Effect of a dietary supplement containing glucosamine hydrochloride, chondroitin sulfate and quercetin glycosides on symptomatic knee osteoarthritis: a randomized, double-blind, placebo-controlled study

Noriyuki Kanzaki,a∗ Kayo Saito,a Akifumi Maeda,a Yoshinori Kitagawa,a Yoshinobu Kiso,a Keita Watanabe,b Akihito Tomonaga,c Isao Nagaokad and Hideyo Yamaguchie

Abstract

BACKGROUND: Oral glucosamine and chondroitin sulfate, alone and in combination, have been used worldwide for the treatment of osteoarthritis (OA), but their efficacy is controversial. This clinical study was aimed at investigating the potential of a dietary supplement containing glucosamine and chondroitin sulfate in combination with derivatives of quercetin, a naturally occurring flavonoid, (GCQ supplement) for knee OA care.

RESULTS: A randomized, double-blind, placebo-controlled study was conducted in 40 Japanese subjects with symptomatic knee OA. Subjects were randomly assigned to GCQ supplement (1200 mg glucosamine hydrochloride, 60 mg chondroitin sulfate and 45 mg quercetin glycosides per day) or placebo and the treatment and follow-up were continued for 16 weeks. The results of symptomatic efficacy assessment based on Japanese Orthopaedic Association criteria showed that scores for two of the four symptom/function subscales, as well as the aggregate scores, were significantly improved at week 16 or earlier in the GCQ group compared to the placebo group. Moreover, analyses of cartilage metabolism biomarkers showed a trend of improvement in type II collagen synthesis/degradation balance in the GCQ group during follow-up.

CONCLUSION: GCQ supplement was thought to be more effective than placebo in decreasing the intensity of knee OA-associated clinical symptoms.

Keywords: dietary supplement; glucosamine; chondroitin sulfate; quercetin glycosides; osteoarthritis; clinical trial

INTRODUCTION

Osteoarthritis (OA), developing as a result of progressive destruction of articular cartilage, is the most common joint disease and the leading cause of pain and physical disability in the elderly. The knee is most frequently affected by OA because it is a weight-bearing joint. In Japan, like many other developed countries, the incidence and prevalence of OA of the knee are now increasing with an increase in the elderly population.1 Thus the management of knee OA, which requires extensive utilization of health care resources, has become a major social and economical burden.

Conventional pharmacological approaches to symptom management in OA involve nonsteroidal anti-inflammatory drugs, selective cycloxygenase-2 inhibitors, and intra-articular injection of hyaluronan or corticosteroids. However, there are accumulating data showing that any of these pharmaceutical drugs frequently produce insufficient benefit, with an associated risk of untoward side effects.2−4 It is therefore no wonder that patients with OA have embraced complementary and alternative approaches to management of OA symptoms, particularly pain.5,6

Glucosamine and chondroitin sulfate are cartilage extracellular matrix components that have been widely used as alternative medicines or nutraceuticals for the management of OA and have
been the subject of a huge number of clinical studies for this purpose. The reason is primarily based on the results of in vitro and in vivo studies demonstrating that glucosamine, used alone and in combination, are capable of stimulating the synthesis or inhibiting the degradation of cartilage matrix molecules, including proteoglycans. Consistent with this, some clinical trials thus far reported support the demonstrated favorable effects of glucosamine and chondroitin sulfate alone or in combination in relieving OA pain. However, none of a larger number of randomized clinical trials gave such positive results, suggesting an ambiguity of the benefit of these two nutraceuticals in OA.

Among other nutraceuticals which have been reported to have the potential for relieving arthritic pain is quercetin (3,3′,4′,5,7-pentahydroxyflavone). It is a member of the flavonoids, which are widely distributed in plants and fruits, and onion is known to contain quercetin in particular high amounts. Quercetin has been shown to possess potent antioxidant and anti-inflammatory activity in vitro and in vivo. Such an anti-inflammatory activity of quercetin may be involved in its efficacy for OA, because evidence for inflammatory and oxidative damage due to overproduction of nitric oxide and other reactive oxygen species (ROS) has been demonstrated in aging and osteoarthritic cartilage and has been correlated with the extent of cartilage damage.

All these data tempted us to develop a new glucosamine/chondroitin sulfate-based preparation supplemented with quercetin for OA treatment. As quercetin molecule per se is lipophilic in nature, we used quercetin 3-(3,3′,4′,5,7-pentahydroxyflavone), it is a member of the flavonoids, which are widely distributed in plants and fruits, and onion is known to contain quercetin in particular high amounts. Quercetin has been shown to possess potent antioxidant and anti-inflammatory activity in vitro and in vivo. Such an anti-inflammatory activity of quercetin may be involved in its efficacy for OA, because evidence for inflammatory and oxidative damage due to overproduction of nitric oxide and other reactive oxygen species (ROS) has been demonstrated in aging and osteoarthritic cartilage and has been correlated with the extent of cartilage damage.

The present clinical study was undertaken to confirm the symptom-improving effect of the short-term (16 weeks) intake of the same GCQ supplement in patients with painful knee OA. In addition, we investigated the effects of this combination supplement on biomarkers of cartilage metabolism, which have been shown to be predictive of radiological progression in knee OA and hip OA.

MATERIALS AND METHODS

Study design
A prospective, randomized, placebo-controlled, parallel-group comparative study was designed to assess the efficacy and safety of GCQ supplement. The study was performed from June 2009 to December 2009, and involved two clinical service organization centers under the control of two medical investigators in Japan. The study protocol was approved by the institutional ethics committee, and was conducted in accordance with the principles of the amended Declaration of Helsinki and ‘Ethical Guidelines for Epidemiological Research’ (recognized by the Japanese Government in 2008). Written informed consent was obtained from all participants prior to enrollment in the study. Those participants who were tentatively judged to be eligible for the study on the basis of a questionnaire about medication status and exercise habit underwent knee radiography at the baseline visit. Radiographical grade was determined with the use of the Kellgren–Lawrence (K/L) grading scale for joint space narrowing. To be selected for the study and to assess the efficacy and safety of the treatment, participants must have undergone a full clinical examination performed by a medical investigator to evaluate symptoms and functions of the knee joints using the assessment criteria for knee diseases and treatments established by the Japan Orthopaedic Association (JOA criteria) and self-recorded 100 mm visual analog scales (VAS) for knee pain.

The JOA criteria basically consist of the following four symptom/function subscales: ‘walking ability and painfullness (walking)’; ‘stairs – ascending/descending ability and painfullness (stairs – ascending/descending)’; ‘range of motion’; and ‘joint swelling’. These four subscales were rated for an individual from 0 to 30, from 0 to 25, from 0 to 35, and from 0 to 10, respectively, in all of which the maximum value indicates no symptom or functional disability and 0 is the worst one, which is extreme difficulty in performing daily living tasks. The sum of scores for these four symptom/function subscales presented the aggregate scores, which were also used to assess symptomatic efficacy. As supplementary outcome measures, the following three pain subscales of 100 mm VAS were used to self-measure knee pain: pain at rest; pain on walking; and pain on ascending/descending stairs. Each subscale was measured from 0 to 100, where 0 indicates no pain and 100 indicates the worst pain thus far experienced. As the present study aimed to assess efficacy of the test supplement for relieving considerably severe pains in the impaired knee, only the knee joints with individual scores for VAS pain subscales of 20 mm or above were used as the assessment target.

At baseline, as well as at weeks 4, 8, 12 and 16 after the start of 16-week treatment, enrolled subjects were required to make clinical visits for undergoing medical and physical examinations. Blood samples (obtained with the subject in a fasting state) and urine samples (second void of the morning) were also collected at baseline and at weeks 8, 12 and 16. Aliquots of serum and urine were stored at a temperature below −40 °C until analyzed.

Subjects
Male and female Japanese subjects, aged 40–85 years, with diagnosed knee OA, in whom presence of knee pain was confirmed by the assessment with scores for the ‘walking’ subscale of the JOA criteria (25 or lower score for either left or right knee joint), were eligible. Major exclusion criteria were: knee OA of grade IV in K/L classification; presence of gout or rheumatoid arthritis that may cause joint pain; surgical treatment of knee joint(s) undergone or its necessity; routine use of health food or medicine containing hyaluronic acid, glucosamine and/or chondroitin sulfate and expected to be continued during the study period; treatment with bisphosphonates, hormones or other medicines that may affect the serum or urine concentrations of biomarkers of bone or cartilage metabolism; intra-articular hyaluronic acid within 2 weeks or corticosteroids within 3 months before inclusion; need to undergo such topical or systemic pharmacological treatments during the study period; occasional taking of hard exercise; a history of osseous or articular diseases other than OA within the past 3 months; treatment with warfarin, undergoing or needed to undergo during the study period; bronchial asthma or potential...
for developing allergy to the test supplement; pregnant women; 
nursing mothers or women of childbearing potential; participation 
in another clinical study; and presence of any medical condition 
judged by the medical investigator to preclude the subject’s 
inclusion in the study.

Treatment and subject assignment

The test supplement (GCQ supplement) was a tablet-form 
commercial product, Gurukosamin & Kondoroohin®11, marketed by 
Suntory Wellness Ltd, Tokyo, which contains 1200 mg glucosamine 
hydrochloride, 300 mg shark cartilage extract (60 mg as chon-
droitin sulfate) and 45 mg quercetin glycosides in a daily dose 
of six tablets. All subjects were sequentially assigned based on 
random number tables to one of the two masked products and 
randomized (1:1) to GCQ supplement (GCQ group) and dummy 
placebo (placebo group). After randomization, it was confirmed 
that the subjects were distributed almost equally, in terms of the 
aggregate scores of the JOA criteria, to the two treatment groups. 
The randomization codes for enrolled subjects were held by an 
appointed person who was not involved in the present study. 
The dummy placebo tablets were also manufactured by Suntory 
Wellness Ltd specifically for the purpose of this study to ensure 
that the placebo tablet was indistinguishable from the GCQ sup-
plement tablet in appearance, as well as the packaging. Allocation 
was pre-assigned on the basis of randomization numbers and was 
concealed from the subjects, the investigators and the researchers 
who recruited and assessed participating subjects. All subjects 
were instructed to take six tablets once a day, which was to be 
self-recorded in the study diary.

Efficacy assessment

Individual scores and the aggregate scores for the four symp-
tom/function subscales of the JOA criteria were used as the major 
efficacy outcome measures. In addition, individual scores for the 
three VAS pain subscales were used as the supplementary outcome 
measures. Data on all of the outcome measures were collected at 
baseline and at weeks 4, 8, 12 and 16 after the start of treatment 
and compared between GCQ group and placebo group.

When all subjects had completed the study, serum and/or 
urine specimens were analyzed for a type II collagen (CII) 
degradation biomarker uCTX-II and a CII synthesis biomarker 
scPII to assess changes in cartilage metabolism. The concentra-
tions of urinary CTX-II (uCTX-II) and serum PII (scPII) were assayed 
by Urine CartiLaPs EIA (Immunodiagnostic Systems, Ltd, Boldon, 
UK) and Procollagen II C Propeptide ELISA (IBEX Pharmaceuticals 
Inc., Mont-Royal, Canada), respectively. Measurements of uCTX-
II were corrected for urinary creatinine (Cr) as measured by a 
standard colorimetric assay and the concentration was expressed 
as ng mmol Cr−1. Within-group comparison of the mean change 
of uCTX-II and scPII levels, as well as uCTX-II/scPII ratios, at weeks 8, 
12 and 16 in each of the GCQ and placebo groups, were performed.

Safety assessment

Safety was assessed on the basis of the incidence and severity 
of treatment-related adverse events reported throughout the 
16-week treatment period, as well as of changes in physical 
parameters and laboratory test variables including hematology, 
blood biochemistry and urinalysis.

| Table 1. Baseline characteristics of the study population |
|--------------------------|--------------------------|
| Variables                | GCQ group (n = 20)       | Placebo group (n = 20) |
| Age (years)              | 55.1 ± 10.9c             | 58.3 ± 7.4             |
| Sex (male/female)        | 4/16                     | 3/17                   |
| Height (cm)              | 157.4 ± 5.6              | 157.3 ± 8.1            |
| Body weight (kg)         | 54.7 ± 9.5               | 56.2 ± 7.5             |
| Body mass index (kg m−2) | 22.0 ± 3.4               | 22.8 ± 3.3             |
| Systolic blood pressure (mmHg) | 118.9 ± 16.5         | 127.8 ± 18.8           |
| Diastolic blood pressure (mmHg) | 73.3 ± 11.1           | 78.5 ± 12.4            |
| Pulse rate (beats min−1) | 68.2 ± 6.6               | 66.9 ± 9.8             |
| Aggregate scores of the JOA criteria | 174.8 ± 13.7         | 178.0 ± 10.6           |
| Scores for VAS pain subscales |                       |                       |
| Pain at rest             | 30.4 ± 37.8              | 22.3 ± 20.9            |
| Pain on walking          | 68.7 ± 43.0              | 61.7 ± 37.5            |
| Pain on ascending/descending of stairs | 86.3 ± 49.1         | 84.7 ± 46.8            |
| Kellgren–Lawrence grades I–II (%) | 90                   | 93                     |
| Urinary CTX-II (ng mmol Cr−1) | 311.7 ± 198.7       | 387.9 ± 147.0          |
| Serum CPII (ng mL−1)     | 1264 ± 237               | 1370 ± 378             |

a A group of subjects receiving GCQ supplement. 
b A group of subjects receiving placebo. 
c All values are expressed as means ± SD except for sex and Kellgren–Lawrence grades.

Statistical analysis

All data were expressed as mean ± standard deviations (SD) unless 
otherwise specified. Baseline data were compared within the two 
groups, using the unpaired t-test for quantitative variables and 
chi-squared test for qualitative variables. Changes in symptomatic 
scores or measurements during the treatment were compared with 
baseline by the Wilcoxon’s signed rank test (for symptom/function 
subscales of the JOA criteria) and the paired t-test (for self-
recorded VAS pain subscales and measurements of CII-related 
biomarkers). Comparisons between groups were performed by 
the Mann–Whitney U test (for symptom/function subscales of the 
JOA criteria), the unpaired t-test (for VAS pain subscales) and 
the analysis of covariance (for measurements of CII metabolism 
biomarkers). Changes from baseline in measurements of physical 
and laboratory test variables at week 16 were analyzed by the 
paired t-test. P-values less than 0.05 were considered significant.

RESULTS

Characterization of study groups

Table 1 presents the baseline characteristics of all of the 40 enrol-
led subjects (7 men and 33 women), among whom 20 each were 
assigned to GCQ supplement (GCQ group) and placebo (placebo 
group). No significant difference was found between the two 
groups on any of demographic, physical, physiological or CII 
metabolic variables, symptomatic scores or a K/L grade-based 
distribution of the knee joint.
Among a total of 20 subjects in placebo group, one female who received a whiplash due to a traffic accident on day 57 after the start of treatment was deleted from the study in week 12. All of the remaining 39 subjects (20 in GCQ group and 19 in placebo group) completed the study. Adherence to GCQ supplement or placebo exceeded 85% in all of the subjects. Thus 20 subjects in GCQ group and 19 in placebo group were judged to be eligible for assessment of symptomatic efficacy by the use of the JOA criteria and effect on the CII metabolism biomarkers. Numbers of knee joints evaluable for individual self-recorded VAS pain subscales in GCQ group and placebo group amounted to 13 and 10 for pain at rest; 29 and 26 for pain on walking; and 30 and 28 for pain on ascending/descending stairs, respectively.

### Efficacy assessment based on the JOA criteria subscale scores

Table 2 shows the changes in individual scores for four symptom/function subscales and their aggregate scores of the JOA criteria during the 16-week treatment period in GCQ group (n = 20) and placebo group (n = 19).

<table>
<thead>
<tr>
<th>Subscale</th>
<th>Groupa</th>
<th>Baseline</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
<th>Week 16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking and painfulness</td>
<td>GCQ</td>
<td>47.8 ± 6.2</td>
<td>49.8 ± 7.0 (4.2)</td>
<td>54.5 ± 5.8** (14.1)</td>
<td>57.0 ± 5.2** (19.4)</td>
<td>58.0 ± 3.4** (21.5)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>51.1 ± 3.9</td>
<td>51.6 ± 4.4 (1.0)</td>
<td>55.0 ± 4.7** (7.7)</td>
<td>55.5 ± 5.2** (8.8)</td>
<td>56.1 ± 4.9** (9.8)</td>
</tr>
<tr>
<td>Stairs – ascending/descending</td>
<td>GCQ</td>
<td>38.0 ± 7.7</td>
<td>38.8 ± 8.4 (2.0)</td>
<td>41.3 ± 5.1* (8.6)</td>
<td>43.5 ± 6.1** (14.5)</td>
<td>45.8 ± 5.4** (20.4)</td>
</tr>
<tr>
<td>descending ability and painfulness</td>
<td>Placebo</td>
<td>39.7 ± 6.1</td>
<td>39.7 ± 6.1 (0.0)</td>
<td>41.8 ± 5.1 (5.3)</td>
<td>42.4 ± 5.6* (6.6)</td>
<td>43.2 ± 5.6** (8.6)</td>
</tr>
<tr>
<td>Range of motion</td>
<td>GCQ</td>
<td>69.0 ± 3.1</td>
<td>69.0 ± 3.1 (0.0)</td>
<td>69.0 ± 3.1 (0.0)</td>
<td>69.0 ± 3.1 (0.0)</td>
<td>69.3 ± 2.4 (0.4)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>68.2 ± 3.4</td>
<td>68.4 ± 3.4 (0.3)</td>
<td>68.7 ± 3.3 (0.7)</td>
<td>68.7 ± 3.3 (0.7)</td>
<td>68.9 ± 3.2 (1.0)</td>
</tr>
<tr>
<td>Joint swelling</td>
<td>GCQ</td>
<td>20.0 ± 0.0</td>
<td>20.0 ± 0.0 (0.0)</td>
<td>20.0 ± 0.0 (0.0)</td>
<td>20.0 ± 0.0 (0.0)</td>
<td>20.0 ± 0.0 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>19.5 ± 1.6</td>
<td>19.7 ± 1.1 (1.0)</td>
<td>19.7 ± 1.1 (1.0)</td>
<td>19.7 ± 1.1 (1.0)</td>
<td>19.7 ± 1.1 (1.0)</td>
</tr>
<tr>
<td>Aggregate</td>
<td>GCQ</td>
<td>174.8 ± 13.7</td>
<td>177.5 ± 14.9* (1.6)</td>
<td>184.8 ± 9.4** (5.7)</td>
<td>189.5 ± 10.2** (8.4)</td>
<td>193.0 ± 7.3** (10.4)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>178.4 ± 10.7</td>
<td>179.5 ± 10.8 (0.6)</td>
<td>185.3 ± 9.6** (3.8)</td>
<td>186.3 ± 10.5** (4.4)</td>
<td>187.9 ± 10.2** (5.3)</td>
</tr>
</tbody>
</table>

* GCQ, a group of subjects receiving GCQ supplement; placebo, a group of subjects receiving placebo.

### Efficacy assessment based on the self-recorded VAS pain subscale scores

Self-recorded VAS-based efficacy assessment was performed on evaluable knee joints using the individual scores for the three pain subscales. As shown in Table 3, knee joints in both groups had reductions in scores for each of the three pain subscales compared with baseline (indicative of pain relief) with increasing weeks of treatment and reached statistical significance at almost all follow-up time points ranging from week 4 to week 16 (> 0.01 or > 0.05). However, the extent of reduction in scores for all three pain subscales appeared to be greater in GCQ group than that in placebo group. The greatest difference in magnitude of scale reduction between groups was seen for ‘pain on walking’ at week 16, with statistical significance (P < 0.05) (Fig. 2).

### Effect on CII metabolism biomarkers

Table 4 shows the changes in mean values of uCTX-II and sCPII, together with those of the uCTX-II/sCPII ratio in GCQ and placebo groups during the 16-week treatment. Compared with placebo group, GCQ group appeared to induce, albeit not achieving significance, substantial decreases in the mean value of the uCTX-II concentration (P = 0.125) and that of the uCTX-II/sCPII ratio (P = 0.167) at week 16.

### Safety assessment

Comparable numbers of subjects in both groups reported experiencing one or more adverse event(s) during the treatment. Relatively frequent adverse events included cold symptoms, myalgia/muscle stiffness, arthralgia, gastric distress, and diarrhea. There were no appreciable differences between the two groups in frequency or pattern of events. All of the self-reported adverse events were mild or intermediate in intensity and occurred only temporarily, and were judged by the investigator as medically unrelated to the treatment.

Routine laboratory tests including those for glucose metabolism (blood glucose level, urinary glucose) did not show any great abnormalities in hematologic or metabolic functions in both groups during the 16-week treatment. Moreover, no adverse
#### DISCUSSION

The present randomized, double-blind, placebo-controlled, parallel group study was conducted to evaluate effects of GCQ supplement containing glucosamine hydrochloride, chondroitin sulfate and quercetin glycosides as the potentially active constituents on knee OA-associated symptoms, as well as on CII metabolism biomarkers, in subjects with mild knee OA (mainly I–II of K/L grades). The effectiveness of GCQ supplement in improving OA-associated symptoms was revealed by the results of efficacy assessment based on the JOA criteria. In GCQ group, magnitude of increases in individual scores for the two symptom/function subscales, ‘walking’ and ‘stairs-ascending/descending’, and the aggregate scores of the JOA criteria at week 12 and/or week 16 were all significantly greater than the corresponding values in placebo group (P < 0.01 or P < 0.05). In contrast, scores for the two other symptom/function subscales of the JOA criteria – ‘range of motion’ and ‘joint swelling’ – did not respond to GCQ supplement. This can be explained by the fact that none of subjects participating in this study scarcely had restricted range of motion nor swelling in any knee joints, as revealed by high baseline scores that were close to the top grade (Table 2).

In the self-recorded VAS-based efficacy assessment, individual scores for the three VAS pain subscales for both groups were almost all significantly decreased from baseline throughout the treatment, suggesting that unexpectedly high placebo effects were self-reported, probably due to the characteristics of VAS in which subject-based measures are used for assessment. However, it was also shown that there was a significant difference between the two groups in magnitude of reductions of scores for pain on walking at week 16 (P < 0.05), indicating the beneficial effects of GCQ supplement above placebo effects. It looks, therefore, likely that the data from the self-recorded VAS-based efficacy assessment may be not contradictory to, but rather consistent with the JOA criteria-based assessment data.
The results obtained from the efficacy assessment based on the JOA criteria strongly suggest that GCQ supplement may have favorable effects on OA-associated symptoms including pain in subjects with knee OA. This possibility is supported by the data on the self-recorded VAS-based assessment, which showed that GCQ supplement can be effective in relieving even considerably severe pain in osteoarthritic knee joints. All these results appear to be basically concordant with those reported by Matsuno et al., who showed through their open-label study the effectiveness of 3-month treatment with the same GCQ supplement prescription as for the present study to relieve knee pains in OA patients. Moreover, these researchers demonstrate that the GCQ supplement also improved synovial fluid properties in terms of protein concentration, molecular size of hyaluronic acid and chondroitin sulfate level, suggesting beneficial effects on synovitis.

There is growing evidence which reveals that biomarkers of cartilage metabolism, particularly those of CII metabolism, could be useful not only for identifying patients at risk for progressing joint destruction in OA but also for monitoring the progression of OA. Various candidates of chondroprotective agents of natural origin, including glucosamine sulfate and chondroitin sulfate, have been investigated for their effects on the level of one or more of several CII degradation biomarkers including CTX-II and CII synthesis biomarkers including CPII, alone and/or in combination, as the disease biomarkers of knee OA. The data on these cartilage metabolic parameters obtained in the present study show that none of them changed significantly in both GCQ and placebo groups during the 16-week treatment period. However, both the uCTX-II level and the uCTX-II/sCPII ratio for GCQ group were likely to decrease at week 16. Thus there is the possibility that GCQ supplement may have some protective effect on the progression of knee OA. A long-term clinical trial, in which a sufficient number of subjects with knee OA are treated with GCQ supplement for 6 months or longer periods, will be needed to obtain the answer to this important issue.

The mechanism by which GCQ supplement exerts a favorable action toward pain and other symptoms in subjects with knee OA remains to be clarified. In connection with this, however, there are several papers reporting anti-inflammatory and/or analgesic activities of not only quercetin (glycosides) but also glucosamine hydrochloride and chondroitin sulfate. Glucosamine hydrochloride has been reported to suppress OA-associated inflammation and pain through inhibiting syntheses of prostaglandin E2, nitric oxide and matrix metalloproteases that depend on interleukin-1β produced in association with OA development, possibly leading to prevention of cartilage destruction. For chondroitin sulfate, Iovu et al., published a paper in which this nutraceutical is active in inhibiting production by synovial cells of proinflammatory cytokines, with resultant improvement of arthritis.

### Table 3. Changes in scores for the three self-recorded VAS pain subcales during the 16-week treatment period in target knee joints in GCQ group and placebo group

<table>
<thead>
<tr>
<th>Subscales</th>
<th>No. of target knee joints (No. of subjects)</th>
<th>Score at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GCQ (13)</td>
<td>Baseline</td>
</tr>
<tr>
<td>Pain at rest</td>
<td></td>
<td>40.9 ± 14.0</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>32.5 ± 7.4</td>
</tr>
<tr>
<td>Pain on walking</td>
<td>GCQ (29)</td>
<td>45.9 ± 18.5</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>44.1 ± 18.2</td>
</tr>
<tr>
<td>Pain on ascending/descending stairs</td>
<td>GCQ (30)</td>
<td>56.0 ± 22.1</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>56.0 ± 22.1</td>
</tr>
</tbody>
</table>

A GCQ, a group of subjects receiving GCQ supplement; placebo, a group of subjects receiving placebo.  
B Values are expressed as means ± SD, with percent changes from baseline in parentheses. Changes in scores during the treatment were compared with baseline by the paired t test. * P < 0.05; ** P < 0.01.

### Table 4. Changes in the concentration of CII metabolism biomarkers, urinary CTX-II (uCTX-II) and serum CPII (sCPII), as well as the concentration ratio of uCTX-II to sCPII (uCTX-II/sCPII) during the 16-week treatment period in GCQ group (n = 20) and placebo group (n = 19)

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Group^a</th>
<th>Concentrations or concentration ratios at:^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Week 4</td>
</tr>
<tr>
<td>uCTX-II (ng mmol Cr⁻¹)¹</td>
<td>GCQ</td>
<td>353.2</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>354.0 ± 23.6</td>
</tr>
<tr>
<td>sCPII (ng mL⁻¹)¹</td>
<td>GCQ</td>
<td>1319</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>1366 ± 37</td>
</tr>
<tr>
<td>uCTX-II/sCPII²</td>
<td>GCQ</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>1.56 ± 0.12</td>
</tr>
</tbody>
</table>

A GCQ, a group of subjects receiving GCQ supplement; placebo, a group of subjects receiving placebo.  
B All values are expressed as means ± SD.  
C The unpaired t test was used to evaluate between-group differences.  
1 Concentration.  
2 Concentration ratios.
and in vivo anti-inflammatory activities have also been reported for quercetin.\textsuperscript{16,17} Although there are no data in this study to explain the actual role of quercetin glycosides in the beneficial effect of GCQ supplement, we are led to the possibility that a composite effect of the three components of GCQ supplement might result in an enhanced efficacy in subject with knee OA. Exogenous glucosamine as an amino sugar has been considered to have the potential for affecting sugar metabolism. In fact, there are papers reporting that intravenously administered glucosamine induced insulin resistance in animals\textsuperscript{25} and humans.\textsuperscript{26} In contrast, contradictory results were provided by Tannis et al.,\textsuperscript{37} who observed that any change in the blood glucose level was not produced after 12-week treatment with glucosamine sulfate. Consistent with the latter paper, the present study showed that the blood glucose level was not significantly changed after 16-week treatment with GCQ supplement, suggesting a lack of any harmful effects of the combination supplement on glucose metabolism in humans. In addition, no abnormalities of any other laboratory tests were observed and no treatment-related adverse events were experienced throughout the follow-up period.

Based on all of the data on efficacy and safety assessments, we are led to the conclusion that GCQ supplement can be safely administered and can relieve pain and improve functions of knee joints in subjects with symptomatic knee OA. GCQ supplement could be a potential candidate for knee OA care.

ACKNOWLEDGEMENTS

We would like to thank to Dr T Yamamoto for his advice and support, and K Yoshimura for her statistical expertise and help in preparation of the manuscript.

REFERENCES


