Research Article Open Access

Safety and Efficacy of NEXT-II®, a Novel Water-Soluble, Undenatured Type II Collagen in Healthy Human Subjects Suffering from Occasional Knee Joint Pain

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Submission Date: June 8, 2015, Acceptance date: July 28, 2015: Publication date: July 31, 2015

ABSTRACT

Background and aim: Oral administration of a novel water-soluble undenatured type II collagen (NEXT-II®) has been demonstrated to ameliorate the signs and symptoms of rheumatoid arthritis (RA) in animal models. In the present investigation, we conducted a pilot study to examine the efficacy and safety of NEXT-II® in borderline subjects defined as healthy and non-diseased state, but with potential risks in knee joint health.

Method: We employed Western Ontario McMaster Index (WOMAC) score and Visual Analog Scale (VAS) scores to assess the extent of improvement in the knee joints in these volunteers following supplementation of 40 mg NEXT-II® (10 mg as undenatured type II collagen) over a period of 12 weeks.

Result: The results demonstrated that NEXT-II® treatment significantly reduced WOMAC and VAS scores compared to subjects at baseline. Specifically, in the evaluation using VAS, the borderline subjects at resting, walking, and going up and down the stairs revealed significant improvement when compared to the baseline.

Conclusion: The results of the studies demonstrated that NEXT-II® might be an ingredient which is safe and effective in the application of dietary supplement in ameliorating joint pain and

symptoms of the borderline subjects without any adverse events.

BACKGROUND

Knee osteoarthritis (OA) is a common chronic disease associated with joint stiffness, pain, and decreased function, which is attributed to increase in obesity, sedentary lifestyle, and lack of exercise and aging (1). Notably, the prevalence of knee OA increases with age (2). By age 65, a significant majority of the population demonstrates radiographic evidence of OA. However, many people suffering from the knee joint pain cannot find suitable drugs and supplements, primarily due to side effects or little efficacy for the pain management. For example, COX-2 inhibitors and NSAIDs have adverse side effects such as renal failure and gastrointestinal bleeding, congestive heart failure, and elevated blood pressure (3, 4). On the other hand, dietary supplements such as glucosamine have shown little efficacy in the amelioration of pain and inflammation (1). Knee OA impairs activities of daily life and quality of life in the elderly. Therefore, preventive approaches in controlling these diseases are important to develop for our increasingly aging society.

There are reports suggesting the ingestion of type II collagen induced prevention of arthritis (5, 6). Furthermore, undenatured type II collagen has been reported to be more effective than the combination of glucosamine and chondroitin on OA subjects (7). Although its mechanism of action is complex and fully understood, it pertains to oral tolerance. However the presence of epitope on the collagen plays an important role in regulating the immunologic activities involved in it (8-10).

We have developed water soluble undenatured type II collagen (NEXT-II®) in light of potential delivery in the liquid form. Recently, we reported safety and efficacy in NEXT-II® on mice, rats and, dogs (11-13). In the clinical pilot study, the aim is to evaluate the efficacy and safety of NEXT-II® in subjects who have health concerns of their knee joints.

METHODS

Investigational product

The investigational study product NEXT-II® is derived from chicken sternums. It is manufactured under low-temperature to preserve its native structure. For the clinical study, test material containing 40mg of NEXT-II®, which provides 10 mg of undenatured type II collagen, was encapsulated in hard capsules with excipients (microcrystalline cellulose). Because 10 mg of undenatured type II collagen has been observed the effect on OA patients in previous study (14).

The study material was prepared in a good manufacturing practice (GMP)-certified facility. Subjects were instructed to take one capsule a day before bedtime.

Study design

This clinical trial was managed by New Drug Research Center (Hokkaido, Japan), and the study was conducted at Miyawaki Orthopedic Hospital (Hokkaido, Japan). This study protocol was approved by Miyawaki Orthopedic Hospital IRB (Hokkaido, Japan) on February 10, 2014 (IRB No. 13170). The study followed the principles outlined in the Declaration of Helsinki.

Twenty-three were screened for eligibility using the inclusion/exclusion criteria defined in Table 1. Only subjects in the borderline between normal and little knee joint pain were included in the study.

The study duration was 12 weeks totaling 5 visits that included: screening, baseline (week 0), weeks 4, 8 and 12.

Table 1. Inclusion-exclusion criteria

Inclusion	● Subject must be ≧60 and <75 years of age ● Body mass index (BMI) must be <25 kg/m^2 ● Subjects have knee joint discomfort after going up and down stairs or walking for a long time ● Their refraction angles to feel pain are less than 140 degrees ● Subjects understand the study procedures and provide signed informed consent to participate in the study and authorize the release of relevant health information to the study investigator
Exclusion	● Subject with any treatment of osteoarthritis and rheumatoid arthritis ● History of allergic reaction to any ingredients in the test product, including chicken ● Excessive smoker ● High alcohol intake ● Subjects whose eating habits are extremely irregular ● History of dangerously ill with lung, heart, liver, and kidney ● History of surgery of gastrointestinal tract and digestive organs (except cecectomy) ● Subject who did the following blood donation before the study: blood donation of 400 ml within 12 weeks, or 200 ml within 4 weeks, or did ingredient blood donation within 2 weeks

	● Worker of graveyard shift or irregularity shift ● Use of concomitant prohibited medication and natural health products ● Subject who participates in other clinical trials, or within four weeks after the end of a study ● Any other condition that, in the opinion of the investigator, would adversely affect the subject's ability to complete the study or its measures

Efficacy and safety assessments

The efficacy and safety methodologies used in the study are well-recognized, reliable, accurate, and also relevant for assessing OA.

For the assessment of the efficacy such as monitoring the range of knee motion, WOMAC and VAS scores were employed in this study. The range of knee motion, flexion, and extension was measured at the times of initial screening, weeks 0, 4, 8 and 12. Furthermore, WOMAC and VAS scores were examined at weeks 0, 1, 2, 3, 4, 6, 8, 10 and 12.

During the study, everyday sleeping and living conditions, intake time of the test sample, other supplement and medicine taken, and adverse effects were recorded in the diary of the subjects. At each visit, the subjects were questioned whether they experienced problems or difficulties. Any adverse events were documented and recorded in the study record and were classified according to the description, duration, severity, frequency, and outcome. The investigator assessed the adverse events and decided causalities.

Physical health analyses including body weight, body mass index (BMI), body temperature, blood pressure, and pulse were determined at every visit (screening, weeks 0, 4, 8 and 12), which were measured at Miyawaki Orthopedic Hospital.

Blood samples were taken from all subjects during screening, week 0, and 12. Blood samples were taken from subjects for the determination of white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet, total protein (TP), albumin (ALB), total bilirubin, aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), ɤ-guanosine triphosphate (ɤ-GTP), fasting blood, cholesterol, triglyceride, uric acid, blood urea nitrogen, creatinine, Na, K, Cl, Ca, C-reactive protein (CRP), matrix metalloproteinase (MMP)-3, rheumatoid factor (RF), and anti-cyclic citrullinated peptide (anti-CCP).

Urine samples were taken from all subjects during screening at week 0 and 12. The analyses

for kidney function were performed as follows: specific gravity, pH, urinary protein, urinary glucose, urobilinogen, bilirubin, ketone body and urine occult blood reaction. All blood and urine samples were analyzed by Daiichikishimoto-kensa center Co. Ltd. (Hokkaido, Japan).

Statistical methods

Each data shows average and standard deviation (SD). WOMAC and VAS scores have been used to perform the statistical analysis by Wilcoxon signed rank test. Values associated with the range of knee motion, physical health, hematologic test, and biochemical examination of blood were analyzed by paired T-test. Urinary semi-quantitative analysis was compared using Wilcoxon signed rank test. $P<0.05$ was considered significant for all data comparisons.

RESULTS

During the course of the study, cancellation and/or drop-outs of subjects was not observed and thus performed analysis using the data of 11 participated subjects. The mean intake rate of the test supplement was 99.8% in 12 weeks.

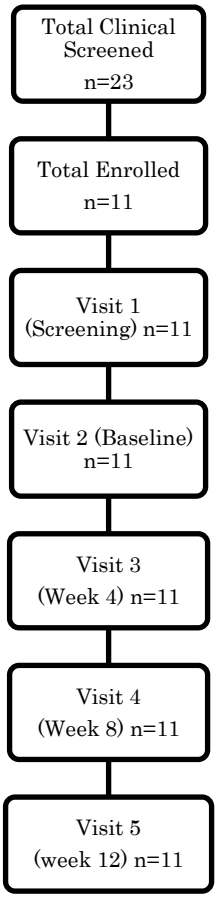

Figure 1. Attrition Chart

Range of knee motion

The range of knee flexion and extension was measured using goniometer. The results are shown in Table 2. Intake of NEXT-II® showed a greater increase in flexion at week 4 and 12 compared to week 0. The increases were noted in 10 of 11 subjects at week 12; one of rest has observed unchanged range between start and end of this study.

There were no differences in the extension.

Table 2. Target knee joint of motion

	Screening	**0 week**	**4 week**	**8 week**	**12 week**
Flexing (°)	136.8 ± 6.8	136.4 ± 5.0	140.5 ± 4.7 **	140.0 ± 5.0	143.6 ± 5.0 **
Extending (°)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Values are expressed as Mean ± SD. ** $p<0.01$ compared with 0 week.

WOMAC score

The results are shown in Fig 2. In the total score, a decrease in the score was significant in all subjects with the ingestion period. In each item of the score, intake of NEXT-II® was more significant, decreasing from week 2 in "the question of the pain of the everyday life", from week 8 in "the question of the tightening" and from week 3 in "the question of the difficulty degree of the everyday life" as compared to baseline.

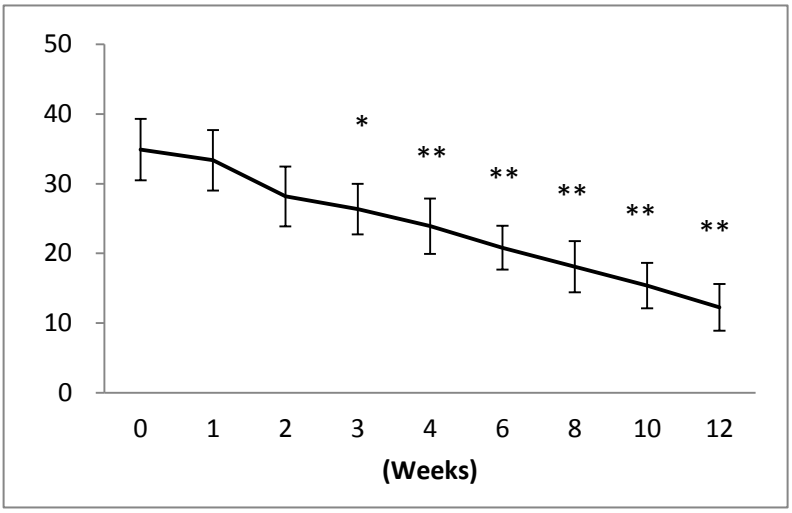

Figure 2. Changes in WOMAC sore at weeks 12 from baseline. Each value presents Mean ± SD. * $p<0.05$, ** $p<0.01$ compared with baseline.

VAS score

VAS score included parameters associated with resting, walking, and going up and down stairs (Fig 3). Decrease was noted in all subjects in each score. Significant decrease was detected in the parameters such as resting and walking at week 2, while observing in going up and down stairs at week 3 compared to baseline.

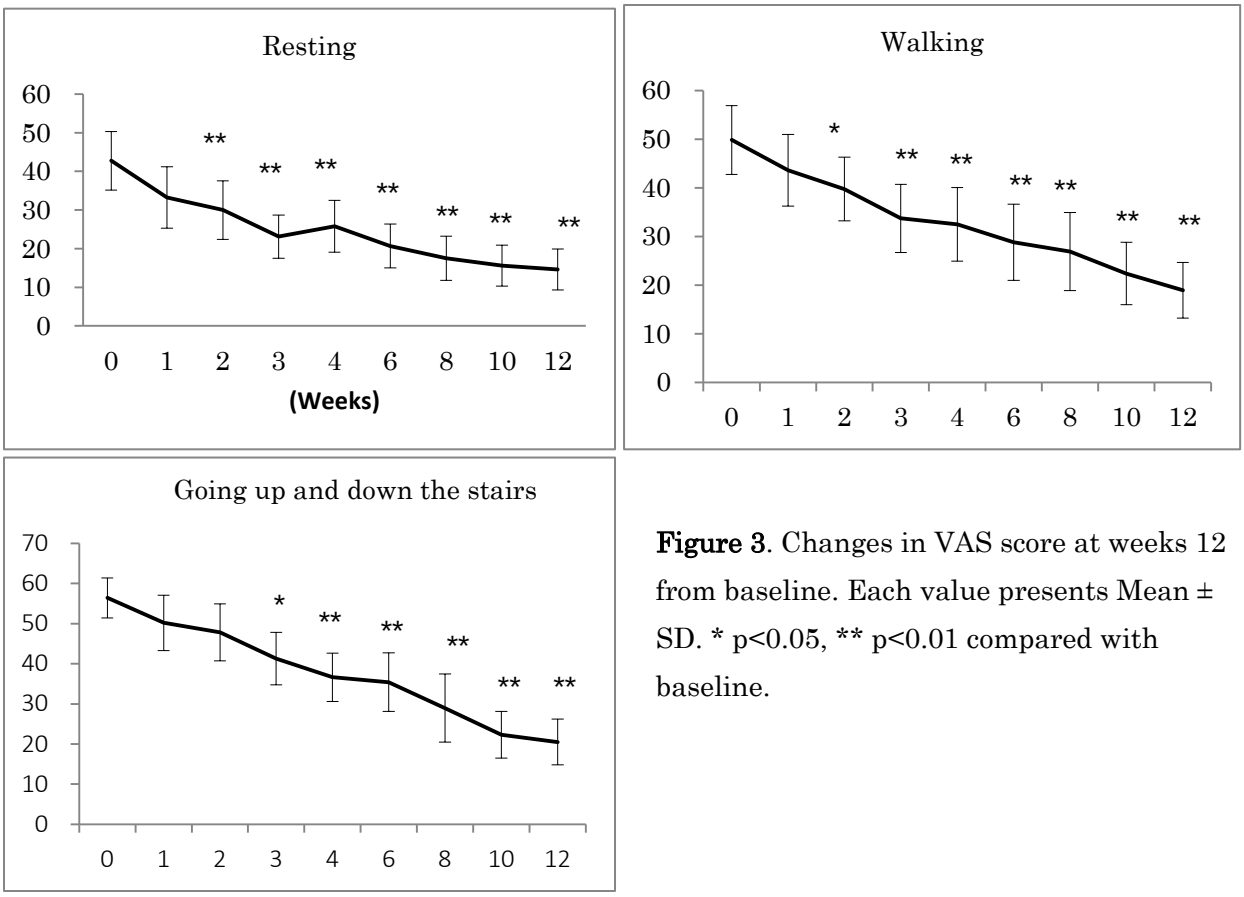

Figure 3. Changes in VAS score at weeks 12 from baseline. Each value presents Mean ± SD. * $p<0.05$, ** $p<0.01$ compared with baseline.

Physical health

The results are shown in Table 3. BMI levels significantly increased at week 8, while significant decrease was noted at week 12 compared to baseline. For body temperature, no apparent decrease was observed at week 8 compared to baseline. Also, apparent change was not noted in the other levels except the aforementioned. However, these changes are not clinically significant.

Table 3. Changes in physical examination at weeks 12 from screening

	Screening	**0 week**	**4 week**	**8 week**	**12 week**
Body weight (kg)	56.71 ± 8.96	56.58 ± 8.49	56.78 ± 8.42	56.75 ± 8.45	56.36 ± 8.21

	Screening	**0 week**	**4 week**	**8 week**	**12 week**
BMI (kg/m^2)	23.02 ± 1.54	22.98 ± 1.49	23.05 ± 1.42	23.06 ±1.52 *	22.91 ± 1.40 *
Temperature (℃)	35.72 ± 0.49	35.78 ± 0.57	35.80 ± 0.37	35.76 ± 0.45 *	35.79 ± 0.64
Systolic blood pressure (mmHg)	132.18 ± 13.95	130.45 ± 15.00	131.59 ± 16.86	122.50 ± 11.51	122.73 ± 14.25
Diastolic blood pressure (mmHg)	76.45 ± 8.01	78.45 ± 7.63	76.77 ± 11.40	71.59 ± 11.72	73.82 ± 12.31
Pulse (beats/min)	66.18 ± 9.33	66.73 ± 10.04	65.55 ± 8.58	67.45 ± 11.30	68.27 ± 12.39

Each value presents Mean ± SD. * $p<0.05$ compared with baseline.

Hematologic analysis

Intake of NEXT-II® decreased the count of red blood cells, hemoglobin content, and hematocrit at week 12 compared to baseline (Table 4). However, these changes are not considered as clinical effects.

Table 4. Changes in hematologic inspection at weeks 12 from screening.

	Screening	**0 week**	**12 week**
White blood cell (/μL)	5214 ± 1301	5204 ± 1448	5021 ± 1032
Red blood cell ($\times 10^4$/μL)	458.0 ± 42.5	459.3 ± 45.9	443.5 ± 46.2 **
Hemoglobin content (g/dL)	14.18 ± 1.45	14.25 ± 1.52	13.85 ± 1.63 *
Hematocrit (%)	43.24 ± 3.32	44.19 ± 3.99	42.43 ± 4.16 **
MCV (fL)	94.6 ± 2.9	96.4 ± 2.8	95.9 ± 2.7
MCH (pg)	30.95 ± 1.05	31.03 ± 1.14	31.20 ± 0.98
MCHC (%)	32.75 ± 1.09	32.21 ± 0.67	32.59 ± 0.82
Platelet ($\times 10^4$/μL)	18.45 ± 4.39	19.21 ± 4.59	18.69 ± 4.55

Each value presents Mean ± SD. * $p<0.05$, ** $p<0.01$ compared with baseline.

Blood chemistry

Treatment of NEXT-II® for 12 weeks significantly decreased the levels of TP and K, while increased the Cl level (Table 5). No apparent change was seen in other levels except the aforementioned. However, these changes are not considered as clinical effects.

Levels of CRP, MMP-3 and anti-CCP were measured for inflammation and rheumatoid (Table 5). These levels except anti-CCP were not significantly different from baseline. The level of anti-CCP was a meaningful decrease, but it is thought that there is no clinical meaning because it is among normal range level.

Table 5. Changes in biochemical examination of blood at weeks 12 from screening.

	Screening	**0 week**	**12 week**
TP (g/dL)	6.99 ± 0.28	7.11 ± 0.34	6.87 ± 0.51 *
ALB (g/dL)	4.21 ± 0.18	4.25 ± 0.20	4.18 ± 0.17
Total bilirubin (mg/dL)	0.89 ± 0.31	0.81 ± 0.20	0.94 ± 0.31
AST (U/L)	20.8 ± 5.3	21.2 ± 6.2	21.7 ± 5.0
ALT (U/L)	19.8 ± 7.6	21.5 ± 9.6	21.0 ± 8.3
ALP (U/L)	246.1 ± 61.8	251.8 ± 65.9	238.0 ± 47.5
LDH (U/L)	185.2 ± 35.0	187.8 ± 32.5	194.0 ± 22.1
ɤ-GTP (U/L)	30.3 ± 19.7	31.0 ± 19.7	29.8 ± 16.2
Fasting blood glucose (mg/dL)	92.7 ± 13.4	94.6 ± 16.2	93.8 ± 12.9
Total cholesterol (mg/dL)	230.1 ± 31.7	226.6 ± 31.1	225.4 ± 22.1
HDL-Chol. (mg/dL)	63.9 ± 21.5	62.3 ± 23.0	64.9 ± 19.6
LDL-Chol. (mg/dL)	138.5 ± 33.0	133.4 ± 34.7	142.0 ± 25.5
Triglyceride (mg/dL)	108.3 ± 54.6	102.5 ± 46.8	101.3 ± 41.9
Uric acid (mg/dL)	4.64 ± 1.07	4.89 ± 1.06	4.65 ± 1.13
Blood urea nitrogen (mg/dL)	14.92 ± 3.49	16.39 ± 4.27	15.65 ± 4.35
Creatinine (mg/dL)	0.632 ± 0.152	0.615 ± 0.137	0.615 ± 0.122
Na (mEq/L)	142.5 ± 1.0	140.8 ± 0.9	141.5 ± 1.4
K (mEq/L)	4.26 ± 0.24	4.23 ± 0.30	3.61 ± 0.21 **
Cl (mEq/L)	102.9 ± 2.2	103.5 ± 1.4	105.3 ± 2.3 *
Ca (mEq/L)	9.34 ± 0.32	9.14 ± 0.32	9.31 ± 0.38
CRP (mg/dL)	0.065 ± 0.087	0.075 ± 0.057	0.039 ± 0.056
MMP-3 (ng/mL)	43.58 ± 23.97	44.98 ± 18.70	43.35 ± 21.37
RF (U/ml)	2.1 ± 3.6	7.3 ± 6.5	7.3 ± 6.5
Anti-CCP (U/mL)	0.93 ± 0.23	0.99 ± 0.21	0.84 ± 0.20 **

Each value presents Mean ± SD. * $p<0.05$, ** $p<0.01$ compared with baseline.

Urinalysis

NEXT-II® significantly decreased the level of specific gravity compare to baseline (Table 6). The meaningful change was not observed in other levels except these. However, these changes are not admitted as clinical problem.

Table 6. Changes in urinalysis at weeks 12 from screening.

	Screening	**0 week**	**12 weeks**
Specific gravity	1.0144 ± 0.0065	1.0142 ± 0.0067	1.0113 ± 0.0070 *
pH	6.05 ± 0.72	6.23 ± 0.79	6.27 ± 0.75
Urinary protein	-	-	-
Urinary glucose	-	-	-
Urobilinogen	±	±	±
Bilirubin	-	-	-
Keton body	-	-	-
Urine occult blood reaction	-	-	-

Each value presents Mean ± SD. * $p<0.05$ compared with baseline.

Subjective symptom

No subjective symptoms were found in the subject's record and the doctor's diagnosis.

DISCUSSION

OA is the most prevalent form of arthritis and is a common chronic disease, resulting in joint stiffness, pain, and decreased function. OA impairs activities of daily life and quality of life. However, because of continued growth of the elderly population around the world, the number of persons affected with OA and the borderline between normal or healthy and arthritis states augment every year. The increase is becoming a major medical and financial problem. Recently, various nutritional supplements including chondroitin, glucosamine, methyl sulfonyl methane, and type II collagen are sold in the market to ameliorate various symptoms of OA.

Type II collagen is available in the form of denatured and undenatured types in the market, while previous studies involved undenatured type II collagen, modulating joint health in OA and RA (5, 7, 15). NEXT-2 has been also examined about safety and efficacy on animal (11-13). We expected that NEXT-II could be higher in human utilization in respect to its high water-solubility

in nature. Because of its water solubility, a wide array of applications of NEXT-II® (such as in beverages) are suitable for the ease of ingestion by elderly people suffering from OA in particular.

The open label study attempted to examine the efficacy of water-soluble undenatured type II collagen, NEXT-II® on subjects with knee joints in uncomfortable condition. The purpose of this study was to examine safety and efficacy in the knee joint of humans. The present study is important to improve our understanding of whether these results support the evidence of healthy knee joints of Japanese subjects.

The subjects are the borderline between normal and patients of arthritis. As we know to date, there has not been any study of type II collagen for those subjects who were diagnosed by physicians at the borderline.

During the early characteristic phase of OA there is an apparent preferential loss of knee motion range (16, 17). And this loss has shown to correlate with WOMAC and VAS scores. Intaking NEXT-II® for 12 weeks improved range of knee flexion compared to baseline. The flexing range of normal people is said to be about 150-160°. From that, even the results in 12 weeks were not normal ranges, however, there was significant increase in comparison with before intake of NEXT-II®. In addition, the range of knee flexion on 10 subjects was improved. Intaking of NEXT-II® showed the improvement in all subjective questionnaires as seen in WOMAC or VAS scores after 2 or 3 weeks of supplementation. From these results, we confirmed improvements in the quality of daily life by orally taken NEXT-II® in majority of the subjects. In this study, the amount of intake a day was 10 mg as undenatured type II collagen; however, we presume that smaller amounts of NEXT-II® potentially are effective based on our understanding of its mechanistic action, in which epitopes on NEXT-II® trigger immunologic response at the small intestine. Furthermore, because NEXT-II® is water-soluble, it seems to exert the immunologic response faster with little amount of NEXT-II®.

In safety, although supplementation of NEXT-II® caused significant changes in physical health, hematologic analysis, blood chemical examination, and urinalysis. But these changes are insignificant, which might not be a clinical significance as each subject is taken into consideration. It seems that there was no harmful observation due to NEXT-II®.

Since NEXT-II® is water-soluble, undenatured type II has only employed in the encapsulated form; dosage form, for NEXT in beverages, seems to be relatively rapid in action attributable to its water-solubility, but substation in bioavailability will have to be considered in future.

CONCLUSION

Supplementation of NEXT-II® for 12 weeks improved the knee range of flexing in 10 subjects compared to baseline. Based on the results of WOMAC and VAS scores, the supplementation for 2 to 3 weeks significantly increased the joint health of all subjects. From these findings, NEXT-II® is thought to be a suitable material for arthralgia. Although significant changes by ingesting NEXT-II® were observed in physical health, hematologic analysis, blood chemical examination and urinalysis, these are not considered as clinically significant because the changes were so negligible. The subjective symptoms were not noted at all, and therefore it was judged there is the absence of harmful incidence due to NEXT-II®.

In brief, intake of NEXT-II was proved to improve the pain of the knee without side effects.

List of abbreviations used: OA: osteoarthritis; RA: rheumatoid arthritis; VAS: visual analog scale; WOMAC: western Ontario McMaster index

Competing interests: The authors have no financial interests or conflicts of interests.

Authors' contributions: All authors contributed to the study.

Acknowledgements and funding: The clinical study was managed by New Drug Research Center, Inc., (Hokkaido, Japan), and was conducted at Miyawaki Orthopedics (Hokkaido, Japan). The study was financially supported by Ryusendo Co. Ltd. (Tokyo, Japan).

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