from the chondrocyte response to the full-length or the globular form of adiponectin.

Results: Adiponectin was not detected in healthy cartilage. Conversely, the adipokine was up-regulated in damaged tissue, but no strong association with the grade of cartilage destruction was found. We showed a positive correlation between adiponectin and mPGES or MMP-13 while AdipoR1 was related to the expression of type 2 collagen, aggrecan and Sox9. The full-length form of adiponectin but not the globular isoform, stimulated the production of PGE_2 and MMP-13 activity in cultured human chondrocytes.

Conclusions: The elevated level of adiponectin found in chondrocytes from OA patients might contribute to matrix remodelling during OA, the full-length isoform being the single active form.

Introduction

Although extensive studies aimed to identify the mechanisms underlying articular cartilage changes associated with osteoarthritis (OA), the pathogenesis of this degenerative joint disease remains unclear. It is well recognized that OA develops in the highly metabolic and inflammatory environment of adiposity. In the last decade, a new hypothesis based on the relation between obesity and OA has emerged[1]. The sole role of biomechanical loading cannot explain the increased risk for OA in non-weight-bearing joints among overweight persons, and recent studies indicate that adiposity rather than simply excess in body mass is detrimental to the joint[2–5]. More especially, some adipokines are thought to be implicated in the development and the progression of OA[6]. In fact, it is now well recognized that adipose tissue plays a critical role as an active endocrine organ through the release of various adipokines in an integrated network that maintains interactions between fat and other organs. These adipose-derived proteins are required for normal physiological homeostasis, but impaired production may be involved in obesity-related disorders.

Adiponectin mediates its biological effects through two receptors, namely AdipoR1 and AdipoR2[7]. This adipokine has a modular structure comprising of an N-terminal collagenous domain with multiple collagen triple helix repeats, followed by a C-

* Address correspondence and reprint requests to: N. Presle, UMR 7365 CNRS-Universite de Lorraine, Biopôle de l'Universite de Lorraine, campus Biologie-Sante, Avenue de la forêt de Haye, BP 184, 54505 Vandoeuvre-les-Nancy, France. Tel: 33-383-68-54-32; Fax: 33-383-68-39-59.

E-mail addresses: pij.francin@hotmail.fr (P.-J. Francin), anne.abot@inserm.fr (A. Abot), cecile.guillaume@univ-lorraine.fr (C. Guillaume), david.moulin@univ-lorraine.fr (D. Moulin), arnaud.bianchi@univ-lorraine.fr (A. Bianchi), pascale.pottie@univ-lorraine.fr (P. Gegout-Pottie), jean-yves.jouzeau@univ-lorraine.fr (J.-Y. Jouzeau), didier.mainard@univ-lorraine.fr (D. Mainard), nathalie.presle@univ-lorraine.fr (N. Presle).

1063-4584/$ – see front matter © 2014 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.joca.2014.01.002

terminal C1q-like globular domain which has similar folding topology with Tumor Necrosis Factor-α (TNFα)[8,9]. A truncated form of adiponectin restricted to the globular domain can be generated by leukocyte elastase secreted from activated monocytes and/or neutrophils[10]. The adipokine exhibits insulin-sensitizing properties[11] and reduced serum levels are found in obesity[12]. Adiponectin is considered as an anti-inflammatory mediator especially with regard to atherosclerosis[13], but in some chronic inflammatory/autoimmune diseases it may have pro-inflammatory effects and its production correlates with inflammatory markers and disease activity. Thus, serum adiponectin levels have been shown to be strongly associated with radiographic changes in patients with rheumatoid arthritis (RA)[14] and to be predictive of radiographic progression in early RA[15]. The adipokine was reported to stimulate the production of IL-6[16], IL-8[17] and prostaglandin E_2 (PGE_2)[18] by human synovial fibroblasts, suggesting its potential contribution to the pathogenesis of synovitis in RA. Adiponectin may also be considered as a marker of joint degradation or local inflammatory processes. Indeed, increased serum levels of adiponectin were detected in female patients with erosive compared with non-erosive OA of the hands[19]. The adipokine is found in the synovial fluid of human OA-affected joints and its articular levels are positively correlated with degenerative fragments of aggrecan[20]. Whether adiponectin plays a pro- or anti-inflammatory role in OA is still the subject of debate and its effects on cartilage are far to be fully elucidated. For Chen *et al.*, adiponectin may have a protective role on cartilage by up-regulating the Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) and down-regulating IL-1β-induced MMP-13[21]. By contrast, the study of Lago *et al.* indicated that adiponectin may trigger cartilage destruction through up-regulation of MMPs and pro-inflammatory mediators[22]. The recent data reporting the stimulating effect of adiponectin on the production of chemokines, cytokines and matrix-degrading enzymes both in synovial fibroblasts and in chondrocytes further support the inflammatory and the destructive activity of adiponectin in the joint[23–25].

In order to further understand the contribution of adiponectin to cartilage changes associated with human OA, we investigated the expression of adiponectin in cartilage obtained from OA patients in relation to the grade of cartilage destruction. We further sought to identify the functional significance of adiponectin expression in chondrocytes. As AdipoR1 functions as a high-affinity receptor for globular and full-length adiponectin[7] and is strongly expressed in chondrocytes[21], we determined whether both forms of adiponectin are able to modulate inflammation and cartilage destruction in OA.

Materials and methods

Patients and samples

Specimens of human OA articular cartilage were obtained from tibial plateaus of patients undergoing total knee replacement surgery ($n = 11$; 2 male and 9 female; ages 64–79 years, mean 70.9 ± 5.3 years; BMI 22–54 kg/m^2, mean 33.0 ± 8.8 kg/m^2). All patients were evaluated by an orthopaedic surgeon and diagnosed for knee OA according to the criteria of the American College of Rheumatology[26]. For comparison with diseased tissues, normal knee joint cartilage was obtained from transplant donors ($n = 2$; ages 56 and 41 years; 1 male and 1 female) through agreement with the Agence de la Biomedecine. The human study described here was conducted in conformity with the declaration of Helsinki principles and was approved by the local Research Institution (Commission de la Recherche Clinique; registration number UF 9607 – CPRC 2005). Written informed consent has been obtained from all participants.

After washing tibial plateaus in sterile phosphate buffered saline (PBS), full-depth standardized cartilage biopsies (between 4 and 9 biopsies for each patient) were collected using a biopsy punch (5 mm diameter). The samples were then cultured for 2 days at 37°C in a humidified atmosphere of 5% CO_2 in Dulbecco's Modified Eagle Medium/Nut Mix F12 (DMEM/Ham's F12 medium) supplemented with L-glutamine (2 mM), penicillin (0.1 U/ml), streptomycin (100 ng/ml) and amphotericin B (250 ng/ml). The tissue specimens were thereafter fixed for 24 h in 4% paraformaldehyde and conditioned media were stored at −80°C prior to analysis.

Adiponectin treatment of human chondrocytes

Cartilage samples collected from six OA patients were washed in sterile phosphate buffer saline (PBS) and then cut into small pieces. Chondrocytes were isolated after a sequential digestion of the extracellular matrix with pronase (0.15%, w/v) for 2 h and collagenase (0.2%, w/v) overnight at 37°C. After centrifugation, cells were suspended in Dulbecco's Modified Eagles Medium/Ham's F-12 (DMEM/Ham's 12) supplemented with 10% (v/v) foetal calf serum (FCS), 2 mM L-glutamine, penicillin (0.1 U/ml), streptomycin (100 ng/ml) and 250 ng/ml amphotericin B (InVitrogen, Cergy-Pontoise, France), then seeded as primary chondrocytes culture in 75 cm^2 culture flasks at high density (2×10^4 cells/cm^2). They were expanded for 10–12 days in monolayer in a humidified atmosphere containing 5% CO_2, and the culture medium was changed every 3–4 days.

Confluent primary chondrocytes were then incubated with human recombinant full-length or globular adiponectin (R&D Systems) at 0.2, 1 and 5 μg/ml for 24 h in 1% FCS containing medium. The culture supernatants were then kept at −80°C until analysed.

Histological assessment

The full-length cartilage biopsies fixed in paraformaldehyde were decalcified in rapid bone decalcifier (RDO, Eurobio, Les Ulys, France) for 1 h, and further fixed in 4% paraformaldehyde. Cartilage specimens were then dehydrated in a graded series of alcohol and embedded in paraffin.

For all cartilage samples, haematoxylin–eosin–safran (HES), and safranin-O-fast-green stainings were performed on serial sections (5 μm) to determine histological grading. The severity of OA cartilage lesions was evaluated for each biopsy by two independent observers, and was graded using the Mankin score[27].

Immunohistochemical analysis

Paraffin sections (5 μm) from cartilage specimens were deparaffinized in Tissue Clear (Bayer Diagnostic, Puteaux, France) and rehydrated in a graded series of ethanol. Antigen retrieval was performed by heating the sections in a citrate buffer (10 mM, pH = 6) up to 60°C for 30 min. The endogenous peroxidase activity was blocked by incubating the sections in H_2O_2 (0.3%). After neutralization of non-specific sites with bovine serum albumin (BSA, 4% (w/v)) for at least 1.5 h, sections were incubated overnight at 4°C with a goat polyclonal antibody of the human adiponectin (R&D Systems). After washing twice in PBS, the corresponding biotinylated rabbit anti-goat IgG (Dako) was applied for 30 min at room temperature. The signal was amplified with preformed avidin-biotinylated horseradish peroxidase complexes for 45 min at room temperature (EnVision kit, Dako), and staining was developed with 3,3′-diaminobenzidine (0.05% in hydrogen peroxide). Counterstaining of nuclei was performed with haematoxylin, and sections were dehydrated in graded ethanol, cleared in