INTRODUCTION

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In addition to COX enzymes inhibition with NSAID, the regulation of the COX enzymes at the posttranscriptional and posttranslational levels also resulted in COX-2 inhibition including rapid degradation of COX-2 mRNA attributed to AU-rich elements (AREs) at its 3’ UTR (9-11), endoplasmic reticulum-associated degradation (ERAD), and proteasomal degradation of cox gene (12, 13). Since COX-2 has a unique C-terminal 19-amino acid cassette (19-aa) located near the C-terminus, but not in COX-1, “19-aa” plays an important role in mediation of the entry of COX-2 into the ER-associated degradation (ERAD) system, where transports ER proteins to the cytoplasm for degradation by the 26S proteasome (12). These

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Abbreviations: NSAID, nonsteroidal anti-inflammatory drugs; GS-HCl, glucosamine hydrochloride; OA, osteoarthritis; COXs, cyclooxygenases; ER, endoplasmic reticulum; AREs, AU-rich elements; ERAD, endoplasmic reticulum-associated degradation; PMSF, phenylmethylsulfonyl fluoride; CTL, non-treated control; CIA, collagen induced arthritis

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID) SPARING EFFECTS OF GLUCOSAMINE HYDROCHLORIDE THROUGH N-GLYCOSYLATION INHIBITION; STRATEGY TO RESCUE STOMACH FROM NSAID DAMAGE

S.H. PARK1,*, H. HONG1,*, Y.M. HAN1,2, N. KANGWAN1,2, S.J. KIM3, E.H. KIM1,2*, K.B. HAHM1,4

1CHA Cancer Prevention Research Center, CHA Cancer Institute, CHA University, Seoul, Korea; 2College of Pharmacy, CHA University, Pocheon, Korea; 3Laboratory of Cell Regulation and Carcinogenesis, CHA Cancer Institute, CHA University, Seoul, Korea; 4Department of Gastroenterology, CHA University Bundang Medical Center, Seongnam, Korea

Gastrointestinal or cardiovascular complications limit nonsteroidal anti-inflammatory drugs (NSAID) prescription. Glucosamine hydrochloride (GS-HCl) alternatively chosen, but debates still exist in its clinical efficiency. COX-2 instability through inhibiting COX-2 N-glycosylation of GS-HCl raised the possibility of NSAID sparing effect. Study was done to determine whether combination treatment of glucosamine and NSAID contributes to gastric safety through NSAID sparing effect. IEC-6 cells were stimulated with TNF-α and compared the expressions of inflammatory mediators after indomethacin alone or combination of indomethacin and GS-HCl by Western blotting and RT-PCR. C57BL/6 mice injected with type II collagen to induce arthritis were treated with indomethacin alone or combination of reduced dose of indomethacin and GS-HCl after 3 weeks. TNF-α increased the expression of COX-2, iNOS and inflammatory cytokines, but GS-HCl significantly attenuated TNF-α-induced COX-2 expression. Decreased COX-2 after GS-HCl was caused by N-glycosylation inhibition as much as tunicamycin. Combination of reduced dose of indomethacin and GS-HCl significantly reduced the expressions of ICAM-1, VCAM-1, IL-8, IL-1β, MMP-2, MMP-7, MMP-9, and MMP-11 mRNA as well as NF-κB activation better than high dose indomethacin alone. These NSAID sparing effect of GS-HCl was further proven in collagen-induced arthritis model. Combination of GS-HCl and 2.5 mg/kg indomethacin showed significant protection from gastric damages as well as efficacious anti-arthritic effect. Taken together, COX-2 N-glycosylation inhibition by GS-HCl led to indomethacin sparing effects, based on which combination of GS-HCl and reduced dose of NSAID can provide the strategy to secure stomach from NSAID-induced gastric damage as well as excellent anti-arthritic effects.

Key words: arthritis, gastric damages, COX-2 N-glycosylation, glucosamine, indomethacin

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modulating actions of COX can provide the chance to rescue stomach from NSAID-induced gastric damage.

Glucosamine (C6H13NO5) is an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. Since glucosamine is a precursor for glycosaminoglycan and glycosaminoglycans are a major component of joint cartilage, glucosamine supplementation has been tried to either prevent cartilage degeneration or alleviate arthritic symptoms (14). However, the real clinical utility of glucosamine hydrochloride (GS-HCl) for arthritis treatment remains still under debates due to lack of variable clinical efficacies. It has been reported that glucosamine could induce COX-2 instability because glucosamine inhibited COX-2 N-glycosylation at “19-aa” (15). Thus, we hypothesized that GS-HCl can synergize NSAID to potentiate COX-2 inhibition.

Based on in vitro and in vivo animal experiments, we confirmed NSAID sparing effects can lead to reduced gastric toxicity under the warranty of anti-inflammatory action.

MATERIALS AND METHODS

Reagents

All chemical reagents were obtained from Sigma (St. Louis, MO). Primers for RT-PCR were synthesized by Bioneer (Daejeon, Korea). Reverse transcriptase, Taq DNA polymerase and 10X PCR buffer were from Promega (Madison, WI). Antibodies for iNOS, COX-2, beta-actin, p-ERK, ERK, p-JNK, JNK, p-p38, p38, IkB-alpha were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-conjugated anti-rat/rabbit/mouse IgG was purchased from Thermo Scientific Pierce (Rockford, IL). All other materials including glucosamine hydrochloride (GS-HCl) were obtained in the highest available grade.

Cell culture and drug treatment

The mouse intestinal cell line IEC-6 was maintained at 37°C in a humidified atmosphere containing 5% CO2. IEC-6 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. IEC-6 cells (1.0×10^4/ml) were plated and incubated for 16 hours after which media were changed with fresh one containing TNF-α. Then the cells were incubated with indomethacin and glucosamine for the indicated times. After treatment, cells were harvested for protein extraction for Western blotting analysis and mRNA extraction for RT-PCR.

Western blot analysis

Briefly, treated cells were washed twice with PBS and then lysed in ice-cold cell lysis buffer (Cell Signaling Technology, Danvers, MA) containing 1 mM phenylmethylsulfonyl fluoride (PMSF). After 15 min of incubation, samples were centrifuged at 12,000 g for 15 min. Supernatants were then collected. Proteins in lysates were separated by SDS-PAGE and transferred to PVDF membranes, which were incubated with appropriate antibodies and then visualized using a West-zol Plus (Intron Biotechnology, Seongnam, Korea).

RNA isolation and RT-PCR

Total RNA was isolated using the TRIzol® reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. One µg of total RNA was reverse transcribed with murine leukemia virus reverse transcriptase (Promega, Madison, WI) at 42°C for 50 min and at 72°C for 15 min. Supernatants were then collected. Proteins in lysates were separated by SDS-PAGE and transferred to PVDF membranes, which were incubated with appropriate antibodies and then visualized using a West-zol Plus (Intron Biotechnology, Seongnam, Korea).

Table 1. Primer sequences for PCR.
NCBI/prime-blast. Oligonucleotide primers were purchased from Bioneer (Daejeon, Korea). The sequences of oligonucleotide primers are listed in Table 1. Amplification products were analyzed on 1.0% agarose gel electrophoresis, stained with ethidium bromide, and photographed under ultraviolet light.

**Animal experimental procedure**

Animals were handled in an accredited animal facility in accordance with Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International) guidelines under the facility named CACU (The Center of Animal Care and Use) of Gachon University Lee Gil Ya Cancer and Diabetes Institute after IRB approval.

Seven weeks old specific pathogen free male C57BL/6 mice (Orient Bio, Sungnam, Korea) were used for the experiments. A total of 40 mice were divided into four groups (n=10, Fig. 3A); Group 1, a non-treated control group; Group 2, collagen induced arthritis group (CIA); Group 3, indomethacin (10 mg/kg) treated CIA group; Group 4, indomethacin (2.5 mg/kg) plus GS-HCl (5 mg/kg) treated group. The CIA group, in which mice were injected with type II collagen (chicken type II collagen, Sigma-Aldrich, St. Louis, MO), was compared with an age-matched control. CIA was induced by an intradermal injection of 100 mg of collagen type II emulsified in complete Freund’s adjuvant into the tail base (16). After 1 week, mice received further hind feet booster injection. The animals were observed twice a week for 3 weeks after primary immunization, then anesthetized and killed at 3 weeks and two days after induction of CIA. CIA development was confirmed by clinical and histological inspection (Figs. 3B and 3C). The severity of arthritis was evaluated by visual inspection. All four legs of the mice were evaluated and scored from 0 to 4 according to the following scale: 0, no signs of arthritis; 1, swelling and/or redness of the paw or one digit; 2, two joints involved; 3, more than two joints involved; and 4, severe arthritis of the entire paw and all digits. Arthritis scoring was performed by two independent observers in a blinded fashion. Animals were deprived of food, but allowed free access to water for 24 hours before administration of drugs. Stomachs were removed, opened along the greater curvature, and rinsed with phosphate-buffered saline. Isolated tissues were subjected to a histological examination and Western blotting, RT-PCR and immunohistochemistry staining. Pathological lesion index was defined and reviewed by two gastroenterology specialists and the first author.

**TdT-mediated biotinylated UTP nick end labeling (TUNEL) assay and immunohistochemistry**

TUNEL assay kit was purchased (Promega) and performed according to manufacturer’s instruction. Briefly, the sections were incubated first with proteinase K (20 μg/ml) for 10 min at room temperature, then with TdT and fluorescein-12-dUTP for 1 h at 37°C, and finally with DAPI for 5 min at room temperature. The samples were mounted and inspected using a confocal microscopy. Immunohistochemistry was performed on replicate sections of mouse colon tissues. Sections fixed in 10% buffered formalin and embedded in paraffin were deparaffinized, rehydrated, and boiled three times in 100 mM Tris-buffered saline (pH 7.6) with 5% urea in an 850 W microwave oven for 5 min each. Sections were also incubated with an appropriate antibody in the presence of 1.0% bovine serum albumin and finally incubated for 16 hours at 4°C. The sections were counterstained with hematoxylin.

**Prostaglandin E2 assay**

Following harvesting of the stomach, and homogenized in 10 mM sodium phosphate buffer, pH 7.4 (1 mL). After centrifugation (9000 × g), the prostaglandin E2 (PGE2) level in the supernatant was measured by ELISA, and the concentration is expressed as pg/mg protein. The processes were performed as prostaglandin E2 express EIA kit manuscript (Cayman, Ann Arbor, MI).

**Statistical analysis**

The data are presented as means ± standard deviations (S.D.). The data were analyzed by ONE-WAY ANOVA, and the statistical significance between groups was determined by Student’s t-test. Statistical significance was accepted when P<0.05.

**RESULTS**

**The effect of GS-HCl, indomethacin, and the combination of glucosamine and indomethacin on TNF-α-induced COX-2 expression**

IEC-6 cell line, non-transformed small intestinal cell line, was used to investigate the influence of GS-HCl alone, indomethacin alone and the combination of GS-HCl and indomethacin on TNF-α-induced COX-2 expression (Figs. 1A-1C). As shown in Fig. 1A, 10 or 20 ng/ml of TNF-α induced overexpression of COX-2 protein, 10 ng/ml of TNF-α induced COX-2 better than 20 ng/ml in this cell line. Co-treatment with indomethacin (50 μM) reduced TNF-α-induced COX-2 around 50% in IEC-6 cells. However, the combination of 5 mM GS-HCl and 25 μM indomethacin was very effective in reducing TNF-α-stimulated COX-2 mRNA and protein, whereas 25 μM indomethacin was not effective in reducing TNF-α-induced COX-2 expression (Fig. 1B). Western blotting showed that GS-HCl alone or combination with 25 μM indomethacin all decreased COX-2 expression, along with reduced molecular size of COX-2, 66 kDa (Fig. 1B). As reported previously (15), these 6 kDa decreases in molecular size of COX-2 might be due to N-glycosylation inhibition of COX-2. Therefore, in order to confirm whether COX-2 decrement after GS-HCl was caused by N-glycosylation inhibition, tunicamycin, a well-known inhibitor of N-glycosylation, was administrated and compared. As seen in Fig. 1C, 1 μg/ml tunicamycin led to COX-2 low molecular mass from 72 to 66 kDa. We inferred that this low molecular mass of COX-2 with glucosamine administration, 66 kDa, might be caused by COX-2 de-glycosylation form of protein. Since N-acetylgalactosamine did not show COX-2 de-glycosylation efficacy, even though it has shown the significant ability to inhibit TNF-α-induced COX-2 protein (Fig. 1C), we concluded that the efficient COX-2 inhibitory mechanism of GS-HCl is operated through N-glycosylation inhibition. In addition, we observed that the combination of GS-HCl and selective COX-2 inhibitor, NS-398 in our study, instead of indomethacin also showed the synergistic effect to inhibit the expression of COX-2 (Fig. 1D). These results suggest that NS-AID sparing effect of GS-HCl was not influenced by COX-2 selectivity.

**The effect of GS-HCl, indomethacin, and the combination of indomethacin and GS-HCl on TNF-α-induced inflammation**

Treatment with TNF-α induced the expressions of IL-8, TNF-α, ICAM-1, and VCAM-1 as well as several kinds of MMP (Fig. 2A) in IEC-6 cells. In addition to COX-2 inhibitory action of glucosamine alone or combination of GS-HCl and indomethacin (Fig. 1), indomethacin alone or combination with GS-HCl apparently inhibited the TNF-α-stimulated IL-8, TNF-α, ICAM-
and VCAM-1 expressions. Additionally, the expressions of IL-1β, IL-6, IFN-γ, though not induced significantly with TNF-α, were significantly decreased with indomethacin alone or combination with GS-HCl. Among the MMPs, MMP-2 and MMP-9 have known to be especially important in collagen degradation, through digestion of denatured collagen generated by thermal denaturation at body temperature after specific cleavage of the triple helix region of the fibrillar collagen molecules by collagenases (17). Indeed, the expressions of MMP-2, MMP-7, MMP-9, and MMP-11 were increased in TNF-α-stimulated inflammation or tissue destruction, which were all decreased with either indomethacin alone or combination with GS-HCl (Fig. 2A). As well as the expression of COX-2 (Fig. 1D), the expression of IL-8, iNOS and VCAM-1 induced by TNF-α were inhibited by treatment with the combination of GS-HCl and indomethacin or the selective COX-2 inhibitor, NS398 (Fig. 2B). These inflammatory responses stimulated by TNF-α were associated with the activation of ERK1/2 and p38 among MAPKs (Fig. 2C). Indomethacin alone or combination with GS-HCl significantly inactivated the phosphorylation of p38 and ERK1/2. JNK was not engaged in either TNF-α stimulation or GS-HCl combination. Additionally, treatment with TNF-α reduced the expression of IkBα, whereas indomethacin alone or combination with GS-HCl preserved the expression of IkBα in spite of TNF-α stimulation, suggesting indomethacin or combination with glucosamine inhibited inflammatory transcriptional activation of NF-κB along with MAPK inactivation. These in vitro experiments led us to investigate whether combination of reduced dose of indomethacin and GS-HCl can exert similar anti-inflammatory effect compared to usual dose of indomethacin, NSAID sparing effect, but rescued gastric toxicity of indomethacin based on reduced dose of indomethacin.

**The NSAID sparing effect of GS-HCl in in vivo animal model**

In order to compare the anti-arthritis effect as well as gastric safety between 10 mg/kg indomethacin and the combination of 2.5 mg/kg indomethacin and 5 mg/kg GS-HCl, collagen induced arthritic model (CIA) was established (Fig. 3A). First, gross and histological assessment of CIA was done according to the group as shown in Figs. 3B and 3C. The joint swelling and severe deformity was shown in the CIA group (white arrow) in Group 2. On pathological findings, severe bone erosion and resorption of cartilage was noted in the CIA group accompanied with high level of inflammation.
of inflammatory cellular infiltration and cellular exudates into the joint space as well as cartilage (Fig. 3C). However, Group 4, the group receiving both 2.5 mg/kg indomethacin and GS-HCl resulted in a significant reduction of joint inflammation \((P<0.05\), Figs. 3B and 3C) and anti-arthritis efficacy of Group 4 was significantly better than Group 3 administered with 10 mg/kg indomethacin \((P<0.05\), Fig. 3D). As shown in Fig. 3E, the pathological scores of Group 4 were significantly decreased than those of Group 3 \((P<0.05)\).

One of key molecular mechanisms of NSAID-induced gastric damage is the robust induction of apoptosis in gastric mucosa (18). Indomethacin (10 mg/kg) induced significant levels of apoptosis assessed with TUNEL staining in erosion area as well as non-eroded area of gastric mucosa (Fig. 4A). TUNEL positive cells were dramatically increased in gastric ulcer margin as well as ulcer base after 10 mg/kg of indomethacin, whereas the combination of 2.5 mg/kg indomethacin and 5 mg/kg GS-HCl led to significant reduction in gastric mucosal apoptosis. Simply lower dose of

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| TNF-α (10 ng/mL) | -   | +   | +   | +   | +   |
| Glucosamine (5 mM) | -   | -   | -   | +   | +   |
| Indomethacin (25 μM) | -   | -   | -   | -   | +   |

| TNF-α (10 ng/mL) | -   | +   | +   | +   | +   |
| Glucosamine (5 mM) | -   | -   | -   | +   | +   |
| Indomethacin (25 μM) | -   | -   | -   | +   | +   |

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Fig. 2. The changes of various inflammatory mediators and MMPs according to drug treatment in TNF-α-stimulated IEC-6 cells. (A) Cells were treated with TNF-α alone and/or indomethacin 25 μM indomethacin and/or 5 mM GS-HCl for 16 hours and then RNA was extracted. RNA was reverse-transcribed and amplified with specific primers and for the housekeeping gene GAPDH to normalize the amount of DNA template used in each PCR reaction. (B) Cells treated with TNF-α were further treated with 50 μM of indomethacin alone or 5 μM of NS-398 and their combinations with 5 mM GS-HCl. Theses samples were analyzed by RT-PCR for inflammatory genes including COX-2. (C) Cells were treated with TNF-α alone and/or 25 μM indomethacin and/or 5 mM GS-HCl for 16 hours. After isolation of protein, immunoblotting was performed using appropriate antibodies for p-ERK, ERK, p-JNK, JNK, p-p38, p38 and IκB-α. Sequential incubation with anti-β-actin confirmed equal protein loading. All these results are representative of three independent experiments.
indomethacin in Group 4 might lead to lower level of apoptosis because the dose of indomethacin was reduced. However, considering similar anti-arthritic efficacy between 10 mg/kg indomethacin and combination of 2.5 mg/kg indomethacin and GS-HCl, NSAID sparing effect of GS-HCl is responsible for with lesser gastric toxicity. The other causative factor associated with NSAID-induced gastric damage is hypoxia caused by increased leukocyte aggregations to supplying blood vessel, reflected with increased levels of HIF-1α and COX-2 as well as increased expressions of MMPs (Fig. 4B-4D). Among MMPs, we have studied MMP-2, of which gastric expressions were significantly increased with 10 mg/kg indomethacin (P<0.05), but statistically significantly decreased with combination of indomethacin and GS-HCl (P<0.05, Fig. 4C). Similar to results as seen in in vitro investigations (Fig. 2B), significant inactivation of MAPK including ERK1/2 and p38 and preservation of IκBα were noted in Group 4.

DISCUSSION

In this study, we have demonstrated NSAID sparing effect of GS-HCl that GS-HCl synergistically induce anti-inflammation by reducing COX-2 mRNA stability and lower dose of indomethacin combined with GS-HCl efficiently attenuated NSAID-induced gastric damage and collagen-induced arthritis. In spite of some reports that GS-HCl showed protective effect against ibuprofen-induced peptic ulcer in rats and inhibition of iNOS and COX-2 in LPS-stimulated macrophage (19), still debates exist regarding real clinical efficacy against arthritis treatment. If our study translated into clinical application, one fourth or one third reduced dose of NSAID can be applied with combination of GS-HCl and these reduction in NSAID dose can afford either preserved anti-arthritic efficacy or reduced risk of thrombotic or cardiovascular complications as well as GI safety. Before our investigation, the clinical efficacy of GS alone or combination with NSAID has been forwarded in patients only with moderate to severe knee arthritis (20). Our study might be the first to support the mechanistic explanation how the combination of GS and celecoxib contributed to significant improvement in the treatment of moderate to severe degree of arthritis. Currently, the best strategy to secure stomach from NSAID-induced gastric damage might be either the coxib, selective COX-2 inhibitor or the combination of NSAID and proton pump inhibitor (PPI) (21, 22). However, coxib carry the another risk of cardiovascular complications and thromboembolic risk in spite of GI safety (23), whereas the combination of NSAID and PPI rather aggravated NSAID-induced enteropathy (24), necessitating further improvement. In addition, it has been reported that the healing of gastric erosion induced by indomethacin may be followed by
other pathological events outside of the stomach, such as intestinal injury, a loss of a normal circadian cycle of heart rate as well as body temperature and locomotion (25). Fortunately, we could open the new hope of NSAID sparing effect of GS-HCl to compensate these limitations.

To secure NSAID-induced gastric damage, additional strategies to inhibit COX-2 might be genetic control of COX-2 by either instabilizing COX-2 gene or increasing proteasome degradation beyond cox inhibition with current NSAID. The uses of natural products to regulation cox gene or genetic manipulation of cox gene are under investigation. In detail, since the maturation of COX-1 and COX-2 first occurs in the endoplasmic reticulum (ER) lumen, where several mechanisms including the cleavage of the N-terminal signal sequence, N-glycosylation at multiple sites, disulfide bond formation, heme incorporation, and membrane insertion or dimerization are operated (1, 26-30), weakening COX-2 stability can be the way to inhibit COX-2, so called posttranslational modification. In detail, the primary structures of COX-1 and COX-2 are very similar, but the difference is that COX-2 has a 19-amino acid (19-aa) insertion near its C-terminal end (Asn-594-Lys-612), a consensus N-glycosylation site at Asn-594. COX-1 is N-glycosylated only at three sites, but COX-2 is glycosylated additionally at Asn-594 in addition to its first three N-glycosylation sites (30, 31), which determines the stability of COX-2 gene and explains why COX-2 inducibly expressed in contrast to constantly expressed COX-1. Relatively rapid rate of protein degradation of COX-2 occurs because COX-2 has these C-terminal 19-aa segments prerequisite for the proteasomal degradation. As results, immunoblotting of a variety of cell lines for COX-2 usually reveals the presence of 72 and 66 kDa variably glycosylated forms of the enzyme due at least in part to alternative glycosylation status at Asn-594 (Figs. 1B-1C). The fact that inhibition of the co-translational N-glycosylation of COX-2 by tunicamycin can eliminate COX and its peroxidase activities, whereas point mutation of the Asn-594 glycosylation site actually enhances the COX-2 (29). COX specific activity clearly supports the biological implication of COX glycosylation. Besides of N-glycosylation-associated COX-2 stability, COX-2 mRNA has a very short half-life due to the presence of multiple copies of the AU-rich element (ARE) within the 3'-UTR of COX-2 mRNA that are known to direct mRNA decay, whereas point mutation of the Asn-594 glycosylation site actually enhances the COX-2 (29). COX specific activity clearly supports the biological implication of COX glycosylation. Besides of N-glycosylation-associated COX-2 stability, COX-2 mRNA has a very short half-life due to the presence of multiple copies of the AU-rich element (ARE) within the 3'-UTR of COX-2 mRNA that are known to direct mRNA decay, whereas point mutation of the Asn-594 glycosylation site actually enhances the COX-2 (29). COX specific activity clearly supports the biological implication of COX glycosylation. Besides of N-glycosylation-associated COX-2 stability, COX-2 mRNA has a very short half-life due to the presence of multiple copies of the AU-rich element (ARE) within the 3'-UTR of COX-2 mRNA that are known to direct mRNA decay, whereas point mutation of the Asn-594 glycosylation site actually enhances the COX-2 (29). COX specific activity clearly supports the biological implication of COX glycosylation. Besides of N-glycosylation-associated COX-2 stability, COX-2 mRNA has a very short half-life due to the presence of multiple copies of the AU-rich element (ARE) within the 3'-UTR of COX-2 mRNA that are known to direct mRNA decay, whereas point mutation of the Asn-594 glycosylation site actually enhances the COX-2 (29). COX specific activity clearly supports the biological implication of COX glycosylation. Besides of N-glycosylation-associated COX-2 stability, COX-2 mRNA has a very short half-life due to the presence of multiple copies of the AU-rich element (ARE) within the 3'-UTR of COX-2 mRNA that are known to direct mRNA decay, whereas point mutation of the Asn-594 glycosylation site actually enhances the COX-2 (29). COX specific activity clearly supports the biological implication of COX glycosylation.
and miR-199a, that can control COX-2 expression (35). These backgrounds suggest that more investigations are needed to achieve higher anti-arthritis efficacy and GI safety with combination or the development of potent NSAID in near future.

Then, we have had the curiosity why GS-HCl is not generally recommended as prime therapeutics in arthritis by many clinicians in spite of these excellent COX-2 inhibitory actions. Though GS-HCl may be effective in treating and possibly slowing the progression of osteoarthritis (OA) because GS-HCl supplements are known to stop cartilage breakdown, build cartilage, and decrease joint swelling (14), but others were proven to be ineffective to recommend with its alone use. Clegg and colleagues performed the multi-center, double-blinded, placebo- and celecoxib-controlled glucosamine/chondroitin arthritis intervention trial (GAIT) in order to evaluate their efficacy and safety of GS-HCl as a treatment for knee pain from osteoarthritis in 1583 patients with symptomatic knee osteoarthritis (36). As result, GS-HCl and chondroitin sulfate were not significantly better than placebo in reducing knee pain. However, the rate of response to combined treatment using GS-HCl and celecoxib was significantly higher (P<0.09) and this improvement was significantly higher (P<0.002). Though clinical trials showing the superiority of combination of GS-HCl and NSAID are all with celecoxib, we identified these NSAID sparing effects of GS-HCl were also seen in the combination of GS-HCl and selective COX-2 inhibitor, NS-398 in our study, instead of indomethacin, telling that the NSAID sparing effect of GS-HCl was not influenced by COX-2 selectivity (Figs. 1D and 2B).

Although GS-HCl has been described as effective when used alone in some study, it is probably reasonable to use the combination with NSAID, irrespective of conventional NSAID or coxib. Our investigation opened the hope to guarantee the combination treatment with NSAID, irrespective of conventional NSAID into the endoplasmic reticulum-associated degradation system.

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Author’s address: Professor Eun-Hee Kim, PhD, CHA Cancer Prevention Research Center, College of Pharmacy, CHA University, 605 Yeoksam 1-dong, Gangnam-gu, Seoul 135-081, Korea.
E-mail: ehkim@cha.ac.kr