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Effects of advanced glycation end products on the expression of COX-2, PGE_2 and NO in human osteoarthritic chondrocytes

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Objective. Advanced glycation end products (AGE) accumulate in articular cartilage with age. We investigated the effects of AGE in primary-cultured human OA chondrocytes.

Methods. Chondrocytes were cultured with/or without AGE-bovine serum albumin (AGE-BSA) and the expression levels of inducible nitric oxide (iNOS), cyclooxygenase (COX)-2 microsomal prostaglandin E synthase-1 (mPGES-1) were evaluated using RT-PCR and western blot analysis. Prostaglandin E_2 (PGE_2) was analysed by ELISA and nitric oxide (NO) was analysed by Griess reaction assay. Pharmacological studies to elucidate the involved pathway were executed using specific inhibitors of MAPK and receptor for AGE (RAGE).

Results. We found that treatment of OA chondrocytes with AGE–BSA increased COX-2, mPGES-1 and iNOS mRNA and protein, as well as elevating production of PGE_2 and NO. Pre-treatment with the MAPK inhibitors SP600125 (JNK inhibitor), SB202190 (p38 inhibitor) or PD98059 (ERK inhibitor) significantly inhibited AGE–BSA induction of COX-2 expression and production of PGE_2. In contrast, SN50, a nuclear factor-κB (NF-κB) inhibitor, had no effect on levels of COX-2 and PGE_2. SB202190 and SN50, but not SP600125 and PD98059, decreased AGE–BSA-induced production of NO. Pre-treatment with soluble receptor for AGE (sRAGE) also reduced AGE-stimulated COX-2, iNOS and PGE_2, implicating the involvement of RAGE.

Conclusions. These results show that AGE may augment inflammatory responses in OA chondrocytes by increasing PGE_2 and NO levels, possibly via the MAPK pathway for PGE_2 and the NF-κB pathway for NO.

Key words: Advanced glycation end products, Osteoarthritis, Chondrocyte, Cyclo-oxygenase-2, Microsomal prostaglandin E synthase-1, Nitric oxide, Prostaglandin E2.

Introduction

OA is a common and disabling chronic condition in the elderly. Although ageing is considered a risk factor for OA [1], its precise contribution to OA development remains largely unknown. One prominent feature of ageing is the accumulation, in long-lived proteins such as cartilage collagens, of advanced glycation end products (AGE) [2]. Once AGEs are formed, they remain in the tissue until the protein involved is degraded [3]. Articular cartilage has been reported to be particularly sensitive to the accumulation of AGE [4]. For example, increased levels of AGE in the knee cartilage of Beagle dogs increased their susceptibility to OA development [5], and accumulation of AGE has been correlated with increased tissue stiffness in articular cartilage [6, 7]. Moreover, an increase in AGE renders articular cartilage tissue increasingly brittle and thus more prone to mechanical damage [8, 9].

In addition to affecting the mechanical properties of tissue, increased AGE decrease the synthesis of proteoglycans and collagens in articular cartilage chondrocytes [6, 9]. The biological activities of AGE are thought to be mediated by specific receptors for AGE (RAGE). Activation of RAGE engages critical signalling pathways linked to pro-inflammatory responses, resulting in activation of various inflammatory genes [10, 11]. Activation of RAGE in OA has been shown to stimulate chondrocytes and synoviocytes, resulting in increased production of matrix metalloproteinase 1 (MMP-1) [12]. Despite the association of AGE with OA, there have been few studies concerning the catabolic functions of AGE in the pathogenesis of arthritic diseases. Recently, we reported that the AGE increase MMP-1, -3 and -13, and TNF-α by stimulating the JNK, p38 and ERK and nuclear factor-κB (NF-κB) activity in human chondrocytes [13].

As well as MMPs, prostaglandin E_2 (PGE_2) plays a critical role in the pathophysiology of OA. Chondrocytes are a major source of PGE_2 in the joint; the production of this prostanoid can be induced by pro-inflammatory cytokines, mitogens, mechanical stress and trauma [14, 15]. The synthesis of PGE_2 from arachidonic acid requires two enzymes acting sequentially. Cyclo-oxygenase (COX)-2 converts the arachidonic acid to PGG_2 and then PGH_2, and PGH_2 is converted to PGE_2 by microsomal prostaglandin E synthase-1 (mPGES-1). So the increasing synthesis of PGE_2 requires these two inducible enzymes acting sequentially [16]. The mPGES-1, originally designated for membrane-bound glutathione *S*-transferase 1-like-1 (GST1-like-1), is especially important in inflammatory response since it is strongly induced by pro-inflammatory stimuli and functionally linked to COX-2 [16, 17]. However, some PGH_2 is converted to PGI_2 by prostacyclin synthase (PGIS), and then PGI_2 is promptly converted 6-keto-$PGF_{1\alpha}$, its stable product. [18].

In human articular chondrocytes, the expression of mPGES-1 is up-regulated by IL-1β [19]. In addition, inducible nitric oxide (iNOS) expression has been demonstrated within the joint fluid of patients with OA chondrocytes [20], and it is up-regulated by diverse pro-inflammatory stimuli such as IL-1β, TNF-α and lipopolysacccharide (LPS). In iNOS-deficient mice, OA was significantly reduced, resulting in diminished cartilage lesions and osteophyte formation [21]. This inducible enzyme generates significantly greater and more sustained amounts of NO [22].

We therefore determined the effects of AGE on the expression of COX-1, COX-2, mPGES-1 and iNOS, and the production of PGE_2 and NO. The underlying mechanism was also investigated using specific inhibitors of the mitogen-activated protein kinases (MAPKs) and NF-κB.

Materials and methods

Reagents

All reagents were purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA) unless otherwise indicated. Fetal bovine

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serum (FBS), DMEM and other culture products were purchased from Gibco-BRL (San Diego, CA, USA). [γ-^{32}P] ATP (6000 Ci/mmol) was obtained from Perkin Elmer Life and Analytical Sciences, Inc. (Boston, MA, USA). Endotoxin-free AGE-bovine serum albumin (AGE-BSA) was purchased from MBL International (Woburn, MA, USA). SP600125 (JNK inhibitor), SB202190 (p38 inhibitor), PD98059 (ERK inhibitor) and SN50 (NF-κB inhibitor) were obtained from Biosource (Camarillo, CA, USA). Soluble RAGE (sRAGE) was obtained from R&D Systems (Minneapolis, MN, USA).

Specimen selection and chondrocyte cultures

With institutional review board (IRB) approval, cartilage was isolated from the knee joints of 32 OA patients aged 59–76 yrs (mean age, 68 ± 2.4 yrs) who underwent knee replacement surgery at Asan Medical Center, Seoul. All studies were carried out according to the Declaration of Helsinki guidelines, and written informed consent was obtained from each patient.

In brief, each cartilage specimen was thinly sliced, minced and digested with agitation for 14–18 h at 37°C in DMEM (Gibco-BRL) containing 1 mg/ml collagenase (Gibco-BRL), 1% antibiotic–anti-mycotic solution (Gibco-BRL) and 10% FBS. After digestion, the chondrocytes were passed through a 70-μm cell strainer (BD Biosciences, Franklin Lakes, NJ, USA), washed with serum-free media, counted and re-suspended in culture media. The released chondrocytes were seeded at 2×10^6 cells in 6-cm culture plates. All experiments were performed within 5 days of primary culture to avoid dedifferentiation of OA chondrocytes.

Cell viability assay

Chondrocytes were seeded at 2×10^4 cells per well in 96-well culture plates and incubated for 48 h. Following treatment with different concentrations of AGE–BSA, the chondrocytes were incubated for 24 h, and their viability was measured using an XTT-based assay kit (Roche, Basel, Switzerland), in which a 50-μl aliquot of XTT solution was added for 16 h and the absorbance was measured at a test wavelength of 450 nm and a reference wavelength of 630 nm.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from OA chondrocytes (2×10^5 cells/ml) treated with/without AGE-BSA using Trizol (Gibco-BRL) according to the manufacturer's protocol. A 1 μg aliquot of each total RNA sample was reverse transcribed for 1 h at 37°C in a reaction mixture containing 40 U RNase inhibitor (Amersham, Piscataway, NJ, USA), 0.5 mM dNTPs (Boehringer Mannheim, Indianapolis, IN), 2 μM random hexamer primers (Stratagene, La Jolla, CA, USA) and 30 U AMV reverse transcriptase (Promega, Madison, WI, USA). PCR was performed using these cDNAs as templates and the primers are shown in the Table 1.

Western blot analysis

The chondrocytes were washed twice with PBS, collected in tubes by the scraper and treated with lysis buffer [50 mM Tris–HCL [pH7.4], 150 mM NaCl, 0.5% sodium deoxycholate, 1% NP-40, 1× proteinase inhibitor (Sigma)]. Cell lysates were centrifuged for 15 min at 13 000 g at 4°C and 30-μg aliquots of proteins were separated by SDS–PAGE gel electrophoresis and transferred onto nitrocellulose membranes (Schleicher & Schuell, London, UK). The membranes were blocked with 5% skim milk in 10 mM Tris–HCl containing 150 mM NaCl and 0.5% Tween-20 (TBS-T), washed with TBS-T and incubated with primary antibodies (1 : 1000) to MAPKs (Cell Signaling, Danvers, MA, USA), COX-2, COX-1 and mPGES-1 (Cayman Chemical, Ann Arbor, MI, USA) and iNOS (R&D Systems). After thorough washing with TBS-T, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Amersham), and the blots were developed using an enhanced chemiluminescence detection kit (Amersham).

Enzyme-linked immunosorbent assay

Concentrations of human PGE_2 and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), a stable product of COX, were measured using specific ELISA kits (Assay Designs, Ann Arbor, MI, USA). Briefly, 2×10^5 cells in 12-well plates were treated with/without AGE–BSA for 24 h, the supernatants were collected and PGE_2 and 6-keto-$PGF_{1\alpha}$ were quantified according to the manufacturer's protocol, using standard curves.

NO quantification

Following a 24 h incubation with AGE–BSA, the chondrocyte culture media were harvested and analysed with a nitrate/nitrite colorimetric assay. Briefly, nitrate was converted to nitrite by nitrate reductase, and Griess reagent (Promega) was added to convert nitrite to a deep-purple azo compound. The absorbance of the latter was measured at 540 nm using a plate reader. The assay had a limit of detection of 1 μM nitrite.

Statistical analysis

Statistical analysis was performed using SPSS 11.0 software (SPSS, Chicago, IL, USA). ANOVA with post hoc test and the Mann–Whitney U-test were used to compare pairs of groups. The accepted level of significance was $P < 0.05$. Data are presented as mean ± S.D.

Results

Effects of age on expression of COX-2, COX-1, mPGES-1 and INOS

To determine whether AGE–BSA influences the viability of OA chondrocytes, cells were treated with 0, 100, 200, 400 and 800 μg/ml AGE–BSA for 24 h, and their viability was measured by a tetrazolium salt XTT assay. We found that, at these concentrations, AGE–BSA had no significant cytotoxic effect on OA chondrocytes (Fig. 1A). We therefore treated OA chondrocytes with 10, 50, 100, 200, 400 and 600 μg/ml AGE–BSA for 8 h and measured the expression of COX-2, COX-1, mPGES-1 and iNOS mRNA. We found that AGE–BSA, as low as 10 μg/ml, markedly increased the level of COX-2 mRNA, with COX-2 mRNA level remaining elevated as AGE–BSA was increased. Consistent with its effects on COX-2, AGE–BSA also increased

TABLE 1. Primers for RT-PCR

	Forward primer	Reverse primer	Product size (bp)
Human GAPDH	5′-ACCACAGTCCATGCCATCAC-3′	5′-TCCACCACCCTGTTGCTGCA-3′	450
Human iNOS	5′-TAG AGG AAC ATC TGG CCA GG-3′	5′-TGG CAG GAT CCC CTC TGA TG-3′	372
Human mPGES-1	5′-CCAAGTGAGGCTGCGGAAGAA-3′	5′-GCTTCCCAGAGGATCTGCAGA-3′	339
Human COX-2	5′-ATCTACCCTCCTCAAGTCCC-3′	5′-TACCAGAAGGGCAGGATACAG-3′	708

GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

the expression of mRNA specific for mPGES-1, which is functionally related to COX-2 [16]. Densitometry showed that AGE–BSA increased mPGES-1 mRNA level ~2.2-fold and COX-2 mRNA level ~4-fold compared with control. AGE–BSA, however, had no effect on COX-1 mRNA level. Lower concentrations of AGE–BSA (0, 10 and 50 μg/ml) had no effect on iNOS mRNA expression, whereas AGE–BSA concentrations of 100 μg/ml and higher increased iNOS message abundance (Fig. 1B). COX-2 and mPGES-1 mRNAs were also slightly increased by BSA alone (400 and 600 μg/ml), but not as much as by AGE–BSA (Supplementary Fig. E1, available as supplementary data at *Rheumatology* Online).

Using western blotting, we also tested the effects of AGE on protein levels of COX-2, COX-1, mPGES-1 and iNOS. Consistent with our RT-PCR results, we found that AGE–BSA increased the levels of COX-2, mPGES-1 and iNOS proteins, but had no effect on the level of COX-1 (Fig. 2).

Stimulation of PGE_2 and NO production by AGE

PGE_2 is coordinately produced by COX-2 and mPGES-1, whereas 6-keto-$PGF_{1\alpha}$, stable product of PGI_2, is a product of COX-2 and PGIS activities. Consistent with its induction of COX-2 and mPGES-1 expression, AGE–BSA stimulated PGE_2 production (Fig. 3A) and 6-keto-$PGF_{1\alpha}$ release (Fig. 3B) in a dose-dependent manner. Moreover, the amount of NO secreted into the extracellular space was increased significantly by AGE–BSA concentrations of 50 μg/ml and higher (Fig. 3C). PGE_2 production was also increased by BSA (200 and 600 μg/ml), but not as much as by AGE–BSA (Fig. 3A), whereas BSA alone (200 and 600 μg/ml) had no significant effect on NO production levels (Fig. 3C).

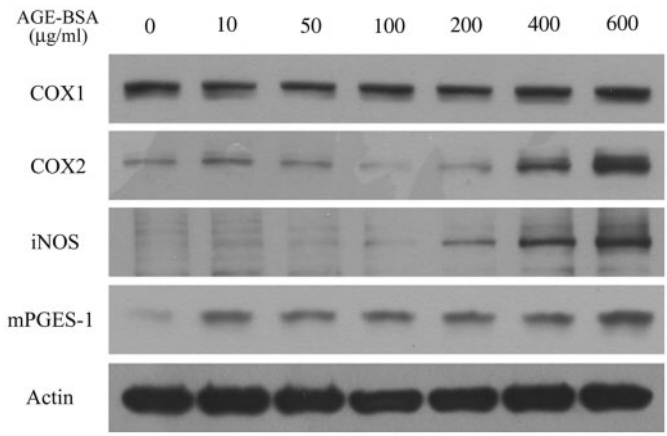

FIG. 2. Effects of AGE–BSA on expression of COX-1, -2, mPGES-1 and iNOS proteins in human OA chondrocytes. Cell lysates were used for western blotting against antibodies to each of these proteins, with antibody to β-actin used as an internal control. OA chondrocytes are obtained from four donors.

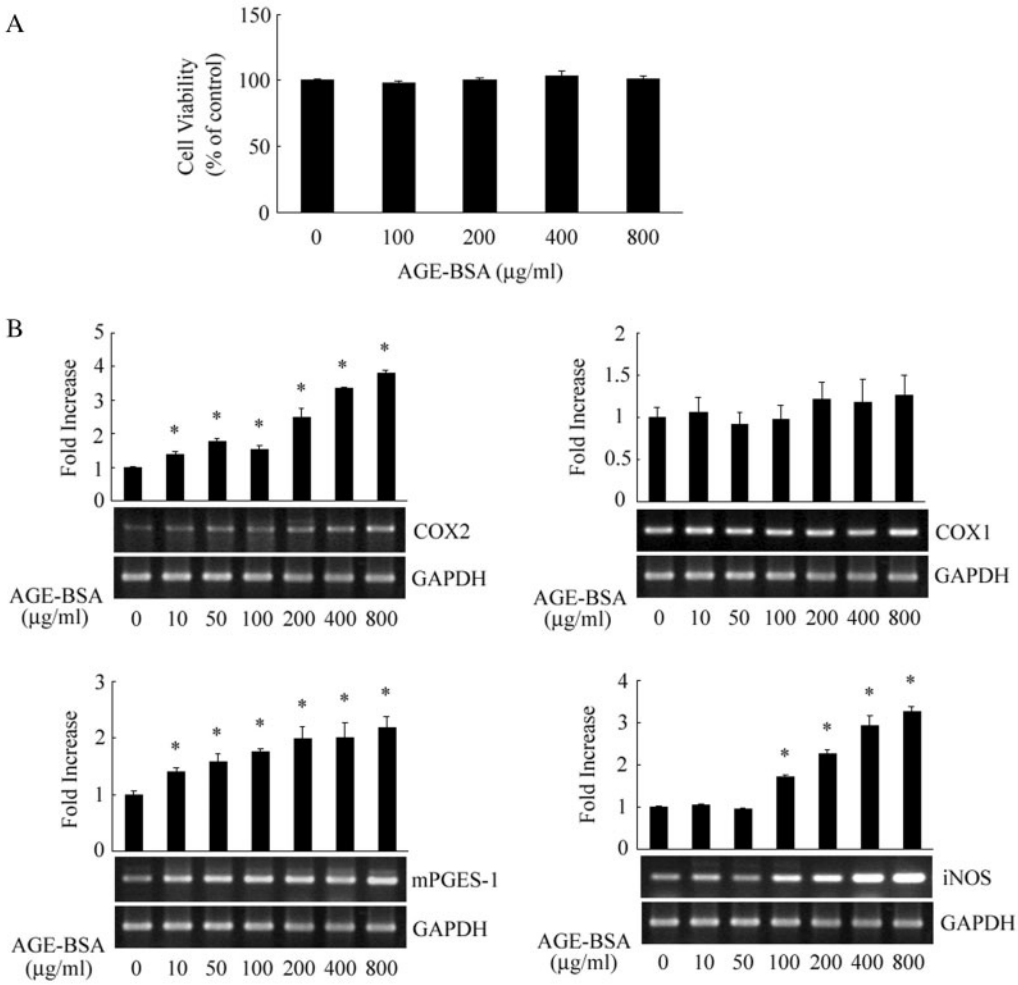

FIG. 1. Effects of AGE–BSA on COX -2, -1, mPGES-1 and iNOS expressions in human OA chondrocytes. (**A**) The cytotoxicity of AGE-BSA was evaluated using the XTT cell-proliferation assay. (**B**) RT-PCR for COX-2, COX-1, mPGES-1 and iNOS. Values are normalized against GAPDH expression. Results are representative of duplicate experiments with OA chondrocytes obtained from four donors. $^*P < 0.05$ *vs* control.

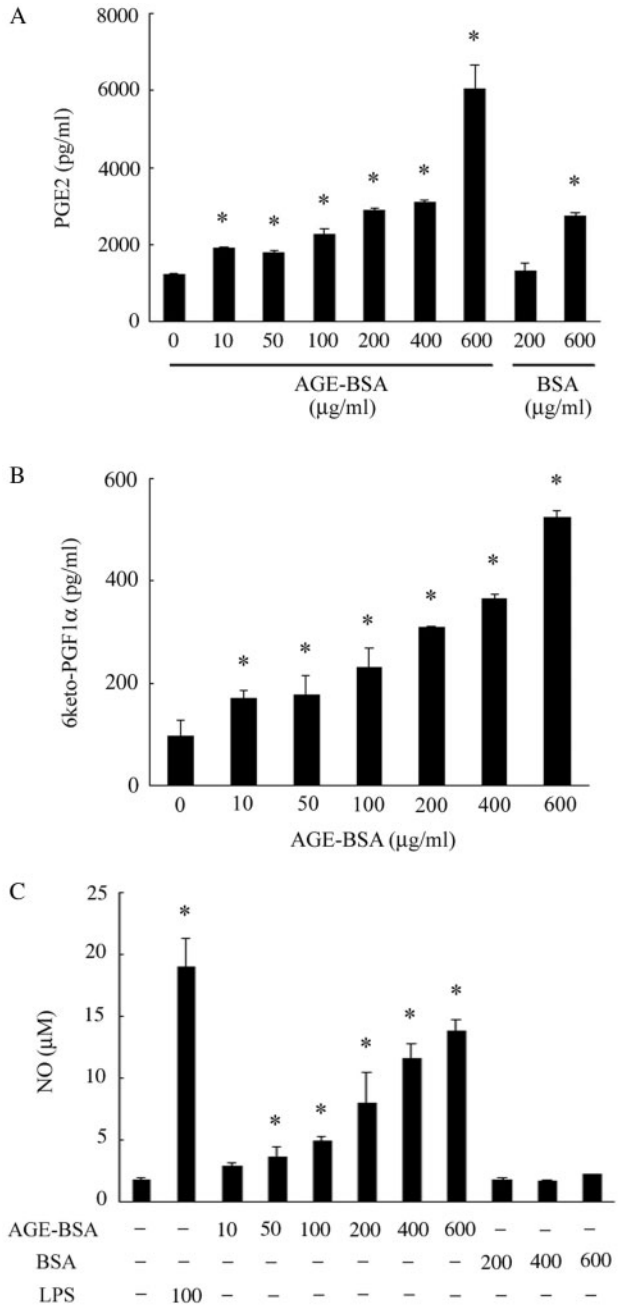

FIG. 3. Effects of AGE–BSA on PGE_2 and NO production in human OA chondrocytes. (**A** and **B**) Measurement of secreted PGE_2 and 6-keto-$PGF_{1\alpha}$ by ELISA. (**C**) Measurement of NO by Griess reaction assay, with 100 ng/ml LPS used as a positive control. Each value represents the mean ± S.D. Results are representative of duplicate experiments with OA chondrocytes obtained from six donors. $^*P < 0.05$ *vs* control.

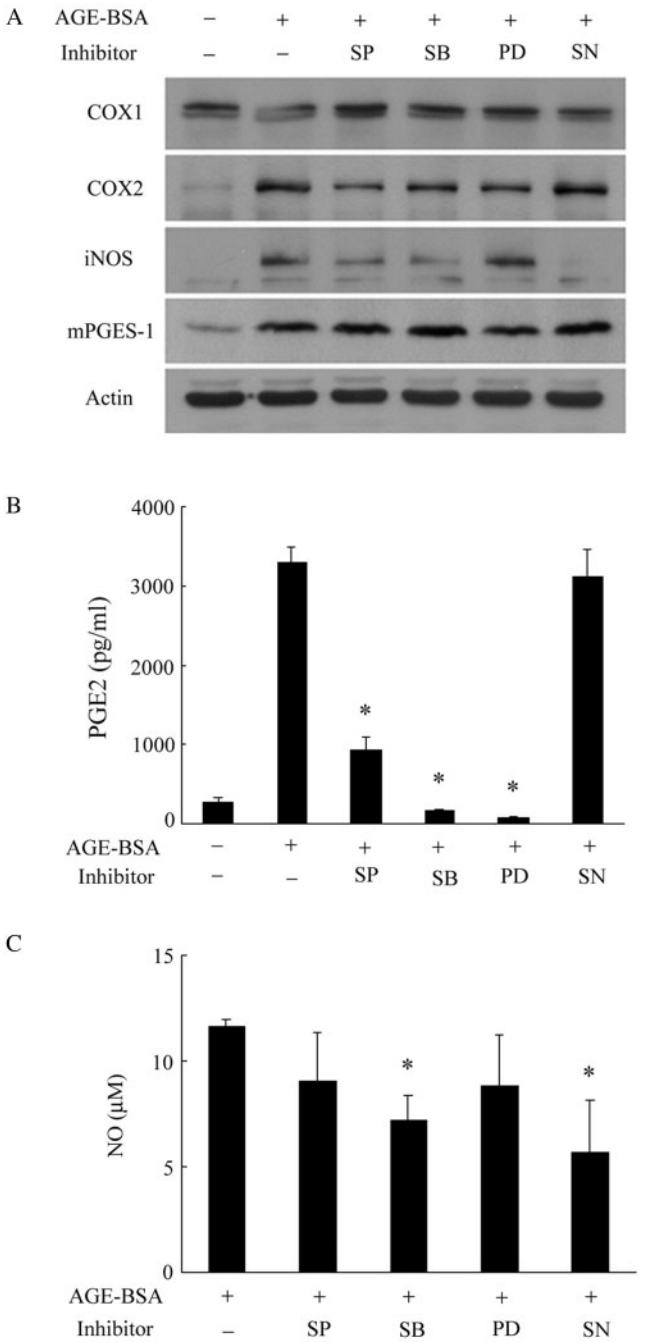

FIG. 4. Pharmacological evaluation of AGE–BSA -mediated MAPKs and NF-κB signalling pathways in human OA chondrocytes. (**A**) Measurement of AGE–BSA-induced expression of COX -2, -1, mPGES-1 and iNOS by western blotting. (**B**) Measurement of secreted PGE_2 by ELISA. (**C**) Measurement of NO by Griess reaction assay. Where indicated, OA chondrocytes were pre-treated with SP600125 (SP), SB202190 (SB), PD98059 (PD) or SN50 (SN) for 1 h, followed by 400 μg/ml AGE–BSA for 24 h. Each value represents the mean ± S.D. Results are representative of duplicate experiments with OA chondrocytes obtained from six donors. $^*P < 0.05$ *vs* control.

Evaluation of MAPKs and NF-κB signalling pathways involved in AGE–BSA-induced PGE_2 and NO productions

Previously, our study revealed that AGE–BSA stimulated phosphorylation of JNK, p38 and ERK-MAPKs, and increased NF-κB activity in a dose-dependent manner [13]. To determine the signalling pathway involved in AGE–BSA-induced PGE_2 and NO production, we tested the effect of pre-treatment with PD98059 (ERK inhibitor), SP600125 (JNK inhibitor), SB202190 (p38 inhibitor) or SN50 (NF-κB inhibitor) in human OA chondrocytes. Western blot analysis showed that pre-treatment with SP600125, SB202190 or PD98059 significantly inhibited AGE–BSA-induced production of COX-2, whereas pre-treatment with SN50 had no effect (Fig. 4A). AGE–BSA increased mPGES-1 expression, but was not inhibited by SP600125, SB202190, PD98059 or SN50, and none of these inhibitors had any effect on COX-1 expression (Fig. 4A). In addition, AGE–BSA-induced production of PGE_2 was significantly inhibited by pre-treatment with SP600125 (71.9%), SB202190 (95.3%) or PD98059 (97.7%), but not by SN50 (Fig. 4B). Taken together,

these results suggest that AGE–BSA-induced production of PGE_2 was associated with increasing COX-2 and mPGEs-1. AGE–BSA-induced activation of COX-2 may be mediated by the MAPK pathways, but not by the NF-κB pathway, whereas AGE–BSA-induced activation of mPGES-1 may not be mediated by either.

AGE–BSA-induced iNOS expression was inhibited by pre-treatment with SP600125, PD98059 or SN50 (Fig. 4A). In addition, the effect of SN50 on AGE–BSA induction of NO level was similar to that of iNOS protein expression (Fig. 4C), suggesting that AGE–BSA-induced NO production may be mainly via NF-κB activation.

Evaluation of the engagement of RAGE

To determine whether RAGE is involved in AGE–BSA-induced expression of PGE_2 and NO, OA chondrocytes were pre-treated with sRAGE for 1 h before the addition of AGE–BSA (400 μg/ml). Using western blotting, the expression levels of COX-2 andiNOS were all significantly reduced with sRAGE, but mPGES-1 was not reduced (Fig. 5A). The expression levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ were all significantly reduced with sRAGE ($P < 0.01$), but the production of NO was not reduced ($P > 0.05$) (Fig. 5B and C). These results indicate that AGE–BSA-induced expression of COX-2, iNOS and PGE_2 is RAGE-mediated.

Discussion

PGE_2 plays a critical role in the pathogenesis of OA [14]. The synthesis of PGE_2 from arachidonic acid requires two enzymes acting sequentially, COX and PGE synthase. Of the PGE synthases, mPGES-1 is especially important since it is functionally linked to COX-2 in articular chondrocytes [16, 17]. In the present study, we demonstrated that AGE–BSA stimulated the expression of COX-2 and mPGES-1, resulting in increased production of PGE_2 in human OA chondrocytes. We also found that AGE–BSA did not affect the expression of COX-1, which was constitutively expressed. These data indicate that AGE–BSA-induced PGE_2 production is related to increased expression of both COX-2 and mPGES-1 in human OA chondrocytes.

The AGE-mediated signalling pathways are linked to pro-inflammatory responses [10]. ERK signalling has been reported to be activated through a direct ERK–AGE interaction [23], and activation of p38 and JNK MAPKs is also thought to occur by similar interactions [24]. We found that AGE–BSA-induced PGE_2 production in human OA chondrocytes was mediated via the JNK, p38 and ERK pathways, a finding in agreement with previous reports showing that MAPKs mediate the effects of AGE in other cells [23–25]. AGE–BSA-induced mPGES-1 expression was not attenuated by any of the inhibitors tested. These results provide compelling evidence that AGE–BSA-induced PGE_2 production via the MAPKs pathway may occur through the activation of COX-2, but may not affect mPGES-1 stimulation, in human OA chondrocytes.

We also observed that AGE–BSA markedly increased NF-κB DNA binding activity in these cells. However, pre-treatment with SN50 did not affect the expression of COX-2 and mPGES-1 and the production of PGE_2, suggesting that NF-κB may not mediate the AGE–BSA induction of COX-2 and mPGES-1 in human OA chondrocytes.

NO is another important inflammatory mediator in the pathogenesis of OA [26]. In chondrocytes and synovial cells, NO inhibits the synthesis of collagen and proteoglycan [27], activates MMPs [28] and increases susceptibility to injury [29]. We found that AGE–BSA increased NO production by stimulating iNOS in human OA chondrocytes. Our results indicate, however, that the underlying mechanism by which AGE–BSA induces NO production is different from that of PGE_2. AGE–BSA-induced

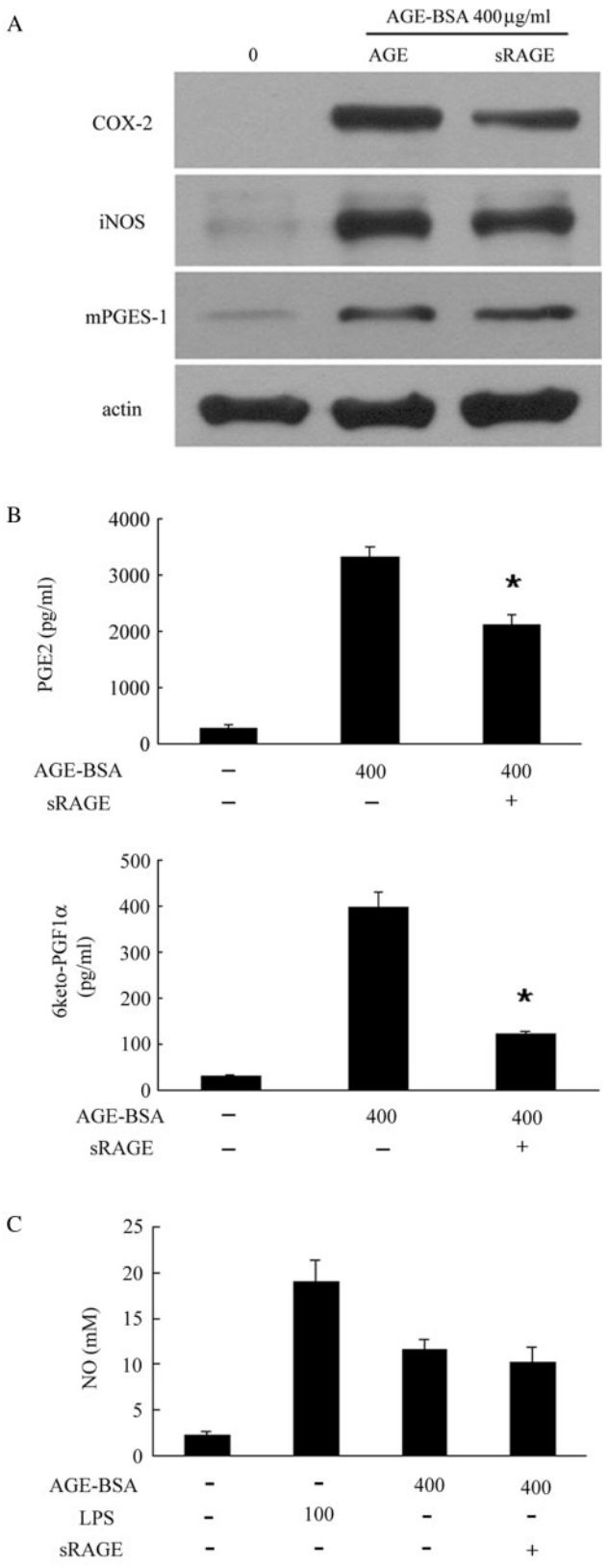

FIG. 5. Pharmacological evaluations of the engagement of RAGE in AGE–BSA-induced PGE_2 and NO production using sRAGE in human OA chondrocytes. (**A**) Measurement of AGE–BSA-induced expression of COX-2, mPGES-1 and iNOS by western blotting. (**B**) Measurement of secreted PGE_2 and 6-keto-$PGF_{1\alpha}$ by ELISA. (**C**) Measurement of NO by Griess reaction assay. OA chondrocytes were pretreated with sRAGE for 1 h and then treated with 400 μg/ml AGE–BSA for 24 h. The culture media were then collected and analysed by ELISA. Results are representative of experiments with OA chondrocytes obtained from six donors. $^*P < 0.05$, AGE–BSA *vs* AGE–BSA with sRAGE-treated group.

iNOS expression was completely inhibited by pre-treatment with SN50, indicating the involvement of NF-κB in NO production.

The mechanisms underlying the effects of AGE involve specific receptor-mediated signalling pathways. A recent report indicated that glycated albumin-stimulated MMP-1 expression was blocked by a neutralizing antibody against RAGE, which is the most characterized AGE receptor, in OA fibroblast-like synoviocytes [12]. The sRAGE has been shown to inhibit the increases in MMP-13 expression seen with S100B, HMGB-1 or RAGE ligands via ERK pathway [11]. However, there have been several questions regarding the involvement of other receptors in mediating the effects of AGE, with galectin-3 [30] and CD36 [31] being likely candidates, and the possibilities of endocytosis of AGE [32] or direct AGE-ERK interaction should be considered [23]. In the current study, sRAGE significantly inhibited the production of COX-2, iNOS and PGE_2, but not mPGES-1 and NO. These findings show that receptors other than RAGE may mediate the effects of AGE, and that the effects of AGE are not solely dependent on RAGE. Based on this result, we cannot exclude the possibility that receptors in addition to RAGE are involved. It should be further determined if inhibition of RAGE expression by siRNA blocks the effect of AGE–BSA.

To exclude the possible effect of BSA, we tested the effect of BSA treatment on the production of PGE_2 and NO in human OA chondrocytes. Although higher concentrations of BSA (600 μg/ml) increased PGE_2 production, this increase was small compared with that induced by AGE–BSA. Moreover, in agreement with previous findings [12], we observed that BSA alone had no significant effect on the level of NO.

Primary human OA chondrocytes in culture undergo extensive morphological changes with sequential passaging, dedifferentiating to a fibroblast-like state [33, 34]. To determine the phenotypic characteristics of the chondrocytes used, we performed RT-PCR analysis for daily change of Col1, and Col2A1 on OA chondrocytes for 14 days. Type II collagen (Col2A1) was not significantly changed till day 14, but type I collagen (Col1) was increased daily and increased more till days 5–14. These results were compatible with the real-time PCR observations [34]. And we stained cells cultured for 7 days with antibodies to collagen types I and II. Type II collagen was detected whereas type I collagen was not, and did not show any morphological changes of dedifferentiation (Supplementary Fig. E2, available as supplementary data at *Rheumatology* Online). We therefore used only OA chondrocytes grown for $\leq$5 days in primary culture.

Although the chondrocytes used in the current study mostly came from the non-lesioned tissue that is similar to normal aged cartilage in terms of the slow steady recruitment of RAGE expression, we cannot exclude the possibility that OA pathology itself might have influenced the chondrocyte responses to AGE. Our results thus may not be extrapolated to the situation in RA or normal cartilage.

Previous studies have used complex AGE generated from fatty-acid-free BSA or a specific AGE, usually pentosidine or N3-carboxymethyllysine (CML). The AGE used in the current study is the commercially available AGE–BSA that was produced by reacting BSA with glycolaldehyde under sterile conditions, followed by extensive dialysis and purification steps; the resulting AGE–BSA is a complex that includes CML, pentosidine and other AGE [35]. Thus, the results of the present study were obtained with a complex AGE rather than a particular AGE.

In conclusion, AGEs increase COX-2, mPGES-1 and iNOS in human OA chondrocytes and the inflammatory responses augment in consequence of PGE_2 and NO production, which is caused by augmentation of COX-2, mPGES-1 and iNOS. In view of the results so far achieved, it is presumed that an augmentation of PGE_2 is regulated via MAPKs pathway and an augmentation of NO production is regulated via NF-κB.

Rheumatology key messages

- AGE are associated with OA with catabolic effects in chondrocytes by increasing PGE_2 and NO levels.
- PGE_2 and NO production is caused by augmentation of COX-2, mPGES-1 and iNOS.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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