

Original article

The efficacy and safety of type E chondroitin-rich giant squid cartilage extract in knee pain symptoms: A placebo-controlled, double blind study

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Abstract

Objective: Decreased function of physical exercises, inherent in locomotive syndrome, is associated with the glycation stress. The prevention from the syndrome has become an important task in maintaining the functionality of physical exercises.

This study was conducted to evaluate the efficacy and safety of type E chondroitin-rich giant squid cartilage extract in the treatment of knee pain symptoms and in the improvement of bone density related with the syndrome by a placebo-controlled, randomized, double blind, parallel-group comparison study design.

Methods: A total of 46 previously diagnosed with knee pain symptoms were subjects in the clinical trial and assigned to either the test group (n=23) or placebo group (n=23). Those subjects in the test group took 250 mg of type E chondroitin-rich giant squid cartilage extract (the test product; IncaCartilago) daily for a period 90 consecutive days. The efficacy and safety in the treatment of knee pain symptoms was measured before and on days 30, 60 and 90 after the trial. The improvement of bone density was measured before and at day 90. The study was approved by an ethical review committee.

Results: Significant improvement in knee pain symptoms and bone density in the test group compared with the control were observed during the study period. By visual analog scale (VAS) score, WOMAC (Ontario & McMaster Universities Arthritis Index) score, Lequesne Index and dual energy X-ray absorptiometry (DEXA) scan on bone density, the between -group differences were observed and statistically significant favoring the test group.

Conclusion: Results of the present study suggest that the test product is effective in the treatment of knee pain symptoms and the improvement of bone density. The safety assessments conducted in this study support the claim that the test product is safe to use in the dose tested.

KEY WORDS: Treatment of knee pain symptoms, Improvement of bone density, Type E Chondroitin, giant squid cartilage extract, Locomotive Syndrome

Introduction

While physical exercises reduce glycation stress, locomotive syndrome which is on the rise in recent years, impedes the effect of the exercises. How to prevent the syndrome has become an important task in maintaining and improving the functionality of physical exercises.

Locomotive syndrome is suggested to be caused by osteoarthritis (OA), osteoporosis and reduced knee muscles. It is estimated that 47 million people¹⁾ in Japan are suffering from this syndrome that may require nursing care some years later. OA is the most common form of arthritis, affecting millions of people worldwide. It occurs when the protective cartilage on

the ends of our bones wears down over time. Approximately 30 million¹⁾ of them have succumbed to OA, and many are assumed to be agonizing over the daily pain²⁾, and taking supplements of chondroitin, glucosamine or collagen. Marketers claim that those supplements replenish the lost cartilage in joints. However, the consumed chondroitin and glucosamine are suggested to get metabolized in or excreted from the body and to have barely worked as direct supplements³⁾. While research papers on chondroitin and glucosamine for OA have exceeded hundreds in number, certain efficacy in ① pain, ② width of articular cavity, or ③ inflammation among subjects in those research papers have not been observed. Negative results, in particular, are reported in large scale clinical studies

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where subjects are plenty^{4,5}). In contrast, the IncaCartilago, a recent item on the market, is primarily comprised of Type E chondroitin extracted from cartilage of giant squid, and differs from the current chondroitin products extracted from sharks or boars. Its effectiveness in high reduction of inflammation⁶⁻¹⁵, osteoblast multiplication¹⁶⁻¹⁹, androgen induction²⁰⁻²² and neural cell multiplication^{23,24} has been recognized while the same efficacy is not observed in other chondroitin products.

Clinical studies that were Japanese-oriented have proven that the Type E chondroitin be superbly effective in reducing of knee joint pains and increasing of bone density²⁵. Though with limited cases, Type E chondroitin has good impact on muscles^{26,27}, which is not observed in any shark or boar-originated chondroitin products. IncaCartilago is the first material in the world that can be expected to bring about effects on locomotive syndrome. We report this great result of a placebo-controlled, randomized double blind study that focused on Americans conducted at Palm Beach Research Center of Florida in the US.

Methods

Subjects

Eligible subjects were selected according to the following criteria.

Subjects must be ambulatory, 40 ~ 80 years of age, inclusive, with a body mass index (BMI) of 18.0 kg/m² ~ 35.9 kg/m², with knee pains, lesser pains than OA of the knee, with mild to moderate pains for target knees (defined as a score of > 30 mm to 70 mm on a 0 ~ 100 mm Visual Analog Scale (VAS)). They have lesser pains than OA of knee for more than three (3) months confirmed by the clinical OA classification criteria of the American College of Rheumatology (ACR), *i.e.* they must have at least three (3) of the following: ① age 50 or above; ② stiffness for < 30 minutes; ③ articular crepitus; ④ bony tenderness; ⑤ bony enlargement; and ⑥ no palpable warmth.

After explanation of the trial, informed consents in written form were obtained from all subjects. A total of 46 subjects participated in the clinical trial. At the first treatment visit (Day 0), Subjects were randomly assigned to receive the placebo or the test product “IncaCartilago” (the test group). One subject in the control group only took the medication once during the study and failed to join the ensuing medical checkups, therefore the subject was excluded from the eligible participants. All of the remaining 45 subjects completed the study and complied with the protocol (per protocol set: PPS). 22 subjects in the placebo group and 23 in the test group were eligible participants in this study. During the trial period, the intake ratio of the test substances and the hospital visit ratio were 88 ~ 95% in the test group and 89 ~ 93% in the placebo group, with no differences observed. **Table 1** shows the subject demographics.

While both groups' demographics showed no differences in age, height, body weight, BMI, blood pressure, heart rate, left and right knee pains and human race, there were more females in the test group as far as gender was concerned.

Study Design

The trial was conducted in a two-group, placebo-controlled, randomized, double blind, and parallel-group comparison study design. Subjects were given either a placebo (the placebo group) or IncaCartilago (the test group) for 90

Table 1. Demographic and baseline characteristics of the trial Subjects.

Items	Placebo (n=22)	Test product (n=23)
Age(years)	54.92±9.53	59.73±9.87
Sex : male/female (%)	57.14% (8/14)	130.0% (13/10)
Height(cm)	169.97±9.55	172.68±11.07
Weight(kg)	85.60±15.08	85.61±16.74
Body mass index	29.66±4.55	28.66±4.41
Systolic Blood Pressure(mm)	128.13±12.41	129.60±16.22
Diastolic Blood Pressure(mm)	78.91±8.55	79.70±9.98
Heart Rate(bpm)	67.59±9.76	67.43±9.64
Target Knee Left n (%)	45.5 (10/22)	52.2(12/23)
Target Knee Right n (%)	54.5 (12/22)	47.8 (11/23)
Race (Black/White) (%)	57.1 (8/14)	53.3 (8/15)

The test product, type E chondroitin-rich giant squid cartilage extract (IncaCartilago).

consecutive days. The test product IncaCartilago, extracted from cartilage of giant squid by the Oishi-method²⁸, is comprised of Type II collagen > 10% and total chondroitin of > 60% in which Type E was > 40% and Types A+C < 20%. Each hard capsule consists of 125 mg test product, 68 mg starch, 4 mg calcium stearate and 3 mg silicon dioxide. The control product (placebo) in each hard capsule contained 200 mg dextrin. Subjects were instructed to take two capsules of either the control or the test product.

The efficacy and safety assessments used in this study were based on common standards for OA and are widely used and recognized as reliable, accurate and relevant. VAS (Visual Analog Scale)²⁸ scores, WOMAC (Ontario & McMaster Universities Arthritis Index)²⁹ scores and the Lequesne index³⁰ were determined at base line, as well as on days 30, 60 and 90. Measurements by DEXA scan (Dual Energy X-ray Absorptiometry Scan) were performed on days 0 and 90 in the improvement of bone density.

The trial was conducted in August–November 2013 at Palm Beach Research Center (West Palm Beach, Florida, USA) supervised by Isaac Narcadis with sufficient attention to following the spirit of the Declaration of Helsinki. The subjects provided informed written consent upon sufficient explanation on the purpose, details of the trial and the participants' rights. They were told that early withdrawal from the trial would not be a detriment.

Ethical Considerations

This trial was conducted at a third-party institution in compliance with ethical principles based on the Declaration

of Helsinki, the Private Information Protection Law, and Ministerial Ordinance on Good Clinical Practice (GCP) for Drugs (Ministry of Health, Labor and Welfare, Ordinance No.28 of March 27, 1997).

The Institutional Review Board of Chesapeake Research Review, Inc. (Columbia, Maryland, USA) reviewed the trial protocol on its ethical aspects and the trial's appropriateness, and approved the protocol by which the trial was executed. The principal investigator and sub investigators, in cooperation with a contract research organization explained the details of the trial to and obtained written consent from each subject based on his or her free will before initiating the study.

Statistical Analysis

Study data were electronically transmitted to a statistician at School of Business, New York University. All data from the qualified subjects were entered into data analysis. Primary efficacy data were analyzed by means of two-group tests. P-values < 0.05 were considered statistically significant.

Results

VAS Score (Visual Analog Scale)

There were 8 items on Pain in this assessment. They were ① Walking Pain, ② Standing Pain, ③ Pain In Climbing Up / Down stairs, ④ Night Pain, ⑤ Resting Pain, ⑥ Total Pain, ⑦ The Most Severe Pain, and ⑧ All-Item Average Pain.

The between-group differences for average improvement from base line scores in ⑧ Average Pain were 5.88 on day 60 for the placebo group as compared to 18.25 for the test group and were 9.45 on day 90 for the placebo as compared to 21.43 for the test group. These difference were found to be statistically significant ($p = 0.018$, $p = 0.022$, respectively, favoring the test group).

There was evidence that the test product had a significant effect on ① Walking Pain, ③ Pain In Climbing Up / Down stairs, ⑥ Total Pain, ⑦ Pain In the Most Painful Knee Movement on day 60 ($p = 0.033$, $p = 0.020$, $p = 0.032$, $p = 0.006$ respectively) and on ② Standing Pain, ⑥ Total Pain, ⑦ The Most Severe Pain on day 90 ($p = 0.044$, $p = 0.039$, $p = 0.008$ respectively) when compared with the placebo. **Fig. 1** shows the result in ⑧ All-Item Average Pain.

WOMAC Score

There were 3 items in this assessment. They were ① Pain, ② Stiffness, and ③ Functionality. The between-group differences for average improvement in ① Pain from base line scores on day 90 were 3.53 for the placebo group as compared to 15.95 for the test group. This differences were found statistically significant, ($p = 0.019$). There was evidence that the test product had a significant effect on ③ Functionality on day 90 ($p = 0.097$, favoring the test group). **Fig. 2** shows the result in ① Pain.

The Lequesne Index

There were 12 items in this assessment. They were ① Pain or Discomfort in Bed in the Morning ② Stiffness or Pain (after get-up), ③ Standing Ability for 30 minutes, ④ Walking Pain,

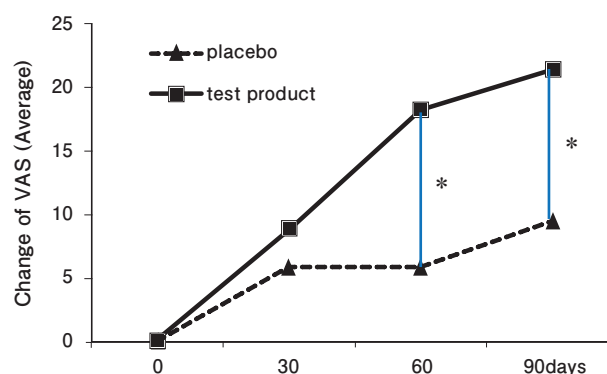


Fig. 1. Changes in VAS score (all-item average pain) from the baseline on day 90. VAS scores from each treatment group are compared to baseline value at specified time points. The values range from 0 (no pain) to 100 (extreme pain). Improvements are obtained in the absolute values, which subtracted base line value (day 0) from the follow-up values, so the differences are improvements. * $p < 0.05$ indicates significant difference between group. VAS, visual analog scale; the test product, type E chondroitin-rich giant squid cartilage extract.

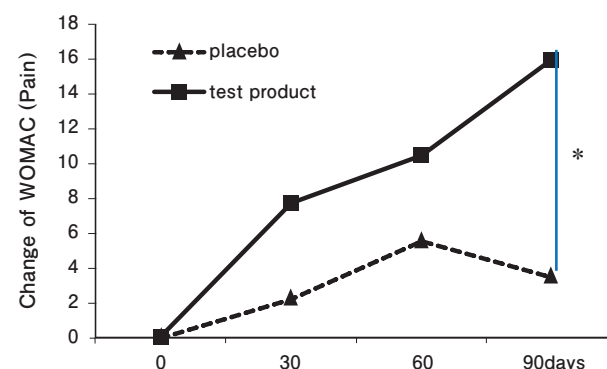


Fig. 2. Changes in WOMAC (Pain) from the baseline scores on day 90. Improvements of WOMAC (Pain) are obtained in the absolute values, which subtracted base line value (day 0) from the follow-up values. * $p < 0.05$ indicates significant difference between group. WOMAC, (Ontario & McMaster Universities Arthritis Index); the test product, type E chondroitin-rich giant squid cartilage extract.

⑤ Pain or Discomfort in Standing up without arms' assistance, ⑥ Maximum Walking Distance, ⑦ Assistance to Walking, ⑧ Daily Activeness, ⑨ Climbing Up Normal Sloped Stairways, ⑩ Climbing Down Normal sloped Stairways, ⑪ Ability to Squat or Bend the Knee and ⑫ Ground Walking.

The between-group differences for average improvement from base line scores in ⑨ Climbing Up Normal Sloped Stairways on day 60 were 0.0294 for the placebo group as compared to 0.3250 for the test group and on day 120 were 0.088 for the placebo group as compared to 0.2857 for the test group. These differences were found statistically significant ($p = 0.011$, $p = 0.019$ respectively, favoring the test group). There was evidence that the test product had a significant effect on ④ Walking Pain, and ⑪ Ability to Squat or Bend the Knee on day 60 ($p = 0.041$, $p = 0.017$ respectively), on ① Pain or Discomfort in Bed in the Morning on day 90 ($p = 0.053$) and on ④ Walking Pain on day 30 ($p = 0.030$) when compared with the placebo.

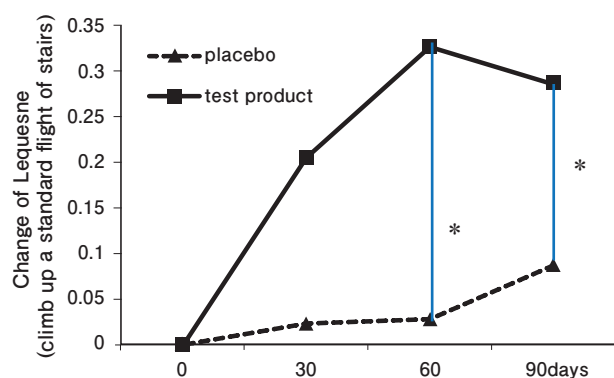


Fig. 3. The Changes in Lequesne Index (climbing up normal sloped stairways) from baseline on day 90. Improvements of Lequesne Index (climbing up normal sloped stairways) are obtained in the absolute values, which subtracted base line value (day 0) from the follow-up values, on the Lequesne's functional index, so the difference represents improvement. * $p < 0.05$ indicates significant difference between group. The test product, type E chondroitin-rich giant squid cartilage extract.

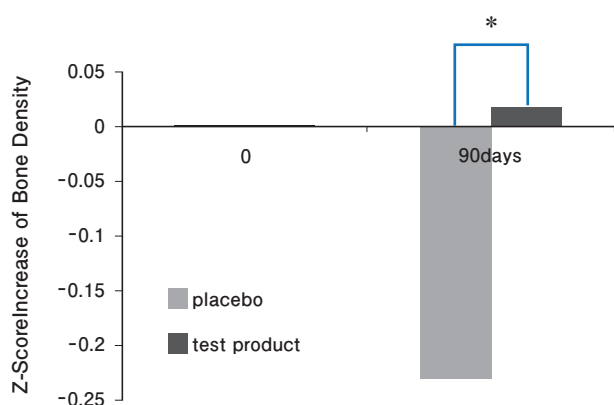


Fig. 4. Changes in Z Scores of DEXA bone density from the baseline on day 90. Z scores of bone density from each treatment group are compared with the baseline value on day 90. * $p < 0.05$ indicates significant difference between groups. DEXA, dual energy X-ray absorptiometry; the test product, type E chondroitin-rich giant squid cartilage extract.

Fig. 3 shows the result in ⑨ Climbing Up Normal Sloped Stairways.

DEXA SCAN (Bone density)

Bone density was evaluated by DEXA scan, with lumbar vertebrae L1-L4 (the petrous parts between 1 and 4 of lumbar vertebrae) examined via radiation prior to the trial and on day 90. The assessment was done by making a comparison among ① bone density, ② increase ratio of bone density, ③ T scores of bone density (% calculated by comparing the subjects' average bone mineral density with that of younger aged people as 100%), and ④ Z scores of bone density (% calculated by comparing the subjects' average bone density with that of peer aged people as 100%).

The between-group differences in change from baseline scores on day 90 were -0.224 for the placebo group as compared to 0.010 for the test group. These differences were found statistically significant, $p = 0.039$, favoring the test

Table 2. Adverse Events in all subjects

Adverse Events	Placebo	Test product
1. Knee pain	1	1*
2. Hip pain		1
3. Head Ache		1
4. Swelling Thumb	1	1
5. Right Elbow Stiffness		1*
6. Left Elbow Stiffness		1*
7. Left Elbow Pain		1*
8. Hand Stiffness		1*
9. Foot Stiffness		1*
10. Upper Respiratory Infection		1
11. Soft Tissue Injury		1
12. Diarrhea		1
13. Abdominal Pain		1*
14. Nausea		1

* This symbol refers to symptoms that took place on one identical subject in the test group. The test product, type E chondroitin-rich giant squid cartilage extract (IncaCartelago).

group. **Fig. 4** shows the result of Z scores of the bone density.

Safety Evaluation

Of the 45 subjects, 10 presented with 16 adverse events of all types (2 subjects in the placebo group with 2 adverse events; 8 subjects in the test group with 14 adverse events). Among those events in the test group, hip pain, headache, swelling thumb, upper respiratory infection, soft tissue injury, diarrhea and nausea were observed (1 event for each) and 7 adverse events happened on one identical subject. Only on 2 subjects was it considered possible that adverse events might be related to study treatment. Nevertheless, all were of mild nature and subjects recuperated without medical treatment. Since no serious adverse events were observed during the trial period, the test product was considered safe. **Table 2** shows adverse events.

Discussion

First action mechanism over knee pain symptoms of Type E chondroitin-rich giant squid cartilage extract (the test product) is suggested to be inflammation control, which means, compared with other chondroitins (Type A from boars, and Type C from sharks), Type E chondroitin controlled the best ① inflammation immunity parameter Th1/Th2 ratio by reducing of the inflammation cell Th2^{14,15}. Type E chondroitin specifically binds ②midkine that aggravate inflammation, and inhibits inflammation around inflamed areas⁶⁻¹⁰. Type E chondroitin cuts characteristically ③CD44, a cell adhesion molecule, and controls inflammation network¹¹⁻¹³.

Second action mechanism of the test product is suggested to be production of bone morphogenetic protein (BMP), osteocarcin and collagen which help multiply the osteoblasts^{16,17}. During these experiments, Type A chondroitin (boar) and Type C chondroitin (shark) were also tested in the meantime using 100 µg added to nutrient media, but both showed the same results as the control itself and neither ended up with any effects. In contrast, a 20 µg addition of Type E chondroitin and the test product led to evident results that demonstrated significant differences in comparison with the control¹⁶. Based on these facts, we can say that Type E chondroitin and the test product are five times as effective as Type A and Type C (100 µg / 20 µg).

In view of the recognized efficacy at 250 mg in the US study or 150 mg in domestic study²⁶ vis-à-vis Types A's and C's common medical dosage of 800 ~ 1560 mg, we can say that the test product is five times as strong in effectiveness both in this placebo-controlled, randomized, double blind American-oriented study and placebo-controlled, randomized, single blind Japanese-oriented study. The good results in the test group at VAS scores, WOMAC scores, the Lequesne index and bone density had been supported by the action mechanisms described above.

In ①VAS scores, significant differences ($p = 0.018$, $p = 0.022$) in Average Pain were observed on days 60 and 90

when compared with the control group. In ②WOMAC scores, significant differences ($p = 0.019$) in Pain were observed on day 90 when compared with the control group. In ③ Lequesne Index, significant differences ($p = 0.011$, $p = 0.019$ respectively) were observed on days 60 and 90 on Normally Sloped Stairway when compared with the control group. In ④ bone density measured by DEXA scan, significant differences ($p = 0.039$) in Z scores were observed when compared with the control group.

Regarding adverse reactions, it seemed that there were more such cases in the test group, but they all were of mild nature and subjects were able recuperate without treatment, thus the test product was safe to use in the dose tested. Due to Type E chondroitin's function produce testosterone (male hormone) by creating more osteocarcin²⁰⁻²², it is expected to show effectiveness in reinforcing male traits, such as bone density enhancement, muscle build-up, and hair growth. Given Type E's function to increase neural cell proliferation^{23,24} and promote insulin secretion³¹⁻³³, it is expected to show effectiveness in preventing aging process with efforts or age-related functional declines.

Conclusion

The results of the present study suggest that the test product (IncaCartilago) may be effective in the treatment of knee pain symptoms and the improvement of bone density. The safety assessments conducted in this study support the claim that the test product is safe to use in the dose tested.

Conflict of interest

This research was mainly supported by Kenko Corporation (Chiyoda-ku, Tokyo).

References

- 1) The Future Way of Surveillance "The Propulsive Meeting for the Care Prevention on the Locomotive Syndromes". Science Council of Japan (2008), Japanese Clinical Orthopedic Association (2009). <http://www.mhlw.go.jp/shingi/2007/08/dl/s0817-3a.pdf> (in Japanese)
- 2) Hannan MT, Felson DT, Pincus T. Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. *J Rheumatol.* 2000; 27: 1513-1517.
- 3) Nagai K, Kawasaki T. The science for carbohydrate chains blazes a new path to the future. Kimpodo, Kyoto, 2005. (in Japanese)
- 4) Wandel S, Jüni P, Tendal B, et al. Effects of glucosamine or chondroitin or placebo in patients with osteoarthritis of hip or knee: Network meta-analysis. *BMJ.* 2010 Sep 16; 341:c4675.
- 5) Wilkens P, Scheel IB, Grundnes O, et al. Effect of glucosamine on pain-related disability in patients with chronic low back pain and degenerative lumbar osteoarthritis randomized controlled trial. *JAMA.* 2010; 304: 45-52.
- 6) Ueoka C, Kaneda N, Okazaki I, et al. Neuronal cell adhesion, mediated by the heparin-binding neuroregulatory factor midkine, is specifically inhibited by chondroitin sulfate E. Structural and functional implications of the over-sulfated chondroitin sulfate. *J Biol Chem.* 2000; 275: 37407-37413.
- 7) Muramatsu T. The function and medical purpose of midkine. *Tanpakushitsu Kakusan Koso.* 2002; 47: 1259-1267. (in Japanese)
- 8) Yamamoto H, Muramatsu H, Nakanishi T, et al. Midkine as a molecular target: Comparison of effects of chondroitin sulfate E and siRNA. *Biochem Biophys Res Commun.* 2006; 351: 915-919.
- 9) Muramatsu T. Midkine and pleiotrophin: Two related proteins involved in development, survival, inflammation and tumorigenesis. *J Biochem.* 2002; 132: 359-371.
- 10) Maeda N, Ichihara-Tanaka K, Kimura T, et al. A receptor-like protein-tyrosine phosphatase PTP ζ /RPTP β binds a heparin-binding growth factor midkine. Involvement of arginine 78 of midkine in the high affinity binding to PTP ζ . *J Biol Chem.* 1999; 274: 12474-12479.

- 11) Kawashima H, Atarashi K, Hirose M, et al. Binding of a large chondroitin sulfate/dermatan sulfate, proteoglycan and vesicant to L-selectin, P-selectin and CD44. *J Biol Chem.* 2002; 277: 12921-12930.
- 12) Taisho Pharmaceutical Co.,Ltd. Patent: Medicines containing low-molecular chondroitin sulfate. P2006-327955A. 2006, December 7. (in Japanese)
- 13) Atarashi K, Kawashima H, Hirata T, et al. Chondroitin sulfate proteoglycan, common ligand of L-selectin and CD44. *Tanpakushitsu Kakusan Koso.* 2002; 47: 2214-2220. (in Japanese)
- 14) Toida T. The metabolism and physiological function of glycosaminoglycan, a functional food. The 4th Meeting of Glucosamine Research. August 31, 2009, Tokyo. (abstract in Japanese)
- 15) Sakai N. The development of fine analysis of chondroitin sulfate and the study of immunological activity of chondroitin sulfate. The doctoral thesis in Chiba University. 2003; 18-41. (in Japanese)
- 16) Miyazaki T, Miyauchi S, Tawada A, et al. Oversulfated chondroitin sulfate-E binds to BMP-4 and enhances osteoblast differentiation. *J Cell Physiol.* 2008; 217: 769-777.
- 17) Tani H, Hattori T, Ohishi H. The effect of the chondroitin sulfate extracted from giant squid on BMP. The Abstract of the 64th Meeting of the Japan Society of Nutrition & Food Science. 2009; 3J-02a. (abstract in Japanese)
- 18) Takada T, Katagiri T, Ifuku M, et al. Sulfated polysaccharides enhance the biological activities of bone morphogenetic proteins. *J Biol Chem.* 2003; 278: 43229-43235.
- 19) David-Raoudi M, Deschrevel B, Leclercq S, et al. Chondroitin sulfate increases hyaluronan production by human synoviocytes through differential regulation of hyaluronan synthases: Role of p38 and Akt. *Arthritis Rheum.* 2009; 60: 760-770.
- 20) Oury F, Sumara G, Sumara O, et al. Endocrine regulation of male fertility by the skeleton. *Cell.* 2011; 144: 796-809.
- 21) Yeh S, Tsai MY, Xu Q, et al. Generation and characterization of androgen receptor knockout (ARKO) mice: An in vivo model for the study of androgen functions in selective tissues. *Proc Natl Acad Sci USA.* 2002; 99: 13498-13503.
- 22) Hattori T, Ohishi H, Tani H. Private communication. Hydrox Incorporation. 2011, November.
- 23) Mizumoto H, Sugawara K. The regulation of neuronal process by chondroitin sulfate / dermatan sulfate. *Experimental Medicine.* 2007; 25: 990-996. (in Japanese)
- 24) Gama CI, Tully SE, Sotogaku NC, et al. Sulfation patterns of glycosaminoglycan's encode molecular recognition and activity. *Nat Chem Biol.* 2006; 2: 467-473.
- 25) Tsuchibe S, Tani H, Someya H, et al. The effects of the subject including E type chondroitin as main ingredient extracted from giant squid cartilage on knee-pain and bone density. *Food Processing and Ingredients.* 2011; 46: 70-72. (in Japanese)
- 26) Numata S, Yasuda H, Tsuchibe F, et al. The total care of knee guaranteed by E type chondroitin. *Japan Food Science.* 2012; 51(Febuary): 9-20. (in Japanese)
- 27) Kenko Corporation, Hydrox Inc. Patent: Oversulfated chondroitin composition. PCT/JP2013/052519. 2013, May 19.
- 28) McCormack HM, Horne DJ, Sheather S. Clinical applications of visual analogue scales: A critical review. *Psychol Med.* 1988; 18: 1007-1019.
- 29) Bellamy N, Buchanan WW, Goldsmith CH, et al. Validation study of WOMAC: A health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol.* 1988; 15: 1833-1840.
- 30) Lequesne MG, Mery C, Samson M, et al. Indexes of severity for osteoarthritis of the hip and knee. Validation-value in comparison with other assessment tests. *Scan J Rheumatol Suppl.* 1987; 65: 85-89.
- 31) Ferron M, Wei J, Yoshizawa T, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell.* 2010; 142: 296-308.
- 32) Fulzele K, Riddle RC, DiGirolamo DJ, et al. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. *Cell.* 2010; 142: 309-319.
- 33) Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell.* 2007; 130: 456-469.