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RESEARCH ARTICLE

Efficacy and Safety of Undenatured Type II Collagen in The Treatment of Osteoarthritis of The Knee: A Randomized, Double-blind, Placebo-controlled Trial

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Abstract

BACKGROUND: Available medication for pain and joint stiffness release in osteoarthritis (OA) often gives considerable side effects. Undenatured type II collagen (UC-II) has been considered as a treatment for OA for its ability to prevent the progress of articular cartilage damage. Hence, this study aimed to evaluate the efficacy and safety of UC-II in modulating knee joint function.

METHODS: This was a randomized, double-blind, placebo-controlled study involving 102 OA subjects. Subjects were randomized into two groups: receiving an oral daily dose of 40 mg/day UC-II or placebo containing microcrystalline cellulose for 90 days. Efficacy was evaluated by using the Western Ontario McMaster Osteoarthritis Index (WOMAC), Lequesne's Functional Index (LFI), and Visual Analogue Scale (VAS) score on day-1, -7, -30, -60, and -90. Safety was evaluated by assessing the adverse events (AEs) and abnormal laboratory findings.

RESULTS: The WOMAC total score showed a significant difference between the UC-II group vs. the placebo group from day-7 ($p<0.05$) to day-90 ($p<0.01$). UC-II was more effective in reducing the WOMAC total scores by 81.6% compared to 19.2% in the placebo group after 90 days. The total LFI and VAS score was significantly reduced in subjects supplemented with UC-II compared to the placebo group (75.8% vs. 7.8%; 67.9% vs. 12.2%, respectively). No significant changes were observed in vital signs and clinical laboratory tests compared to the placebo. The UC-II had a good safety profile with no serious adverse events among participants.

CONCLUSION: UC-II significantly improved the knee pain, stiffness, and functional mobility of OA patients and was well-tolerated.

KEYWORDS: osteoarthritis, undenatured type II collagen, WOMAC, VAS, LFI

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Introduction

Osteoarthritis (OA), a degenerative joint disease mostly occurred in women aged 35-75 years old, is the leading cause of pain and disability.(1,2) The incidence of OA rises with age, increasing the prevalence and burden of OA.(3,4) This has become a global problem, and novel therapeutic intervention is warranted. Drugs, including acetaminophen

and nonsteroidal anti-inflammatory drugs (NSAIDs), tramadol, and intraarticular corticosteroid injection, are essential medication for OA patients with moderate to severe pain.(5,6) These drugs effectively reduce pain associated with OA, however, they do not modify the progression of the disease.(7) Safety in selecting the drugs should be a major consideration since it has been reported to be associated with severe side effects such as liver toxicity, gastrointestinal and cardiovascular complications.(8) For that, a lot of natural

nutraceuticals, such as glucosamine and chondroitin, was given to OA patients to ease their pain and discomfort. But the studies only showed the small-to-moderate efficacy of these supplements.(9,10) Recent studies have focused on collagen derivative supplementation. As the progressive destruction of articular cartilage characterizes OA, collagen supplementation may induce cartilage matrix synthesis by stimulating the chondrocytes.(11) Previous preclinical studies have shown promising supplementation results with collagen derivatives.(11,12)

Undenatured type II collagen (UC-II) is a chicken sternum cartilage-derived supplement that has been considered as an important treatment to prevent the progress of articular cartilage damage.(7,12,13) UC-II improved knee joint function in knee OA and was well-tolerated.(13-16) Since there is not much evidence from clinical studies on the benefits of UC-II in OA and cartilage repair, further research of UC-II in OA needs to be done. Therefore, this research aimed to evaluate the efficacy and safety of UC-II in modulating knee joint function by assessing the change of Western Ontario McMaster Osteoarthritis Index (WOMAC) subscales, Lequesne's Functional Index (LFI), and Visual Analogue Scale (VAS) score from baseline through day 90 between the UC-II and placebo groups.

Methods

Study Design and Subjects Recruitment

The study was a randomized, double-blind, placebo-controlled trial conducted at the Faculty of Medicine, Universitas Tarumanagara, Jakarta, between 2017 to 2019. Total of 102 subjects were randomized at a 1:1 ratio into two groups: receiving an oral daily dose of 40 mg/day UC-II® (PT Pharos, Jakarta, Indonesia) or placebo containing microcrystalline cellulose (PT Pharos) for 90 days. The UC-II product was obtained from chicken sternum and was encapsulated in red and white, size "00" capsules identical to the placebo. Follow-up visits were carried out on day-1 (baseline), -7, -30, -60, and -90. The efficacy measurements were assessed at all visits, which includes the measurement of WOMAC, LFI, and VAS indices.

Inclusion criteria for subjects were male or female aged 40-75 years old with mild to moderate OA in one or both knees, having a body-mass index (BMI) of 18-30 kg/m², Kellgren and Lawrence radiographic grading of 2 or 3 (17), VAS score during the most painful knee movement between 4-7, and LFI score between 4.0-7.5 points after seven days withdrawal of excluded medications. Subjects

were not suggested to take any pain relievers during the study, and for subjects who have taken omega-3 fatty acids dietary supplements should undergo a 2-week washout period before the treatment begin. Subjects were excluded from the study if they had history of hypersensitivity to poultry products (eggs), chicken or fowl or shellfish, and NSAIDs. Subjects were also excluded if they had history of underlying inflammatory arthropathy or severe rheumatic arthritis (RA) or OA, congestive heart failure, systemic lupus erythematosus (SLE), psychiatric disorder, hyperuricemia, gout, injury in the knee area affected by OA (for past 4 month), clinically significant organ disorders or malignancies (within the last 5 years). Subjects who were taking corticosteroid or other NSAID or glucosamine and chondroitin (within 3 months prior to the treatment period), intra-articular injections with corticosteroid or hyaluronic acid (within 6 months prior the treatment period), and any pain relievers within (7 days prior to the screening visit), as well as subjects who had history of alcohol intake or use of recreational drugs, planning of surgery in the next 4 months, pregnant or lactating female, were also excluded. The study protocol has been approved by The Ethics Committee of Universitas Tarumanagara (Study Protocol No. PP220172009).

Assessment of WOMAC Score, LFI, and VAS Score

WOMAC score was used to evaluate the pain, physical functioning, and stiffness of the joints of patients with knee and hip OA.(17,18) The scale consisted of 3 subscales comprising 24 questions. Each question was assessed rated from 0 to 4 points (0 indicates no pain and 4 extreme pain). Higher scores indicate a worse clinical condition.(18,19) LFI was used to assess the effectiveness of therapeutic interventions for patients with knee osteoarthritis. It consisted of three sections about pain or discomfort, maximum distance walked, and activities of daily living. The score ranges from 0 (no pain, no disability) to 24 (maximum pain and disability).(20) The VAS score was a subjective measure of acute and chronic pain, with a higher score indicating greater pain intensity.(21) The scores were recorded by measuring the distance on a 10-cm line representing a continuum between no pain and worst pain.

Subject diaries and study products were provided on day-1 (baseline), -7, -30, and -60 and were collected on day-1 (baseline), -7, -30, -60, and -90. Subject diary was used to evaluate subject compliance by counted and recorded remaining capsules. Blood samples and urine were collected on screening day and day 90 for clinical laboratory tests (hematology, chemistry and urinalysis parameters).

Subjects were instructed to report adverse events and the use of rescue medication. Pregnancy testing was done on screening and follow-up visits. Vital signs were checked every visit. Adverse events (AEs) were recorded using each case report form (CRF).

Measurement of Liver and Kidney Function, Hematology, and Glucose

The biochemical markers include serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, urea, creatinine and uric acid, were assessed to monitor the liver and kidney function. The serum AST and ALT levels were analysed using the NADH (without P-5'-P) methodology using Alinity Abbott (Chicago, IL, USA) with Abbott reagents. Bilirubin was measured by the diazo method. The colorimetric and enzymatic assay techniques were used for creatinine and uric acid quantification, respectively. Urinalysis was evaluated by visual exam and microscopic exam. Routine hematology analysis was also conducted to obtain red blood cell count, erythrocyte sedimentation rate, platelet count, and white blood cell count using Sysmex Hematology (Kobe, Japan) reagents. All blood and urine samples were analyzed by Bio Medika Laboratory (Jakarta, Indonesia). In addition, random blood glucose was also measured using Autocheck Glucare glucometer (Medical Technology Promedr, St. Ingbert, Germany). The vital signs (pulse rate, blood pressure, respiratory rate, and body temperature) were measured at the baseline and the end of the study. Subjects must report any adverse events to the investigator, who would record them in the case record form.

Rescue Medication Usage

All subjects had been given acetaminophen at 500 mg thrice a day, *i.e.*, 1.5 grams per day, as a rescue medication. After consulting with the investigator, subjects may use rescue medication if necessary, but not within 48 hours of evaluations. The investigator recorded the use of rescue medication in the subject's diary.

Statistical Analysis

All statistical analyses were done by using SPSS v.20 statistical software (IBM Corporation, Armonk, NY, USA). Subjects' characteristics and baseline values were analyzed using the summary statistics procedure and either an independent Student t-test (for continuous indicators) or Pearson Chi-square/Fisher exact test (for categorical indicators). The Generalized Estimating Equations (GEE) statistical procedure was applied to identify whether the two

treatment groups differed in their alteration patterns from baseline in the outcome measures over the 90-day of follow-up as well as to identify the difference between groups averaged across follow-up occasions (between-group effect) and the change from one follow-up occasion to the next averaged across treatment groups (within-group effect). Additional analyses to identify the changes from baseline at each follow-up day (the individual between-subject effect) were done by using baseline-adjusted ANCOVA within the Generalized Linear Model (GLM) statistical procedure, and the changes from baseline in each treatment group (the individual within-subject effect) were identified by using paired t-test. A statistically significant difference was defined at the level of $p < 0.05$.

Results

Demographics and Baseline Characteristics

A total of 102 subjects, including 50 subjects in the UC-II group and 52 subjects in the placebo group met the eligibility criteria. Overall, the subjects' profiles with respect to age, sex, height, weight, blood pressure, pulse rate, body temperature and target knee were similar between both groups (Table 1).

UC-II Reduces Subjects' WOMAC Total Score and WOMAC Subscales

The WOMAC total score showed a significant difference between the UC-II group vs. the placebo group since day-7 ($p < 0.05$) and remains significant with different means throughout the study (day-30, -60, -90; $p < 0.01$) (Figure 1A). Change from baseline within the UC-II group showed significant results since day-7 ($p < 0.01$) and remains significant during the study period. In comparison, the placebo group showed a significant difference from baseline on day-30 and day-60 ($p < 0.05$). On day-30, approximately 10% of subjects from the UC-II group reported no pain in both knees, which was doubled on day 60 (20%) and quadrupled on day 90 (38%) compared to the baseline. UC-II was more effective in reducing the WOMAC total scores by 81.6% (from 18.58 on day-1 to 3.41 on day-90) compared to 19.2% (from 18.31 on day 1 to 14.78 on day-90) in the placebo group after 90 days.

The WOMAC A score showed a significant difference between the UC-II group vs. the placebo group from day-30 ($p = 0.03$) to day-90 ($p < 0.001$) (Figure 1B). Change from baseline in the UC-II group also showed significant results from day-30 ($p = 0.01$) to day-90 ($p < 0.001$) (Table 2). The

Table 1. Baseline characteristics of the subjects.

Parameters	UC-II (n=50)	Placebo (n=52)	p-value
Age (years), mean±SD	53.12±7.09	51.83±7.11	0.360
Sex, n (%)			
Men	7 (14.0)	6 (11.5)	0.710
Women	43 (86.0)	46 (88.5)	
Anthropometrics, mean±SD			
Height (cm)	154.33±6.91	154.69±6.29	0.780
Weight (kg)	59.64±8.49	58.50±8.27	0.490
BMI (kg/m ²)	25.05±3.15	24.47±3.32	0.370
Physical exams, mean±SD			
Systolic blood pressures (mmHg)	116.84±14.57	116.08±15.50	0.790
Diastolic blood pressures (mmHg)	75.38±9.34	75.06±8.22	0.850
Pulse rate (times/min)	73.96±9.48	79.75±10.48	0.004*
Respiratory rate (times/min)	18.16±1.89	18.42±2.90	0.590
Temperature (°C)	36.33±0.28	36.38±0.34	0.490
Knee pain, n (%)			
One knee	43 (86.0)	46 (88.5)	0.920
Both knees	7 (14.0)	6 (11.5)	

Independent Student T-test was used to analyze the numerical data, while Pearson Chi-square/ Fisher exact-test was used to analyze the categorical data). *Significant if $p < 0.05$.

WOMAC B score showed a significant difference between the UC-II group vs. placebo group B from day-30 ($p=0.002$) to day-90 ($p < 0.001$) (Figure 1C). Change from baseline in UC-II group also showed significant results from day-7 ($p=0.03$) to day-90 ($p < 0.001$). The WOMAC C score showed a significant difference between the UC-II group and the placebo group from day-7 ($p=0.04$) throughout the

study period (day-30, -60, and -90; $p < 0.001$) (Figure 1D). Change from baseline in the UC-II group also showed significant results from day-7 ($p=0.02$) to day-90 ($p < 0.001$). The WOMAC subscales scores showed significant reductions in all three WOMAC subscales in UC-II groups, compared to the placebo group, with 77.3%, 89.9%, and 81.8% reduction, respectively. Analysis of the WOMAC

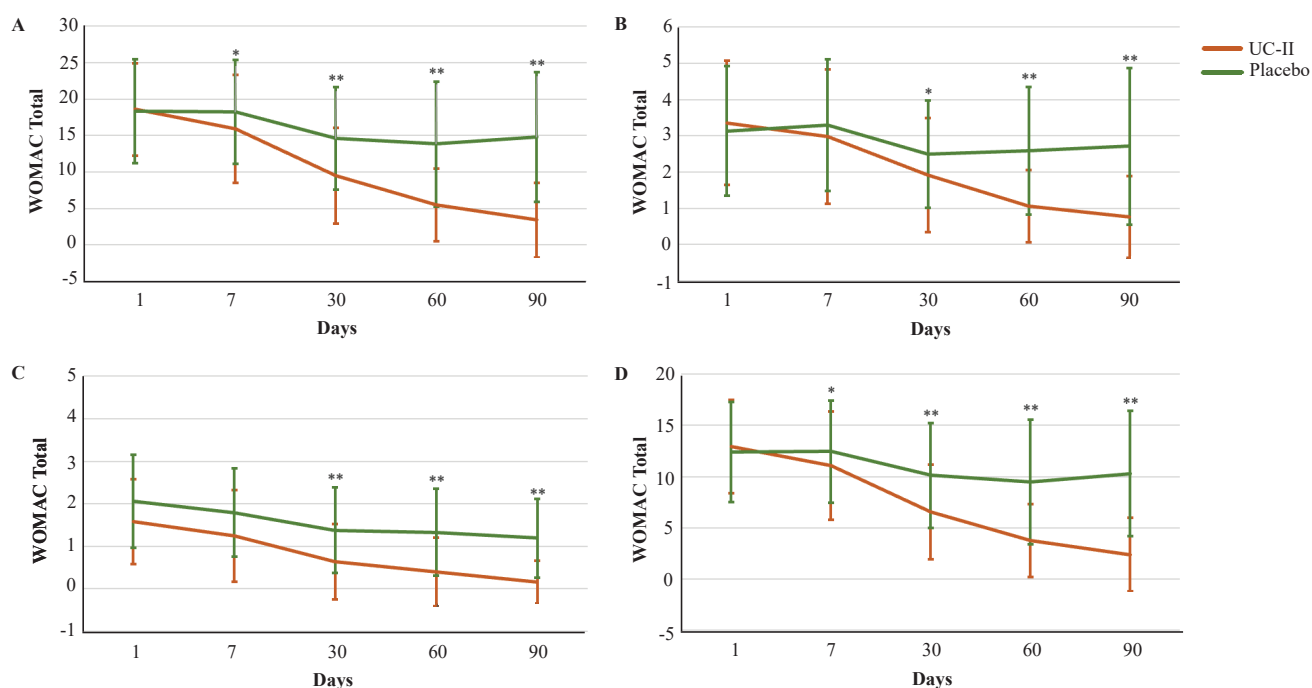


Figure 1. Changes in WOMAC total score and WOMAC subscales. A: WOMAC total score; B: WOMAC subscale A; C: WOMAC subscale B; D: WOMAC subscale C. Scores were assessed over 90-day of study period. Values are presented as mean±SD. Significant difference between the UC-II and the placebo group indicated by * $p < 0.05$, ** $p < 0.01$.

Table 2. Changes from baseline for WOMAC subscale A, B, C (pain, stiffness, and physical function) by study groups and within-groups over 90 days of study period.

Day	Descriptive		Change from Baseline			
	UC-II (n=50)	Placebo (n=52)	UC-II vs. Placebo ¹ (n=102)		Within-group ² Follow-up vs. Baseline	
	Mean±SD	Mean±SD	Mean (95% CI)	p-value	Mean (95% CI)	p-value
WOMAC 1						
D-1	3.36±1.71	3.13±1.79	-	-	-	-
D-7	2.98±1.85	3.29±1.82	-0.45 (-1.03; 0.12)	0.120	-0.38 (-0.96; 0.20)	0.600
D-30	1.92±1.58	2.50±1.48	-0.63 (-1.21; -0.04)	0.030*	-1.44 (-2.33; -0.55)	0.010*
D-60	1.06±1.00	2.58±1.76	-1.51 (-2.08; -0.94)	0.000*	-2.30 (-3.14; -1.46)	0.000*
D-90	0.76±1.12	2.71±2.16	-1.95 (-2.63; -1.26)	0.000*	-2.60 (-3.48; -1.72)	0.000*
WOMAC 2						
D-1	1.58±0.99	2.06±1.09	-	-	-	-
D-7	1.24±1.08	1.79±1.04	-0.26 (-0.61; 0.08)	0.130	-0.34 (-0.66; -0.02)	0.030*
D-30	0.64±0.88	1.38±1.01	-0.55 (-0.89; -0.20)	0.002*	-0.94 (-1.35; -0.53)	0.000*
D-60	0.40±0.81	1.33±1.02	-0.83 (-1.20; 0.47)	0.000*	-1.18 (-1.65; -0.72)	0.000*
D-90	0.16±0.51	1.19±0.93	-0.98 (-1.28; -0.68)	0.000*	-1.42 (-1.84; -1.00)	0.000*
WOMAC 3						
D-1	12.94±4.57	12.40±4.89	-	-	-	-
D-7	11.10±5.29	12.44±5.00	-1.68 (-3.33; -0.03)	0.040*	-1.84 (-3.53; -0.15)	0.020*
D-30	6.54±4.60	10.13±5.13	-3.75 (-5.60; -1.90)	0.000*	-6.40 (-8.86; -3.94)	0.000*
D-60	3.76±3.58	9.48±6.07	-5.79 (-7.76; -3.83)	0.000*	-9.18 (-11.63; -6.73)	0.000*
D-90	2.36±3.59	10.29±6.12	-8.03 (-9.99; -6.07)	0.000*	-10.58 (-12.90; -8.26)	0.000*

¹Baseline-adjusted ANCOVA within the GLM procedures for change from baseline (95% confidence interval) between UC-II vs. placebo group at individual follow-up day, significant at the level of $p < 0.05$; ²Paired t-test for change from baseline (95% confidence interval) between individual follow-up day (7, 30, 60, or 90) vs. day 1 (baseline) in each study group, significant at the level of $p < 0.05$.

subscales in subjects supplemented with UC-II showed that reductions in all three WOMAC subscales contributed to improving the overall WOMAC score.

UC-II Reduces Subjects' LFI Subcategory

Significant differences in the LFI total between groups had already been shown from day-30 to day-90 ($p<0.001$) (Figure 2), as well as the within-group analysis in the UC-II group. While in the placebo group, there were no significant differences in change from the baseline during the study period. The LFI total was significantly reduced in subjects supplemented with UC-II with a 75.8% reduction compared to the placebo groups, which showed nearly no change (7.8%). The subcategories analysis of LFI measurement showed significant differences between study groups from day-30 to day-90 (Figure 2A, 2B, and 2C) and the within-group analysis in the UC-II group (Table 3). No significant differences were found in the placebo group. The improvement in the UC-II group's total LFI score was attributed to a significant decrease in the LFI subcategory for daily activities.

UC-II Reduces Subjects' VAS Score

Significant differences between groups for the VAS score were already shown from day-7 ($p=0.02$) to day-90 ($p<0.001$) (Figure 3). As for the within-group analysis, the

UC-II group had significant differences from the baseline from day-7 to day-90 ($p<0.001$). While in the placebo group, no significant differences in change from the baseline were found during the study period. The VAS score was significantly reduced in subjects supplemented with UC-II with a 67.9% reduction, compared to the placebo groups, which showed only a 12.2% reduction (Figure 3).

Safety, Rescue Medications and AEs Assessments

No significant changes were reported in any of the hematology and blood biochemistry results (Table 4), as well as the vital signs and the urinalysis results (Supplementary 1, Supplementary 2), confirming the safety of UC-II treatment. The percentage of participants requiring rescue medication throughout the 90 days of the study period was between 12% (day-90) and 30% (day-30) in the UC-II group and between 21.2% (day-7 and day-90) and 28.8% (day-30) in the placebo group. No subject withdrew from the trial due to an AEs related to the investigational product. The most commonly reported AEs were headache, common cold, and fever in both study groups (less than 10%). The average day of all AEs was less than one day throughout the observation days. All AEs were mild, did not require in-patient hospitalization, and had mostly recovered. All AEs had no relationship and did not require any action to the investigational product.

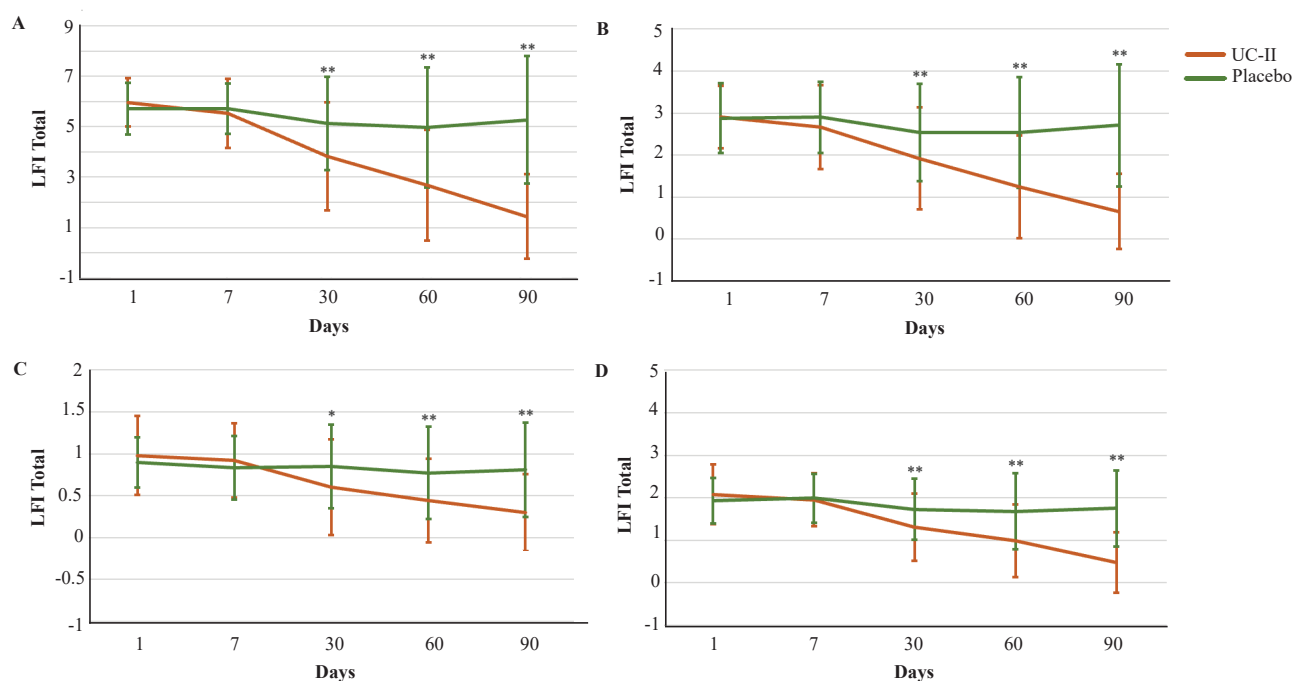


Figure 2. Changes in LFI. A: LFI total; B: LF1; C: LF2; D: LF3. Scores were assessed over 90-day study period. Each bar presents mean±SD. Significant difference between the UC-II and the placebo group indicated by * $p<0.05$, ** $p<0.01$.

Table 3. Changes from baseline for LFI by study groups and within-groups over 90 days of study period.

Day	Descriptive		Change from Baseline					
	UC-II (n=50)	Placebo (n=52)	UC-II vs. Placebo ¹ (n=102)			Within-group ² Follow-up vs. Baseline		
	Mean±SD	Mean±SD	Mean (95% CI)	p-value	Mean (95% CI)	p-value	Mean (95% CI)	p-value
LFI 1								
D-1	2.90±0.74	2.88±0.83	-	-	-	-	-	-
D-7	2.66±1.00	2.90±0.85	-0.25 (-0.58; 0.07)	0.130	-0.24 (-0.62; 0.14)	0.700	0.02 (-0.34; 0.38)	0.990
D-30	1.92±1.21	2.54±1.16	-0.63 (-1.07; -0.18)	0.006*	-0.98 (-1.49; -0.47)	0.000*	-0.35 (-0.82; 0.12)	0.350
D-60	1.24±1.22	2.54±1.32	-1.30 (-1.80; -0.80)	0.000*	-1.66 (-2.24; -1.08)	0.000*	-0.35 (-0.91; 0.22)	0.770
D-90	0.66±0.89	2.71±1.45	-2.06 (-2.52; -1.59)	0.000*	-2.24 (-2.71; -1.77)	0.000*	-0.17 (-0.75; 0.41)	0.990
LFI 2								
D-1	0.98±0.47	0.90±0.30	-	-	-	-	-	-
D-7	0.92±0.44	0.83±0.38	0.05 (-0.09; 0.18)	0.480	-0.06 (-0.22; 0.10)	0.990	-0.08 (-0.24; 0.08)	0.990
D-30	0.60±0.57	0.85±0.50	-0.27 (-0.48; -0.06)	0.010*	-0.38 (-0.64; -0.12)	0.001*	-0.06 (-0.28; 0.16)	0.990
D-60	0.44±0.50	0.77±0.55	-0.35 (-0.56; -0.15)	0.001*	-0.54 (-0.77; -0.31)	0.000*	-0.14 (-0.38; 0.11)	0.990
D-90	0.30±0.46	0.81±0.56	-0.52 (-0.73; -0.32)	0.000*	-0.68 (-0.92; -0.44)	0.000*	-0.09 (-0.34; 0.15)	0.990
LFI 3								
D-1	2.08±0.70	1.93±0.53	-	-	-	-	-	-
D-7	1.95±0.62	1.99±0.57	-0.10 (-0.32; 0.11)	0.360	-0.13 (-0.41; 0.15)	0.990	0.06 (-0.20; 0.32)	0.990
D-30	1.31±0.79	1.73±0.72	-0.48 (-0.76; -0.19)	0.001*	-0.77 (-1.16; -0.39)	0.000*	-0.20 (-0.48; 0.08)	0.370
D-60	0.99±0.86	1.68±0.89	-0.70 (-1.05; -0.36)	0.000*	-1.09 (-1.55; -0.63)	0.000*	-0.25 (-0.65; 0.15)	0.710
D-90	0.48±0.71	1.75±0.89	-1.28 (-1.61; -0.96)	0.000*	-1.60 (-2.03; -1.17)	0.000*	-0.18 (-0.57; 0.20)	0.990

¹Baseline-adjusted ANCOVA within the GLM procedures for change from baseline (95% confidence interval) between UC-II vs. placebo group at individual follow-up day, significant at the level of $p < 0.05$; ²Paired t-test for change from baseline (95% confidence interval) between individual follow-up day (7, 30, 60, or 90) vs. day 1 (baseline) in each study group, significant at the level of $p < 0.05$.

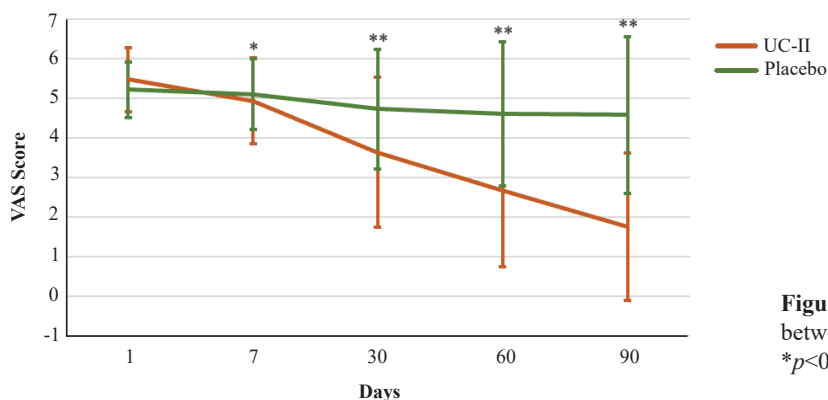


Figure 3. Changes in VAS. Significant difference between the UC-II and the placebo group indicated by * $p < 0.05$, ** $p < 0.001$.

Discussion

This study assessed the efficacy and tolerability of UC-II compared to placebo in modulating knee joint function in patients with mild to moderate OA. The result showed that UC-II significantly reduced the total and subscales WOMAC for pain, stiffness, and physical function compared to the placebo group after 90 days. Similar results were shown for LFI and VAS scores.

UC-II supplementation improved daily activities by modulating knee joint function. Previous studies have shown the efficacy of UC-II in treating OA. Treatment with UC-II for 90 days was more efficacious in reducing the WOMAC and VAS scores than glucosamine and

chondroitin.(13) Other studies evaluated the efficacy and tolerability of UC-II for knee OA pain compared to placebo and to glucosamine hydrochloride plus chondroitin sulfate for 180 days and 12 weeks, respectively.(14,15) Treatment with 40 mg/day UC-II significantly reduced the overall WOMAC score (14,15) and all WOMAC subscales (14) compared to placebo and glucosamine-chondroitin. The other one found a significant reduction in the overall and all subscale WOMAC score, LFI, and VAS with the treatment of 40 mg/day UC-II over 120 days.(16) Another study evaluated the safety and efficacy of 40 mg/day UC-II in Indian patients with OA for 90 days, and showed that UC-II is safe and efficacious in OA patients by improving the WOMAC and VAS scores.(21) Contrary to the current study, an open-label clinical trial showed no significant

Table 4. Safety parameter assessment at baseline and day-90.

Parameters	UC-II (n=50)			Placebo (n=52)		
	Mean±SD Baseline	Mean±SD at Day-90	p- value	Mean±SD Baseline	Mean±SD at Day-90	p- value
Hemoglobin (g/dL)	13.02±1.24	13.09±1.20	0.488	12.76±1.06	12.84±1.20	0.400
Red blood cell count ($\times 10^6/\mu\text{L}$)	4.52±0.39	4.56±0.40	0.133	4.43±0.38	4.46±0.43	0.504
Erythrocyte sedimentation rate (mm/h)	25.24±15.35	25.70±17.22	0.795	23.42±12.90	24.44±13.87	0.438
Platelet count ($\times 10^3/\mu\text{L}$)	317.32±63.34	308.48±57.58	0.056	303.00±64.09	296.33± 62.00	0.169
White blood cell count ($\times 10^3/\mu\text{L}$)	7.26±1.81	7.30±1.51	0.244	7.56±1.77	7.28±2.01	0.332
Total bilirubin (mg/dL)	0.55±0.18	0.55±0.16	0.977	0.57±0.20	0.54±0.20	0.189
Direct bilirubin (mg/dL)	0.21±0.06	0.22±0.06	0.729	0.22±0.07	0.22±0.08	0.981
Indirect bilirubin (mg/dL)	0.34±0.2	0.33±0.11	0.836	0.35±0.14	0.32±0.13	0.052
AST (U/L)	19.34 ±5.05	18.94±4.11	0.482	19.94±10.17	19.73±8.35	0.771
ALT (U/L)	17.76±9.06	17.84±7.80	0.927	17.02±13.20	16.81±12.23	0.782
Urea (mg/dL)	21.40±6.51	21.51±6.69	0.944	21.96±6.13	20.73±6.30	0.090
Urea Nitrogen (mg/dL)	9.99±3.03	9.93±3.26	0.186	10.25±2.85	9.75±3.04	0.064
Creatinine (mg/dL)	0.74±0.11	0.73±0.11	0.301	0.73±0.09	0.73±0.10	0.661
Uric acid (mg/dL)	4.31±1.03	4.51±1.29	0.026	4.27±0.95	4.37±0.95	0.335
Random glucose (mg/dL)	96.38±17.57	98.34±2095	0.569	93.71±15.74	93.46±19.20	0.931

differences after four weeks of undenatured type II collagen supplementation.(22)

In this study, the improvement of the knee joint was observed periodically on day-7, -30, -60, and -90. The significant improvement of all parameters had been shown earlier compared with other studies using the same dose of 40 mg/ day of UC-II. WOMAC total had been significantly improved from day-7 (13) and at day-30 (14). UC-II was found to be more effective with a 67.9% reduction for VAS score after 90 days of treatment compared to a 40% reduction in previous study.(13) The significant reduction of VAS score and differences in the LFI total between groups had been shown from day 30 compared with other study at day 180.(14) The result was contrary to another previous study in that no between-group differences existed for LFI scores.(13)

There was no statistically significant difference between the groups in any safety parameters during baseline and day-90. Vital signs and laboratory biomarkers did not change beyond the normal range. These results were supported by the previous studies (14,15,22), suggesting that UC-II is safe for patients. The most commonly reported AEs were headache, common cold, and fever in both study groups. All the AEs were mild and considered unrelated to the investigational product. A previous study reported 12.7 % of subjects reported AEs, less than 5% of which were related to gastrointestinal disturbances, and none were considered related to UC-II.(14) Conversely, another study reported 43% of mild and 54% of moderate AEs, with 11.4% of subjects possibly related to UC-II, with the most common being intermittent constipation and headache.(13) Another study reported less than 5% AEs possibly related to UC-II, with the most common AEs being gastrointestinal disturbances.(21)

Many theories were postulated to explain the mechanisms of collagen products to ameliorate articular cartilage health.(12) Type II collagen is one of the main and most abundant collagen in articular cartilage.(23,24) Degradation and reduction of type II collagen are frequently observed in osteoarthritic cartilage. Supplementation with collagen derivatives may improve cartilage repair by providing adequate nutrients for the repair and maintenance. (25) Numerous hypotheses were suggested to clarify the precise mechanisms by which collagen products enhance articular cartilage health. A study in the mouse model showed an action attributed to oral tolerance.(26) Another study in the rat model showed that UC-II provided symptom relief by an act attributed to oral tolerance and modulating inflammatory pathways.(27) UC-II can alleviate

inflammatory T-cell response and activate T-regulatory cells via its oral tolerance mechanism, eventually reducing cartilage damage. The consumption of UC-II will be taken up by the Peyer's patches, activating immune cells. It transforms naive T-cells into T regulatory (Treg) cells targeting type II collagen. Treg cells then migrate through the circulation. When they recognize type II collagen in joint cartilage, Treg cells secrete anti-inflammatory mediators (cytokines), including the transforming growth factor (TGF)- β , interleukin (IL)-4, and IL-10. This action helps reduce joint inflammation and promotes cartilage repair. Collagen derivatives could be considered for the prevention or treatment of OA as they can improve articular cartilage health and are safe for patients. Further research is needed to explore the oral tolerance mechanism and modulation of inflammatory pathways.

Conclusion

The results of this study demonstrated that UC-II is effective in treating patients with mild to moderate knee OA by alleviating subjective symptoms and has a good safety profile with no serious adverse events among participants.

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Authors Contribution

OT and AL were involved in the conception, planning, and coordination of the research. OT, SG, J, and FF performed the data acquisition/collection. MER calculated the study data and performed the analysis. OT drafted the manuscript and SG designed the figure. OT, AL, FF, and SG were collected literature. SG, and J contributed to the review, and editing of the manuscript.

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