EXPERIMENTAL STUDY

Dose-dependent effects of adiponectin on ADAMTS-9 gene expression in human chondrocytes

Yaykasli KO1, Hatipoglu OF2, Yaykasli E3, Kaya E4, Ozsahin M5, Uslu M6

Duzce University, Faculty of Medicine, Department of Medical Genetics, Duzce, Turkey.

ABSTRACT

OBJECTIVE: A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), comprising of 19 members is a family of peptidases. They have several vital functions in physiological and pathological processes in organisms. ADAMTS-9 has aggrecanolytic activity and is responsible for degradation of aggrecan mainly in articular cartilage. It is known that adiponectin is the most abundantly secreted adipokine (adipocytokines), and the characteristics of adiponectin have not been elucidated yet. It was assumed that adiponectin has anti-inflammatory effect before. However, an inflammatory feature of adiponectin was shown in researches. In our study, the effect of adiponectin on ADAMTS-9 gene expression in primary human chondrocytes was investigated.

METHODS: Primary human chondrocytes were exposed to adiponectin at 1, 4, 8 and 12 μg/ml doses for certain time period. Total RNA was isolated and reverse-transcribed by random primer after incubation. ADAMTS-9 and β-actin genes expression levels were determined using real-time polymerase chain reaction (qRT-PCR).

RESULTS: The highest upregulation of ADAMTS-9 gene expression level was found at 12 μg/ml dose of adiponectin and 48 h incubation.

CONCLUSION: Adiponectin is the key element in the maintenance of cartilage homeostasis. Similarly, the involvement of adiponectin in articular inflammatory diseases was demonstrated in detail. These findings bring adiponectin into central place in the research to develop adiponectin based new therapy methods for arthritic diseases. Together with these findings, our results suggest that adiponectin may be involved in the degradation of articular cartilage by increasing ADAMTS-9 gene expression (Tab. 1, Fig. 3, Ref. 35). Text in PDF www.elis.sk.

KEY WORDS: adiponectin, ADAMTS-9, human chondrocytes.

Introduction

A member of proteases family called ADAMTS (A disintegrin and metalloproteinase with thrombospondin motifs) has 19 members. It has been demonstrated that ADAMTSs are involved in numerous vital physiological and pathological processes especially connective tissue degradation in the organism since characterization of the first member of ADAMTS (ADAMTS-1) in 1997 (1, 2). The structures and functions of ADAM and ADAMTS are similar. However, ADAMTSs have several thrombospondin type-1 motifs at C-terminal region, while ADAMs having transmembrane and cytoplasmic domains. Nowadays, ADAMTSs entitled as a family of metalloproteinases containing one or more thrombospondin type 1 motifs interact with the component of extracellular matrix (ECM) (3, 4). ADAMTS-9 is a member of zinc-dependent metalloproteinase ADAMTS family and characterized in 2000. The location (3p21.1-p14.3) of ADAMTS9 gene has shown to have pivotal role in several types of cancers especially renal cancer (5, 6). Besides, ADAMTS-9 has aggrecanolytic activity, and is responsible for the degradation of aggrecan, mainly in articular cartilage (7, 8).

Adiponectin, one of the early characterized adipokines was discovered by Scherer et al in 1995. Adiponectin, a 244-aminoacid-long polypeptide is the most abundantly secreted adipokine from adipose tissue and has biological effects through two distinguished receptors called AdipoR1 and AdipoR2 located in the joint cartilage, bone and synovial tissue (9, 10). Adiponectin plays an important role not only in insulin sensitivity but also maintenance of cartilage homeostasis. It was clarified that adiponectin has potential to act in matrix degradation and inflammation in the human joint by our group (11). So, adiponectin gains importance in arthritic disease investigations. However, there is no research...
to investigate putative relationship between adiponectin and ADAMTS gene family. In this study, we aimed to clarify the relationship between ADAMTS-9 and adiponectin of primary human chondrocytes.

**Materials and methods**

**RNA isolation and cDNA synthesis**

Recombinant human adiponectin was purchased from Enzo Life Sciences (Farmingdale, NY) and dissolved in PBS (phosphate buffered saline) (Sigma, St. Louis, MO). The obtained stock solution was stored at –80 °C until usage. Prior to use, the solution was diluted to the desired concentrations.

Normal human chondrocyte (NHAC-kn) cells were obtained from Lonza (Walkersville, MD), and plated in growth medium (CC-3216, Lonza, Walkersville, MD) at 37 °C and 5% CO2 conditions. After incubation for 4–7 days, the cells were divided into 6 or 12 well flask using subculture kit (CC-3233, Lonza, Walkersville, MD). Confluent chondrocytes (Fig. 1) were exposed to certain concentration of recombinant adiponectin (1, 4, 8 and 12 (μg/ml)) for 6, 12, 24 and 48 hours.

After the chondrocytes were treated with adiponectin for a certain period of time, the medium was discarded, the cells were washed 3 times with PBS, and total RNA was isolated with TriPure Reactive (Roche Diagnostics, Mannheim, Germany). The obtained total RNA concentration was measured and the complementary DNA (cDNA) was re-transcribed from 2 μg total RNA using random primer and High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA).

**Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)**

The expression levels of ADAMTS-9 and β-actin were measured by RT-PCR methods as describe before (12). Briefly, a total of 20 μl mixture consisting of 1x LightCycler FastStart DNA Master SYBR Green (Roche Diagnostics, Mannheim, Germany) obtained cDNA, proper primers and H2O was subjected to RT-PCR amplification using LightCycler® Nano System (Roche Diagnostics, Mannheim, Germany). For the RT-PCR protocol, a 3-step cycle (20 s at 95 °C, 20 s melting temperature (Tm) and 20 s at 72 °C) was repeated 45 times after 95 °C for 600 s pre-incubation. Primers and Tm values used in the amplifications are given in Table 1. Negative controls were used to check samples. In negative control, nuclease-free water was added to reactions tubes instead of RNA templates. The obtained experimental data were analyzed using absolute standard curve method (Fig. 2). The amplification of housekeeping gene, β-actin, was used as internal standard to normalizing ADAMTS-9 gene expression level. For each samples, the copy numbers of ADAMTS-9 and β-actin were calculated using template based standard curve, and ADAMTS-9/β-actin ratios were given to relative gene expression level.

**Statistical analysis**

PASW 18 (version 18.0 for Windows; SPSS Inc., Chicago, IL, USA) program was used for data evaluation. For statistical analysis, Mann Whitney U test was used. The values are given as mean value ± SEM. The difference was accepted statistically significant if p values were less than 0.05.

**Results**

To analyze the putative role of adiponectin on ADAMTS-9 gene expression, primary human chondrocytes were treated in the presence of certain doses (1, 4, 8 and 12 (μg/ml)) of adiponectin for 6, 12, 24 and 48 hours. At the end of the incubation, ADAMTS-9 gene expression level was analyzed by RT-PCR and standardized according to β-actin expression level. The relative increase in ADAMTS-9 gene expression was obtained by com-
paring ADAMTS-9 gene expression levels in chondrocytes with adiponectin-free conditions.

There was no statistically significant increase in ADAMTS-9 gene expression level for 1 and 4 μg/ml adiponectin treatment (Figs 3A and 3B). However, ADAMTS-9 gene expression level was increased at the dose of 8 and 12 μg/ml (Figs 3C and 3D). The highest up-regulation was obtained at 12 μg/ml adiponectin incubation for 48 hours.

**Discussion**

Adiponectin effectively plays vital roles in a number of metabolic processes especially in inflammatory pathological circumstances in organisms. The involvement of adiponectin in diseases such as cardiovascular diseases, endothelial dysfunction, type 2 diabetes, metabolic syndrome, and rheumatic diseases has been proven up to date (13, 14). Although, the hypothesis that adiponectin shows anti-inflammatory characteristic was suggested, the catabolic effect of adiponectin with inflammatory features in arthritic diseases was proven by several groups (15, 16). In addition, Lee et al (17) has suggested that adiponectin takes more important role than IL-1β in joint destruction. Therefore, the underlying mechanism for the pleiotropic action of adiponectin should be clarified. For this aim, primary human chondrocytes were exposed to recombinant adiponectin at physiological and pathological doses (18). The highest ADAMTS-9 upregulation was obtained at 12 μg/ml dose for 48 h incubation.

Irreversible destruction of joint cartilage components such as aggrecan is a critical event for not only rheumatoid arthritis (RA) but also osteoarthritis (OA). It was clarified that aggrecan destruction is the result of elevated aggrecanases activity by inflammation (19, 20). Obesity identified as accumulation of excessive amounts of adipose tissue is assumed one of the risk factors for RA and OA. Adipose tissue contributes to the development of inflammation in diseases by secreting adipokines. The mechanism of adipokines induced-ADAMTS gene expression in chondrocytes and chondrosarcoma was demonstrated before by our group (21–23). An up-regulated adiponectin level in both RA and OA patients was reported (24, 25). After these findings, the researchers have focused on the mechanism of adiponectin caused joint destruction. It was
demonstrated that adiponectin increased inflammatory interleu-
kin-6 (IL-6) (26) and interleukin-8 (27) in synovial fibroblast cells.
Besides, the upregulation of matrix metalloproteinase (MMP) -1
and -13 genes expression level in fibroblast-like cell was demon-
strated by Choi et al (28). The relationship between adiponectin
and other MMPs (MMP-2 and MMP-3) was also showed by other
researchers (29, 30).

However, there is no investigation apart from our data to demo-
strate the putative effect of adiponectin on ADAMTSs gene ex-
pression level in literature. The dose-dependent catabolic effect of
the other adipokines (leptin, resistin and visfatin) has been proven
by our group (31–33). It was also hypothesized that adiponectin
plays an important role as much as cytokines like IL-1β in the
cartilage destruction. To clarify the putative effects on ADAMTS
expression level, the primary human chondrocytes were incubated
with adiponectin for certain time points. The highest upregulation
(nearly 8 times more) was obtained at 12 μg / ml dose for 48 h
incubation. Similar data was obtained before by Lee et al (17).
The human endothelial cells and osteoblasts were stimulated with
adiponectin (1 or 10 μg / ml) by them. The upregulation of IL-6,
IL-8, MMP-1, and MMP-13 was much higher than with IL-1β
(0.1 ng / ml) stimulation. As a result of these experimental data, it
may be speculated that adiponectin may be involved in inflamma-
atory arthritic diseases by increasing ADAMTS-9 expression level.
However, the other ADAMTSs should be investigated to clarify
the mechanism. Anyway, our results supported the hypothesis
that adiponectin has a potential to be a marker for arthritic disease
(34, 35). So, adiponectin might be targeted to get new therapy for
arthritic diseases.

References

K. Molecular cloning of a gene encoding a new type of metalloproteinase-
disintegrin family protein with thrombospondin motifs as an inflammation
2. Hurskainen TL, Hirohata S, Seldin MF, Apte SS. ADAM-TS5, AD-
AM-TS6, and ADAM-TS7, novel members of a new family of zinc metal-
loproteinases. General features and genomic distribution of the ADAM-TS
3. Takeda S. ADAM and ADAMTS Family Proteins and Snake Venom
Metallloproteinases: A Structural Overview. Toxins (Basel) 2016; 8 (5).
4. Yaykasli KO, Ohashi T, Hirohata S, Hatipoglu OF, Inagawa K,
Demircan K, Ninomiya Y. ADAMTS9 activation by interleukin 1 beta
via NFATc1 in OUMS-27 chondrosarcoma cells and in human chon-
5. Clark ME, Kelner GS, Turbeville LA, Boyer A, Arden KC, Maki
RA. ADAMTS9, a novel member of the ADAM-TS/metalloproteinase gene
6. Apte SS. A disintegrin-like and metalloprotease (reprolysin-type) with
thrombospondin type 1 motif (ADAMTS) superfamily: functions and
7. Yaykasli KO, Yaykasli E, Ozsahin M, Uslu M, Kaya E. Leptin in-
creases ADAMTS9 gene expression in human chondrocytes. Curr Opinion
8. Murphy G, Nagase H. Reappraising metalloproteinases in rheumatoid
arthritis and osteoarthritis: destruction or repair. Nat Clin Pract Rheumatol
protein serum similar to C1q, produced exclusively in adipocytes. J Biol
11. Yaykasli K, Dogan M, Hatipoglu O, Yaykasli E, Kaya E, Ozsahin
M, Uslu M. Effect of adiponectin on a disintegrin and metalloproteinase
with thrombospondin motifs-9 gene expression in human chondrocytes
12. Hatipoglu OF, Hirohata S, Yaykasli KO, Cilek MZ, Demircan K,
Shinohata R, Yonezawa T et al. The 3’-untranslated region of ADAMTS1
13. Ye R, Scherer PE. Adiponectin, driver or passenger on the road to
Paul G et al. The potential of adiponectin in driving arthritis. J Immunol
2006; 177 (7): 4468–4478.
15. Chen TH, Chen L, Hsieh MS, Chang CP, Chou DT, Tsai SH. Evi-
dence for a protective role for adiponectin in osteoarthritis. Biochim Bio-
P, Jouzeau JY et al. Association between adiponectin and cartilage degra-
17. Lee YA, Ji HI, Lee SH, Hong SJ, Yang HI, Chul Yoo M, Kim KS.
The role of adiponectin in the production of IL-6, IL-8, VEGF and MMPs
in human endothelial cells and osteoblasts: implications for arthritic joints.
B. Serum adiponectin concentrations during a 72-hour fast in over-
19. Yaykasli KO, Yaykasli E, Ataoglu S, Ozsahin M, Memisoğullari
R, Çelebi E, Üçgun T et al. The frequency of omentin Val109Asp poly-
morphism and the serum level of omentin in patients with Rheumatoid
20. Inagawa K, Oohashi T, Nishida K, Minaguchi J, Tsubakihita T,
Yaykasli KO, Ohtsuka A et al. Optical imaging of mouse articular carti-
lage using the glycosaminoglycans binding property of fluorescent-labeled
21. Hatipoglu OF, Yaykasli KO, Doğan M, Yaykasli E, Kaya E, Özsa-
hin M, Uslu M. Leptin induction of aggrecanases-1 and -2 genes ex-
pression in human chondrocytes is mediated by p38 mitogen-activated protein
22. Yaykasli KO. Visfatin promotes a disintegrin and metalloproteinase
with thrombospondin motifs-5 gene expression through p38 MAPK and
23. Hatipoglu OF, Yaykasli KO, Yaykasli E, Kaya E, Özsahin M, Uslu
M, Yildirim K et al. Resistin induces a disintegrin and metalloproteinase
with thrombospondin motifs-4 gene expression in human chondrosarcoma
24. Lee YH, Bae SC. Circulating adiponectin and visfatin levels in rheu-
matoid arthritis and their correlation with disease activity: A meta-analysis.


Received January 30, 2017.
Accepted March 6, 2017.