Effects of an Oral Administration of Glucosamine-Chondroitin-Quercetin Glucoside on the Synovial Fluid Properties in Patients with Osteoarthritis and Rheumatoid Arthritis

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The effects of an orally administered combination of a glucosamine-chondroitin-quercetin glucoside (GCQG) supplement on the synovial fluid properties of patients with osteoarthritis (OA) and rheumatoid arthritis (RA) were investigated from the clinical nutrition view point. In this study, forty-six OA and twenty-two RA patients were administered with the GCQG supplement orally for 3 months. Several parameters of the knee joints were monitored before and after supplementation. The OA patients showed a significant improvement in pain symptoms, daily activities (walking and climbing up and down stairs), and visual analogue scale, and changes in the synovial fluid properties with respect to the protein concentration, molecular size of hyaluronic acid, and chondroitin 6-sulphate concentration were also observed. However, no such effects were observed in the RA patients. These results suggest that the GCQG supplement exerted a special effect on improving the synovial fluid properties in OA patients.

Key words: glucosamine; chondroitin; rheumatoid arthritis; osteoarthritis; synovial fluid

Complex sugars such as glucosamine sulphate (GS) and chondroitin sulphate (CS) amino sugars have been found to have a clinical effect on patients with osteoarthritis (OA).11 GS is a common constituent of glycosaminoglycans in the cartilage matrix and synovial fluid, and it exerts various pharmacological effects on the articular cartilage and joint tissue. Several clinical trials have demonstrated its symptom-modifying10 and radiographic (joint space-narrowing) effects in OA patients.3,4 This radiographic alteration could be induced by the biological effect of GS, whereby GS stimulates proteoglycan production, leading to suppression in both the activation of collagenase and secretion of matrix metalloproteinases from chondrocytes and/or synoviocytes.3,4,5 However, these studies have been conducted under in vitro conditions and there is no biological evidence regarding the effects of GS under in vivo conditions. Moreover, no clinical trials have examined the effect of glucosamine in patients with rheumatoid arthritis (RA), although both symptomatic and histological improvements have been observed in RA animal models.7,10

Orally administered CS has been used for treating OA patients with beneficial results.11 CS has variable effects, including an increase in the pool of substrates available for cartilage matrix deposition due to the inhibition of lysosomal enzymes and the stimulation of glycosaminoglycans and collagen synthesis.12,13 Therefore, GS and CS are considered to share a common function in the management of damaged articular cartilage. Recent animal studies have reported that CS had potential in RA therapy.10,14 However, there are no clinical reports on the use of CS in patients with RA. The effects of a combination of GS and CS have also been examined; a combination of sugars was used because a mixture of these complex sugars was more effective.3,4

Here, we investigated the clinical effect of an orally administered combination of GS and CS on patients with OA and RA in terms of changes in the biochemical characteristics of the synovial fluid.

Materials and Methods

Patients. Prior to enrolment in this study, we manually confirmed the presence of synovial fluid in the knee joints of OA and RA patients and obtained written informed consent from the patients according to the Declaration of Helsinki. Any patients with a history of synovial fluid drainage or who had been administered with intra-articular steroid injections within 2 months before the initiation of the study were excluded. Twenty-two RA patients who fulfilled the revised criteria laid down by the American College of Rheumatology for the classification of OA of the knee were enrolled in this study. Roentgenogram studies revealed that these patients had painful arthritis in their knee joints and in more than half of the remaining normal joint space (stage II).16 Both groups of patients were matched for age, gender, and disease duration (RA patients: age, 58.0 ± 10.0 years; females: males, 20:2; disease duration, 7.9 ± 10.3 years; OA

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Abbreviations: ADL, activities of daily living; CS, chondroitin sulphate; C4S, chondroitin 4-sulphate; C6S, chondroitin 6-sulphate; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GCQG, glucosamin-chondroitin-quercetin glucoside; GS, glucosamine sulphate; HA, hyaluronic acid; OA, osteoarthritis; QOL, quality of life; RA, rheumatoid arthritis; RF, rheumatoid factor; VAS, visual analogue scale
patients: age, 62.5 ± 9.4 years; females:males, 39:7; disease duration, 8.5 ± 6.7 years). With regard to the activities of daily living (ADL) and quality of life (QOL), these patients achieved scores of ninety-five points or higher based on the Barthel Index,\(^\text{17}\) or eighty-nine points or lower based on the walking item in the Sickness Impact Profile.\(^\text{18}\) If both knees of a patient were affected, the knee that was more severely affected was examined.

To evaluate the effects of our nutritional supplement, the administration of any oral drug or additional treatments (surgery, rehabilitation, and braces) was prohibited from 2 months prior to the beginning of the clinical trial and throughout the trial period.

Test supplement. During the 3-month study period, both the OA and RA patients were administered with the nutritional supplement orally; the nutritional supplement consisted of glucosamine hydrochloride (1200 mg/d), shark cartilage powder (300 mg/d) containing 75–111 mg of chondroitin, and quercetin 3-4’-(O-α-glucosyl)l-α-O-β-glucoside (45 mg/d) (Suntory Ltd., Osaka, Japan). The combination of these constituents is abbreviated as GCQG. Rutin, quercetin-3-rutinoside, is known as a cofactor that reinforces collagen to form a strong matrix,\(^\text{16}\) although the bioavailability of rutin is low. Instead, quercetin 3-4’-(O-α-glucosyl)l-α-O-β-glucoside, which is an enzymatically converted glycoside of rutin and is easily absorbed into the blood flow, was added for this purpose.

Evaluation of knee symptoms. As in our previous report, the knee scoring system of the Japan Orthopedic Association (JOA) was used for clinical evaluation.\(^\text{19}\) This scoring system evaluates four items: ability to walk (thirty points), ability to climb up and down the stairs (twenty-five points), range of motion (ROM; thirty-five points), and joint swelling (ten points). Each knee joint can achieve a maximum score of 100 points. X-ray images and the visual analogue scale (VAS) were also evaluated both before and after the oral administration of the GCQG supplement. The knee joints were monitored before and 1 week after GCQG supplementation. To evaluate the systemic arthritis in RA patients, the following laboratory and clinical parameters were also evaluated both before and after the oral administration of the GCQG supplement. The knee joints were monitored before and 1 week after GCQG supplementation. To evaluate the systemic arthritis in RA patients, the following laboratory and clinical parameters were assessed: C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) titre, duration of morning stiffness, joint score, grip strength, and Lansbury activity index of RA.\(^\text{19}\)

Analysis of synovial fluid. As in our previous reports, the synovial fluid obtained from the knee joints of patients with OA and RA was analysed before and 1 week after the end of the trial.\(^\text{19,20}\) In brief, each synovial fluid sample was collected in a plastic tube and centrifuged at 10,000 × g for 15 min to remove cell and tissue debris. The supernatant was stored at −80 °C until needed. The following eight items were measured by their respective methods: (i) volume (ml) of aspirated synovial fluid by volumetry; (ii) protein concentration (mg/ml) by Lowry’s method, using bovine serum albumin as the standard; (iii) viscosity (mPa s) by using a rotatory viscometer at 37 °C, rotation of 29 rpm, and shear speed of 40/s; (iv) stringing (mm) by Phillips’s method; (v) hyaluronic acid (HA) concentration (mg/ml) by the Morgan-Elsion method; (vi) molecular size of HA in terms of reduced viscosity by calculating the intrinsic viscosity ([h]) by the computed Laurent’s equation, where the molecular weight of HA = ([h] × 10/3.6)\(^\text{1.78}\); (vii) type II procollagen peptide (pCOL-II-C, Chondrocalcin) by the ELISA method (Teijin, Tokyo, Japan); and (viii) the levels of chondroitin 4-sulphate (CS4) and chondroitin 6-sulphate (CS6) in the synovial fluid by high performance liquid chromatography according to the method of Shimmie et al.\(^\text{21}\)

Statistical analysis. Quantitative variables are each presented as the mean ± SD, with the data considered significant at P < 0.05. The statistical significance was determined by the Wilcoxon signed rank test.

**Results**

**Clinical efficacy of GCQG**

There was no improvement in the systemic arthritis of the RA patients after GCQG supplementation. No intragroup differences were observed with regard to the RA activity index, pain score, joint swelling score, grip strength, duration of morning stiffness, ESR, CRP level, or RF titre at the baseline and after supplementation (Table 1). There were no changes (narrowing of the joint space, osteophyte, etc.) in the X-ray images of both the OA and RA groups obtained at the end of the trial as compared to those at the beginning of the trial (data not shown). There was no change in the ADL and QOL scores of the patients (data not shown).

In the OA patients, the scores for the ability to walk and the ability to climb up and down stairs improved significantly after the supplementation (Figs. 1 and 2). The improvement in these scores was reflected in the reduced joint pain. The reduced joint pain also influenced the total JOA knee score (Fig. 3A). The improvement of the JOA scores was reflected in the reduced joint pain. The reduced joint pain influenced the total JOA knee score (Fig. 3A). However, despite the significant decrease in the VAS levels (Fig. 3B), there was no considerable difference in the ROM and joint swelling pre- and post-supplementation (Fig. 4). Further, there were no significant changes in the clinical symptoms of the RA patient (Figs. 1–4). Moreover, no adverse reactions were apparent in either the RA or OA patients.

**Changes in properties of the synovial fluid**

In both groups of patients, there was no statistically significant difference in the total amount of synovial fluid before and after GCQG administration (Fig. 5A). In the OA patients, the protein concentration significantly decreased after GCQG supplementation; however, GCQG supplementation had no effect on the protein concentration in the RA patients (Fig. 5B). The viscosity and stringing of synovial fluid remained unchanged in the OA and RA patients (Fig. 6).

There were no significant differences in the concentration of HA before and after GCQG supplementation in either group of patients (Fig. 7A). In the OA patients, the molecular weight of HA significantly increased after GSCG supplementation (Fig. 7B). The concentration of chondrocalcin remained statistically unaltered in both groups (Fig. 7C).

For both groups, there was no significant difference in the C4S concentration of the synovial fluid after the GCQG treatment. However, the C6S concentration significantly decreased in the OA patients after GCQG supplementation (Fig. 8). In the RA patients, there were no significant differences in the C4S and C6S concentrations of the synovial fluid pre- and post-supplementation.
Discussion

Several clinical studies on GS (an amino monosaccharide) and CS (a disaccharide polymer) have revealed that both these sugars shared a common function, namely, management of damaged articular cartilage, and that these sugars have been administered as supplements to OA patients with satisfactory results.1) These nutritional supplements have demonstrated their ability to reduce the progression of osteoarthritis and are termed disease-modifying OA drugs (DMOADs).4,22) These saccharides were generally administered in isolation, probably because they exerted greater effects when administered under such conditions.8) Long-term radiographic studies have revealed that GS and CS played a role in articular cartilage repair.3,4) Several experimental data also support nutritional supplements as exhibiting both chondroprotective and metabolic

Fig. 1. Effects of Oral Administration of Glucosamine-Chondroitin-Quercetin Glucoside on Ability to Walk (Japan Orthopedic Association [JOA] score).

Fig. 2. Effects of Oral Administration of Glucosamine-Chondroitin-Quercetin Glucoside on Ability to Climb up and down Stairs (JOA score).

Fig. 3. Effects of Oral Administration of Glucosamine-Chondroitin-Quercetin Glucoside on Total JOA Score and Joint VAS Level. A, Total JOA score; B, VAS level.

Fig. 4. Effects of Oral Administration of Glucosamine-Chondroitin-Quercetin Glucoside on Range of Motion (ROM) and Joint Swelling (JOA score).

Fig. 5. Effects of Oral Administration of Glucosamine-Chondroitin-Quercetin Glucoside on Total Amount of Aspirated Synovial Fluid and Protein Concentration. A, Total amount of aspirated synovial fluid; B, Protein concentration.

Fig. 6. Effects of Oral Administration of Glucosamine-Chondroitin-Quercetin Glucoside on Viscosity and Stringing of Synovial Fluid. A, Viscosity; B, Stringing.
synergistic effects. However, these experimental studies were conducted on animals, and there is no in vivo biological evidence in humans which verifies the abovementioned effects. Moreover, studies on the differences in pain reduction and radiological score of the knee joint in comparison with placebo controls remain to be conducted. To clarify the biochemical effects of the nutritional supplement on the condition in vivo, we conducted a study from a clinical nutrition viewpoint in order to investigate the effects of a combination of GS and CS on synovial fluid properties.

In addition, RA and OA share similar pathological characteristics that include varying degrees of inflammation and degeneration. Therefore, it may be expected that the supplement could improve the condition of both OA and RA patients. Recent animal studies have indicated the potential of nutritional supplements as novel anti-RA drugs. This is why we investigated the usefulness of the GCQG nutritional supplement to patients with RA. The administration of GCQG resulted in satisfactory improvements in the walking score, stairs score, JOA total score, and VAS level in OA patients; these results are consistent with previous data. Although the X-ray images did not reveal any changes, because the supplementation was short term, they demonstrated significant changes in the synovial fluid properties of the protein concentration, molecular weight of HA, and C6S concentration; the decrease in protein concentration suggests that the administered supplement had anti-inflammatory effects. Generally, the molecular weight of HA decreases in joints with inflammatory arthritis. In this study, we could not identify the effects of glucosamine and chondroitin on the molecular weight of HA. High molecular weight HA enhances chondrocyte HA and proteoglycan synthesis, reduces the production and activity of proinflammatory mediators and matrix metalloproteinases, and maintains joints in good condition. In this study, we observed a reduction in the synovial fluid C6S level but not C4S level in OA patients post GCQG supplementation. The synovial fluid C6S level can be expected to increase with articular cartilage degradation, because most of the C6S is produced by damaged articular cartilage. C4S exists not only in articular cartilage but also in platelets; therefore, not all change in the C4S level would be due to articular cartilage degeneration. Data regarding the improvement in synovial fluid properties post supplementation with GCQG suggests that this supplement was beneficial for OA patients. Previously, we had investigated the changes in the synovial fluid properties without GCQG supplementation and had obtained no positive results. However, in the present study, GCQG supplementation resulted in a significant improvement in the OA patients, thus revealing its beneficial effect. Although a further study is required, not only a combination of glucosamine and chondroitin, but also of quercetin 3-O-beta-D-glucopyranoside might contribute to the improvement.

However, there were no significant changes in the clinical symptoms and synovial conditions of the RA patients, although the supplementation tended to slightly decrease the VAS level, joint pain score, and swelling score. These results suggest that GCQG has potential for treating arthritis. However, the effect would be mild, because the inflammatory condition is more severe in RA patients than in OA patients. In addition, previous reports have described that supplementation with neither glucosamine nor chondroitin was any more effective than supplementation with a placebo control under...
severe arthritic conditions. In order that a slow-acting dietary supplement can generate effects, it should be administered over a long term.

In conclusion, GCQG had a desirable nutritional effect on OA patients in terms of their synovial fluid properties. Further experimentation is required to confirm the effect of this dietary supplement specifically in patients with osteoarthritis, which is one of the diseases with increasing incidence.

References


