Soluble receptor for advanced glycation end products (sRAGE) in plasma and synovial fluid is inversely associated with disease severity of knee osteoarthritis

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ARTICLE INFO

ABSTRACT

Objectives: The aim of this study was to measure soluble receptor for advanced glycation end products (sRAGE) in plasma and synovial fluid of knee osteoarthritis (OA) patients and to determine the correlation between sRAGE levels and disease severity.

Design and methods: Thirty-six OA patients and 15 healthy controls were enrolled in this study. OA grading was performed using the Kellgren–Lawrence classification. sRAGE levels in plasma and synovial fluid were analyzed by enzyme-linked immunosorbent assay.

Results: Plasma sRAGE levels were significantly lower in OA patients than in healthy controls ($P = 0.01$). sRAGE levels in plasma were remarkably higher with regard to paired synovial fluid ($P = 0.001$). Additionally, sRAGE concentrations in plasma and synovial fluid showed significant inverse correlation with disease severity ($r = -0.65$, $P < 0.001$ and $r = -0.55$, $P = 0.001$, respectively). Further analysis showed that there was a strong positive correlation between plasma and synovial sRAGE concentration ($r = 0.81$, $P < 0.001$).

Conclusions: sRAGE levels were significantly lower in OA patients compared with controls, and sRAGE levels in plasma and synovial fluid also decreased significantly as the disease severity increased. Accordingly, sRAGE levels could be used as a biochemical marker for assessing the severity and progression of knee OA.

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Introduction

Osteoarthritis (OA) is a chronic progressive degenerative joint disease that involves not only articular cartilage, but also synovium, subchondral bone, and surrounding muscles and ligaments. OA is characterized by cartilage destruction, subchondral bone sclerosis or cyst, and osteophyte formation. Knee osteoarthritis is the major cause of severe pain, joint stiffness, limited motion, and disability. At present, a conventional method to determine the affected joint is radiological study which reflects disease severity by grading the joint destruction. Despite the uncertain pathophysiology, several risk factors are considered as major contributors to the development of OA. Among these circumstances, aging and obesity are of great importance [1,2]. A principal feature of aging is the modification of proteins by non-enzymatic glycation.

Advanced glycation end products (AGE) are formed by a non-enzymatic glycation of proteins, initiated by a reaction between reducing sugars and free amino groups in lysine or arginine residues [3,4]. Once being formed, AGEs permanently attach to the protein, therefore the amount of AGEs accumulation is highly dependent on the rate of protein turnover [3,5]. Due to a remarkably long half-life of collagen, an abundance of AGEs accumulate in articular cartilage, and their amount increases with age. AGEs lead to cartilage stiffness and impair matrix synthesis in articular cartilage [3,4,6]. Thus accumulation of AGEs might explain an age-determined predisposition to the development of OA.

Receptor for advanced glycation end products (RAGE) is a multi-ligand cell surface receptor of the immunoglobulin (Ig) superfamily. Its structure is composed of 3 regions: an extracellular region comprising one Ig-like V domain and two Ig-like C type domains, a transmembrane region, and a short cytoplasmic tail [7]. RAGE is expressed in low level in different types of adult cells, such as macrophages, endothelial cells, neurons, synoviocytes, and articular chondrocytes [7–9]. Under an inflammatory condition with the present of its ligands, RAGE is rapidly up-regulated. RAGE can bind with varied ligands including AGEs, S100/calgranulins, high mobility group box-1 protein (HMGB-1), β-sheet fibrils, and β2-integrin Mac-1 [10,11]. RAGE activation leads to the generation of reactive oxygen species, which in turn increase the expression of the transcription factor NF-kβ. This pathway stimulates the production of matrix metalloproteinase and inflammatory mediators, which plays an important part in cartilage damage [8,11,12]. Moreover, ligand–

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receptor interaction promotes the expression of RAGE on the cell surface, thus acting as a positive feedback mechanism [13].

Soluble receptor for advanced glycation end products (sRAGE) is a C-truncated form of full-length RAGE, which lacks a cytoplasmic tail and a transmembrane domain [7,9]. sRAGE can be formed by 2 main mechanisms: the alternative splicing of RAGE mRNA (also called endogenous secretory RAGE or esRAGE), and the proteolytic cleavage of membrane-bound RAGE (also called cleaved RAGE or cRAGE). Because of its ability to bind with the same ligands, sRAGE acts as a competitive inhibitor of RAGE, and precludes the cell-bound RAGE signaling. RAGE adversely involves in several pathological processes of human diseases, such as diabetes, Alzheimer's disease, and chronic inflammatory diseases, namely rheumatoid arthritis (RA) [10], while sRAGE plays a protective effect. A number of clinical studies have been conducted to explore the possibility of using sRAGE as a disease marker in such disorders [7].

Recently, previous studies have shown that several cytokines are related to clinical parameters of disease severity in knee OA and could play essential roles in the pathogenesis of OA [14–17]. Therefore, we hypothesized that sRAGE in plasma and synovial fluid may be correlated with the severity in knee OA patients. Accordingly, the purpose of this study was to determine both plasma and synovial fluid levels of sRAGE in knee osteoarthritic patients, and examine the association between plasma and synovial fluid sRAGE with the disease severity.

Materials and methods

Subjects

Thirty-six patients (28 females and 8 males), who met the criteria of the American College of Rheumatology for knee osteoarthritis, and 15 healthy individuals (12 females and 3 males) were enrolled in this study. None of the participants had the underlying diseases of diabetes or other chronic inflammatory diseases. The present study was conducted in agreement with the guidelines of the Declaration of Helsinki. Every patient and healthy subject had signed written informed consents before participating in the study. This research was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University.

The Kellgren and Lawrence (KL) grading system was used to determine the disease severity [18]. Osteoarthritis was divided into 5 grades (0–4) according to the structural changes presented in weight-bearing anteroposterior radiographs of the affected knee: grade 0 (normal findings), no radiological changes; grade 1 (questionable), doubtful narrowing of joint space and possible osteophyte lipping; grade 2 (mild), definite osteophytes and possible joint space narrowing; grade 3 (moderate), multiple moderate osteophytes, definite narrowing of joint space, bone sclerosis and possible deformity of bone contour; grade 4 (severe), large osteophytes, marked joint space narrowing, severe sclerosis, and deformity of bone contour. OA patients were defined as having radiographic knee OA of KL grade ≥ 2 in at least 1 knee. Controls were defined as having neither radiographic hip OA nor knee OA, as indicated by KL grades of 0 for both hips and both knees. The grading of more severely affected knee in each patient was used for data analysis.

Laboratory methods

Venous blood samples taken from the cubital vein of each participant were centrifuged and stored immediately at −80 °C for later measurement. Synovial fluid was collected from the affected knee during a total knee arthroplasty. The specimen was then centrifuged to remove cells and joint debris and stored at −80 °C until analysis.

Double-blind quantitative assessment of sRAGE concentration in plasma and synovial fluid was performed using commercially available enzyme-linked immunosorbent essay (ELISA) (Quantikine, R&D Systems, Minneapolis, MN, USA). According to the manufacturer's protocol, 50 μL of recombinant human sRAGE standards, plasma, and synovial fluid samples were added into each well, which has been pre-coated with mouse monoclonal antibody specific for sRAGE. After incubating for 2 h at room temperature, every well was washed thoroughly with wash buffer for 4 times. Then 200 μL of polyclonal antibody against sRAGE conjugated to horseradish peroxidase was pipetted into each well and incubated for a further 2 h at room temperature. After 4 washes, substrate solution was added into the wells and then a microplate was incubated for 30 min at room temperature with protection from light. Lastly, the reaction was stopped by the stop solution and the optical density was determined using an automated microplate reader at 450 nm. A standard optical density–concentration curve was drawn for the determination of sRAGE concentration. Twofold serial dilutions of recombinant human sRAGE with a concentration of 78–5000 pg/mL were used as standards. The manufacturer reported precision was 4.8–6.2% (intra-assay) and 6.7–8.2% (inter-assay). The sensitivity of this assay was 4.12 pg/mL.

Statistical analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS) software, version 16.0 for Windows. Demographic data between patients and controls were compared by Chi-square tests and unpaired Student's t tests, where appropriate. Comparisons between the groups were performed using one-way analysis of variance (ANOVA) with Tukey post hoc test if ANOVA showed significance. Correlations between plasma and synovial fluid RAGE and disease severity were assessed using Pearson's correlation coefficient (r). Data were expressed as a mean ± standard error of the mean. P-values < 0.05 were considered to be statistically significant for differences and correlations.

Results

Thirty-six knee OA patients whose ages ranged from 51 to 81 years and 15 healthy individuals whose ages ranged from 59 to 83 years were recruited in the present study. The age of OA patients did not significantly differ from that of controls (68.8 ± 1.4 vs. 72.1 ± 1.9 years, $P = 0.6$). As shown in Fig. 1, plasma sRAGE levels were lower in OA patients than in healthy participants (596.8 ± 52.8 pg/mL
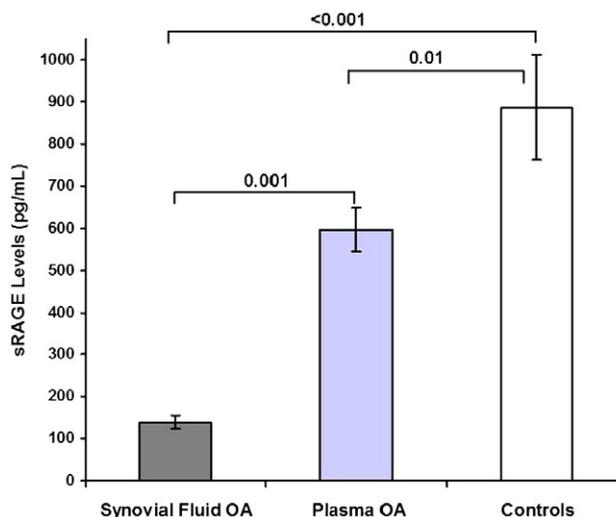

Fig. 1. sRAGE levels in plasma and synovial fluid of patients with OA and healthy controls.

vs. 887.5 ± 124.8 pg/mL, $P = 0.01$). sRAGE levels in synovial fluid of OA patients (138.2 ± 15.5 pg/mL) were 4-fold lower than in corresponding blood samples ($P = 0.001$), and were 6-fold lower than in the plasma of healthy controls ($P < 0.001$). There was a strong positive correlation between plasma and synovial sRAGE concentration ($r = 0.81$, $P < 0.001$) (Fig. 2).

With regard to the radiological KL classification, patients were categorized into 3 groups in relation to OA grading. Ten patients were classified as grade 2, 13 as grade 3, and 13 as grade 4. As illustrated in Table 1, sRAGE concentrations in plasma and synovial fluid decreased significantly as the disease severity increased.

In addition, the associations between plasma and synovial fluid levels of sRAGE and the disease severity of osteoarthritis were statistically investigated. The plasma concentrations of sRAGE inversely correlated with knee OA severity ($r = -0.65$, $P < 0.001$) (Fig. 3). Subsequent analysis demonstrated that synovial fluid sRAGE levels of knee OA patients also negatively correlated with OA grading ($r = -0.55$, $P = 0.001$) (Fig. 4).

Discussion

RAGE and its ligands are believed to play a role in OA pathogenesis in aging population. Despite low expression in adult cells, RAGE is up-regulated in inflammatory condition including rheumatoid arthritis and osteoarthritis [10,11,19]. Moreover, RAGE expression is increased in the presence of its ligands, such as AGEs, S100/calgranulins, and HMGB-1. While RAGE promotes synovial inflammation, cartilage destruction and matrix degradation, sRAGE prevents them. sRAGE is a truncated form of cell surface RAGE. It not only competes with RAGE in binding the same ligands, but also precludes them from interacting with other receptors, such as toll-like receptors-2 and 4, α7-nicotinic acetylcholine receptor, CD36, and proteoglycans [10].

In this study, we found that plasma sRAGE levels were significantly lower in OA patients compared with those in healthy controls. Concurrently, decreased sRAGE levels have been recently observed in rheumatoid arthritis (RA) [7,9,13]. Circulating and synovial fluid sRAGE levels in RA patients were lower than those in patients with non-inflammatory joint diseases and sRAGE levels increased after treating with DMARDs [13]. Furthermore, Stewart and colleagues conducted a study in patients with Sjogren's syndrome, and found that patients with primary Sjogren's syndrome had significantly lower serum sRAGE levels compared with secondary Sjogren's disease patients [20].

The mechanism by which plasma and synovial fluid sRAGE levels are decreased in knee OA patients is unknown. In the light of these

Table 1

Plasma and synovial fluid sRAGE in knee osteoarthritis patients. Data are expressed as mean and SEM. *P*-values for differences among KL subgroups.

	Total	KL grade 2	KL grade 3	KL grade 4	*P*
n	36	10	13	13	
SF sRAGE (pg/mL)	138.2 ± 15.5	213.5 ± 35.2	133.0 ± 18.6	85.4 ± 17.1	0.002
Plasma sRAGE (pg/mL)	596.8 ± 52.8	865.6 ± 119.1	632.3 ± 62.5	354.6 ± 27.8	0.001

SF = synovial fluid; sRAGE = soluble receptor for advanced glycation end products.

considerations, several possible mechanisms could contribute to the reduction of circulating sRAGE in knee OA patients. One possibility is that sRAGE production is inhibited by enhanced AGEs and/or cytokines. Alternatively, an imbalance between sRAGE production and sRAGE clearance could be responsible for decreased sRAGE levels. In advanced OA stage, lower sRAGE concentrations may be ascribed to an increased clearance of sRAGE-ligand complexes [21]. The accumulation of RAGE ligands either by aging or inflammation might lead to the increased consumption of sRAGE. In rheumatoid arthritis, many RAGE ligands strongly expressed in inflamed synovial tissues, such as HMGB-1, and S100/calgranulins [22,23]. Similarly, levels of AGEs in serum, synovial fluid, and urine from OA patients were increased compared with controls [3]. High levels of these ligands promoted the production of sRAGE-ligand complexes, which were subsequently eliminated by the reticuloendothelial system, thus decreasing levels of sRAGE in synovial fluid and blood samples [13]. Moreover, RAGE activation by its ligands might inhibit the formation of sRAGE either by decreasing the cleavage of cell surface receptor or by altering the expression of soluble isoform [11]. Additionally, an oxidant environment, which is produced by reactive oxygen species, might decrease the splicing of RAGE mRNA that formed sRAGE. Another possible explanation may be genetic variant in RAGE gene. Recent study revealed that RAGE Gly82Ser polymorphism generated a soluble isoform of RAGE with higher affinity to RAGE ligands [24], which could compete with membrane-bound RAGE even better. Therefore, patients with this polymorphism might display more decreased levels of sRAGE. Furthermore, this genetic variant also affected the splicing of RAGE mRNAs, and the susceptibility of cell-bound RAGE to the cleavage by matrix metalloproteinase [25].

In the present study, synovial fluid sRAGE levels showed a strongly positive correlation with plasma sRAGE levels; however, sRAGE levels in synovial fluid were dramatically lower than those observed in paired plasma samples. These findings implied that sRAGE was secreted
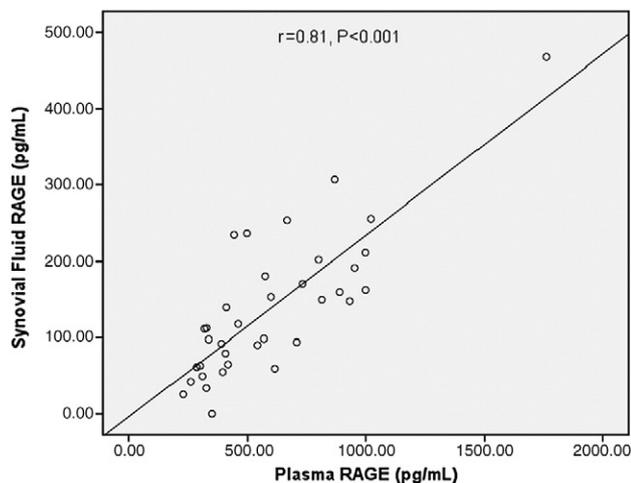
Fig. 2. Scattergram showing the positive correlation between plasma and synovial fluid sRAGE concentrations ($r = 0.81$, $P < 0.001$).
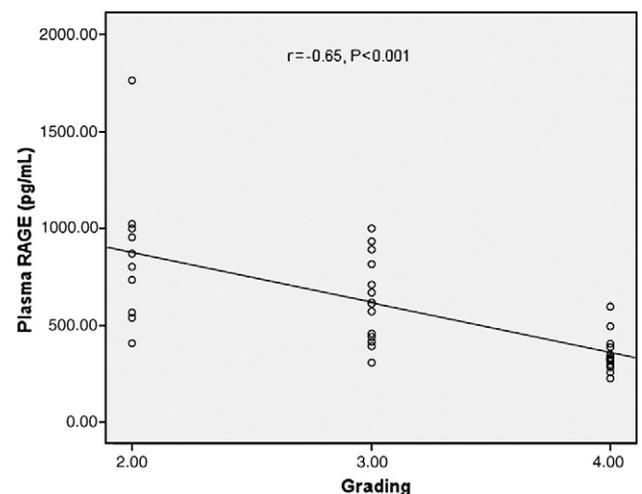
Fig. 3. Scattergram showing the negative correlation between plasma sRAGE levels in patients with OA severity classified according to Kellgren and Lawrence grading scale ($r = -0.65$, $P < 0.001$).
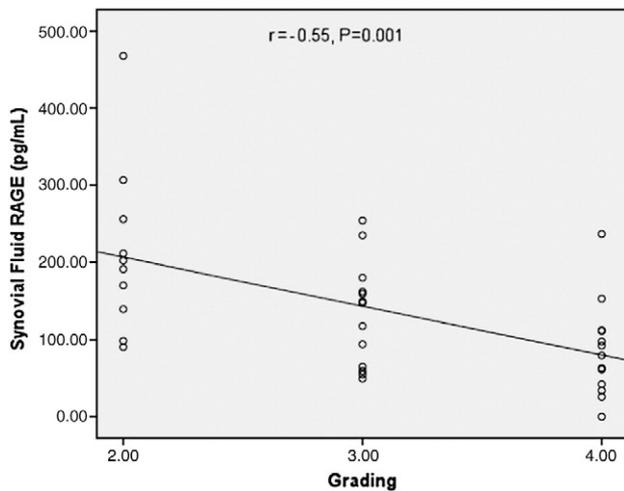
Fig. 4. Scattergram showing the negative correlation between synovial fluid sRAGE levels in patients with OA severity classified according to Kellgren and Lawrence grading scale ($r = -0.55$, $P = 0.001$).

elsewhere into a systemic circulation, and entered the joint through synovial membrane. The reason for decreased levels of sRAGE in synovial fluid could be the greater consumption of sRAGE intra-articularly, due to the higher levels of RAGE ligands accumulated in the inflamed joints. Alternatively, it is presumably attributed to the fact that sRAGE's structure and molecular weight prevented it from entering the synovial fluid. The mechanisms of sRAGE reduction in the plasma and synovial fluid of knee OA patients remain to be further investigated.

The current study demonstrated that sRAGE levels in plasma and synovial fluid declined as the severity of knee OA increased. This could be explained by the hypothesis that greater inflammatory response in more severe disease created more amounts of RAGE ligands resulting in the consumption of sRAGE. Moreover, recent study showed that OA severity scores were positively correlated with RAGE expression on articular chondrocytes [8]. As previously mentioned, RAGE activation lowered the production of soluble isoform. Nevertheless, whether the low level of sRAGE was a cause or an effect of OA progression remained unclear. Genetic variant that produced less amount of sRAGE might put a particular person in a greater risk of developing more severe diseases, or sRAGE levels might decrease as the disease progressed to a more advanced stage. Although there is a growing body of evidence supporting the protective role of sRAGE in inflammatory joint diseases, some study results were against it. Pullerits and coworkers demonstrated that rheumatoid arthritic patients with less severe disease had higher levels of anti-RAGE antibodies [26], which reacted with sRAGE. This result suggested that sRAGE might act as a pro-inflammatory molecule.

It should be mentioned, however, that this study inevitably has some inherent limitations. Firstly, the ELISA assay for sRAGE used in this study assesses all soluble forms, including soluble RAGE cleaved from cell surface RAGE, endogenous secretory RAGE, and new splice variants of RAGE [27]. Moreover, the system developed for sRAGE determination may not distinguish among diverse sRAGE splice variants. Due to this limitation, we could not indicate the definite source of sRAGE detected by the assay we used. sRAGE may originate from alternative splicing of RAGE mRNAs and/or from proteolytic release of the RAGE proteins from the cell surface [27,28]. Since the proportion of various isoforms of sRAGE could be distinct among individuals, the development of novel specific assays will help improve the measurement of sRAGE concentrations. In this regard, we speculate that each soluble variant plays a specific role within the RAGE pathway, being expressed in a highly regulated pattern [29]. Secondly, sample size of patients enrolled in the present study was relatively small. Further investigation with a random sample of a

larger population will be warranted to make a more definite conclusion. Thirdly, our study was designed as a cross-sectional study; therefore, definite cause and effect relationships may not be concluded. However, our study provides evidence for the role of sRAGE in the development and severity of knee osteoarthritis. Prospective longitudinal studies are required to define the cause-effect relationships and clarify the exact role of sRAGE in knee OA. Lastly, it was impossible to differentiate whether low levels of sRAGE reflected low levels of circulating AGEs and tissue RAGE expression, or resulted from the sRAGE capture of high circulating AGEs levels while functioning as a decoy receptor for RAGE. Additional studies for circulating AGEs and tissue RAGE expression could render more valuable information on the pathogenic role of RAGE in OA.

Taken together, the present study showed that plasma sRAGE in knee OA patients was significantly lower compared with that of healthy volunteers. Synovial fluid sRAGE was greatly reduced with respect to paired plasma sRAGE. Whether low sRAGE levels in OA patients are the consequence or a potential contributing factor of the disease needs to be elucidated. Furthermore, sRAGE levels in plasma and synovial fluid were inversely associated with the disease severity in OA patients. These findings indicated that sRAGE levels could be used as a biochemical marker for assessing disease activity in knee osteoarthritis. Prospective and intervention studies are necessary to evaluate the possibility of using sRAGE as a treatment option in retarding the progression of osteoarthritis.

Acknowledgments

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