ORIGINAL RESEARCH

In Vivo Osteoinduction: Evaluating 2-Beta Coxatene as an Immunoinductive Compound and Novel Ingredient for Joint Support

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Abstract

Context: Osteoarthritis (OA) is a degenerative joint disease characterized by progressive loss of articular cartilage. Many treatments lack the ability to stimulate the growth of native cartilage tissue while they simultaneously increase joint comfort. For the past few decades, dietary supplements have been investigated for the ability to both address joint inflammation and stimulate cartilage tissue.

Objectives: The present study intended to examine the supplement's in vivo osteoinductive capabilities and clinical efficacy for overall joint health.

Design: The research team designed a randomized, double-blind, comparative clinical trial.

Setting: The study took place via telephone interviews.

Participants: Participants had self-reported OA of a weight-bearing joint (ie, of the knee, hip, spine, or ankle). Patients were recruited using the Health Science Institute, a consumer supplement newsletter.

Intervention: Participants in the intervention group were blindly given 135 mg of 2-Beta Coxatene (2BCT) orally, which contained (1) a custom blend of low-dose Cyplexinol, an osteoinductive protein complex derived from bovine bone tissue, and (2) *Boswellia serrata* resin enriched to 65% 3-*O*-Acetyl-11-keto-β-Boswellic acid. A positive control group was blindly given 1500 mg of glucosamine hydrogen chloride and 1200 mg of chondroitin sulfate. Participants took the supplements for 3 mo.

Outcome Measures: A histological evaluation was performed on an athymic rat to test the supplement's in vivo osteoinductive capabilities. A negative control, commercially purchased, unhydrolyzed type 2 collagen was used for that test. Participants were evaluated for parameters of pain and joint function at baseline (day 0) and at days 7, 30, and 90 using the Western Ontario and McMaster Universities (WOMAC) OA index and a visual analogue scale (VAS).

Results: The histological evaluation of the athymic rat confirmed that the Cyplexinol component of the 2BCT was positive for de novo bone tissue and collagen synthesis, corroborating osteoinduction. In the clinical trial, the intervention group reported significant decreases of 57.4%, 52.5%, and 58% in normalized WOMAC scores for pain, stiffness, and joint functionality, respectively, from baseline to postintervention. The control group reported a decrease of 17.5%, 18.1%, and 23.9% for pain, stiffness, and joint functionality, respectively. For the intervention group, pain intensity and frequency, as measured by the VAS, also decreased 57.1% and 56.3%, respectively, from baseline to postintervention, whereas the control group showed a decrease in VAS scores of 18.0% and 14.8%, respectively. In total, an average of 81.2% of participants administered the 2BCT had reported a statistically significant improvement from baseline to postintervention, compared with 22.9% of participants administered glucosamine and chondroitin.

Conclusions: In vivo studies confirmed that the bioactive proteins (Cyplexinol) within the 2BCT stimulated de novo bone and cartilage tissue production, demonstrating osteoinduction. The intervention group reported greater improvements in the psychometric evaluations that assessed joint comfort when compared with participants given the glucosamine and chondroitin. The results suggest that 2BCT may provide a novel and synergistic response to preserving joint homeostasis and improving quality of life.

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Osteoarthritis (OA) is one of the most common a degenerative joint diseases, characterized by a progressive breakdown of the articular cartilage. OA presents as a variety of interrelated symptoms, including pain, stiffness, reduced mobility and range of motion, swelling, inflammation, and crepitus.[1] In 2002, an estimated 40 million Americans were diagnosed with OA.[2] Studies have suggested that 70% to 90% of individuals 75 years or older are affected by OA. However, OA is not limited to older populations.[2] Approximately 13.9% of adults 25 years and older have clinically diagnosed OA in at least 1 weight-bearing joint.[3] Those numbers are expected to rise, in part due to the increase in the risk factors for OA, such as obesity and sedentary lifestyles.[2,4]

The pathophysiology of OA is multifactorial and includes both a genetic predisposition[5] and mechanical factors that have long-term deleterious effects on the structure and function of synovial joints, ultimately leading to joint failure.[2] Recent findings have expanded scientists' understanding of OA pathogenesis, as new evidence has demonstrated that key cytokines—bone morphogenetic proteins (BMPs) 4 and 5—are decreased or are missing in the synovium of patients with OA or rheumatoid arthritis.[6]

Multiple treatments may be employed in a stepwise approach to provide relief for patients with OA. First-line nonpharmacological approaches involve a change in lifestyle that includes physiotherapy to strengthen and stabilize a joint coupled with weight-loss programs to reduce the overall load.[7] Although those approaches have demonstrated long-term improvements after 6 to 18 months, short-term relief as well as stimulation of cartilage regrowth has not been within the scope of those approaches.

In conjunction with lifestyle changes, many patients use medications and supplements to provide short-term relief of OA symptoms.[8] Medical interventions popularly known as nonsteroidal anti-inflammatory drugs (NSAIDs) target key components of the inflammatory pathway, such as cyclooxygenase (COX) pathways,[2,4] Although effective as a pain management tool, prolonged NSAID use has serious health consequences.[9,10]

One main side effect of the nonselective COX inhibitors in the NSAID class is the increased risk of gastropathies associated with chronic use.[11-13] Second-generation COX inhibitors have attempted to reduce the inhibition of nonspecific pathways by selectively inhibiting COX-2.[14] Unfortunately, prolonged use of that class of NSAIDs has been linked with an increased risk of cardiovascular-based adverse reactions.[13,15] In addition, although NSAID use is an effective tool for reducing inflammation and limiting further cartilage destruction, also known as chondroprotection, the drugs lack signaling capabilities to stimulate production of de novo cartilage.[16]

For the past few decades, dietary supplements have been investigated for their ability to both address joint inflammation and stimulate cartilage tissue.[8] The basis of that approach relies on scientific findings that a physiological decrease in the concentration of key nutritional components, such as vitamin D, has been linked to an increased rate of progression of OA.[17] Therefore, direct supplementation of key nutrients is believed to restore joint homeostasis.

However, literature reviews had provided little evidence for any disease-modifying activity for popular supplements.[8] Glucosamine sulfate has been shown to provide only a mild, longer-term symptomatic relief in specific cases.[18] Another popular supplement that is usually paired with glucosamine is chondroitin. Chondroitin has been shown to produce a small change in the rate of decline in joint space width as well as to reduce pain and improve overall function in OA of the knee.[19]

Collagen supplements are another commonly used ingredient in joint-specific supplements. Although compelling evidence has supported its ability to improve joint comfort by reducing inflammation and swelling and, thereby, protecting the joint from further degradation,[20] collagen does not stimulate de novo tissue growth, which is necessary to preserve overall tissue integrity. Furthermore, differences in the quality of the ingredients of supplements, limited research, and poor design of clinical studies have hindered the development of recommendations for widespread use of a supplement for OA management.[21]

The final, most invasive approach to treating symptoms of OA includes surgical interventions. Arthroscopic surgery is one of the most common surgical practices for individuals with OA in the knee.[2,4] However, recent studies have questioned the validity of that process, indicating that its benefits have been no better than sham procedures.[22] Conversely, total joint replacements are an effective tool for replacing damaged joint tissue, yet the invasive nature of the procedure and the limited life span of the prosthetics make that approach ill suited for patients with significant comorbidities.[23] Therefore, multifocused approaches in disease modification may be the most beneficial long-term treatment approach.

Bone Morphogenetic Proteins

Studies have shown that BMPs are essential for the growth and preservation of joint homeostasis as well as for the control of localized inflammation[24] and are necessary for the growth of joint cartilage and bone tissue.[25] By activation of both the Similar to Mothers Against Decapentaplegic (SMAD) and mitogen-activated protein kinase (MAPK) pathways, BMPs stimulate the differentiation of mesenchymal stem cells (MSCs) into osteoblasts or chondrocytes, which synthesize and regrow bone and cartilage tissue.[26-29]

Furthermore, the BMPs elicit an antagonistic effect on key cytokines that promote inflammation and cartilage destruction, specifically interleukin (IL)-6, IL-1, and numerous transcription factors that have been associated

with the inflammatory process.[30-34] That mechanism of action has implications in joint health because recent studies have shown that BMPs are lacking in the synovial fluid of individuals with OA in the knee.[35]

The effects of MSC activation are not only limited to joints; they are essential for replenishing the pool of endogenous cells within the bone, skin, muscles, teeth, and gastrointestinal tract.[24] Recent evidence within the literature has indicated that BMPs are essential for maintaining the pool of healthy cells, which in turn support overall tissue homeostasis as well as structure and function.

Boswellia serrata Gum Resin

Boswellia serrata gum resin has been used for hundreds of years and is currently used in the traditional medications of India and the Middle East.[36] Modern biochemical analysis of the resin has revealed a total of 6 boswellic acids.[36] The most potent of the 6 acids is 3-O-Acetyl-11-keto-β-Boswellic acid (AKBA), which has shown both historical and scientifically validated, signaling capabilities that provide immune support. It occurs from 1% to 3% naturally.[36] Through use of chemically based enrichment processes involving sequential oxidation and acetylation, the less potent boswellic acids can be converted to AKBA, increasing the total composition to a minimum of 65%. The main mechanism of AKBA is the inhibition of inflammatory mediators, such as nuclear factor-κB,[37] tumor necrosis factor-α,[38] IL-1β,[38] MAPK pathways,[39] and 5-lipoxygenase,[40,41] which have been shown to provide clear clinical benefits in addressing joint pain.[35]

In clinical research, 2 AKBA extracts have been successful in the symptomatic treatment of OA. A study by Sengupta et al[42] researched a standardized extract with an AKBA content of 30%, which they gave to participants in divided dosing (twice daily) for a period of 90 days, with a comparison with a placebo. The study found that the extract had clinical effectiveness in reducing the participants' scores on the Western Ontario and McMaster Universities (WOMAC) OA index as compared with the placebo, in a dose-dependent manner.

A second AKBA standardized extract with an AKBA content of 20% was also shown to have clinical efficacy in the symptomatic management of knee OA when compared with a placebo control. Participants' scores on the WOMAC OA index for all parameters were significantly better in the treatment group than in the placebo group in a 30-day, double-blind, placebo-controlled trial.[43]

Osteoinduction Assay

Osteoinduction was first proven by Urist, as published in his seminal work in 1965. The standard osteoinduction assay in vivo is to induce bone and cartilage tissue ectopically in an athymic rat model.[44] One main function of that family of assays is assessing the osteoinductivity of commercial bone-paste products, which mainly are composed of demineralized bone matrix (DBM).

The in vivo assay also has become the standard for evaluating the true osteoinductive potential of materials being used for surgical tissue growth. That assay model assesses exogenous osteoinductive capabilities of materials by quantifying (1) induced growth of bone and cartilage tissue via activation of MSCs, (2) differentiation, and (3) specific tissue proliferation.

On the other hand, in vitro testing, such as that using alkaline phosphatase, employs testing methodologies that can produce results that are open to interpretation and have been found erroneously to be osteoinductive. The model developed by Urist[45] using in vivo testing has been validated for more than 40 years and has found that no agent other than BMPs is capable of the inducing de novo tissue.

Methods

Participants

The current study was a double-blind, controlled trial. Participants had self-reported OA of a weight-bearing joint (ie, of the knee, hip, spine, or ankle). The study took place via telephone interviews and used recruitment via Health Science Institute, a consumer supplement newsletter. A rolling recruitment model was used beginning in June 1, 2014, and ending on September 1, 2014, when the recruitment limit of 80 participants was met. In total, 88 individuals were recruited. Eight participants were excluded due to concurrent neuromuscular disorders and chronic pain medication use. Inclusion in the study required that participants (1) were older than 55 years; (2) had had pain persisting for a minimum of 1 year before joining the trial; (3) experienced spontaneous occurrences of pain ≥4 times per week; (4) had scored ≥4 on a scale of 0 to 10 for pain and stiffness in weight bearing joints—hip, knee, or ankle; (5) weighed not more than 108 kg; (6) were willing to stop all joint- and inflammation-related supplements and medication for joint pain 2 weeks before the initiation of the study, including rescue medications; (7) were willing and able to abide by the study's parameters; and (8) were able to provide consent.

Prospective participants were excluded if they (1) were pregnant or nursing; (2) had pain or discomfort only in nonweight-bearing joints; (3) had joint pain associated with neuromuscular disorders; (4) had been diagnosed with rheumatoid arthritis; (5) had had an injury within the 12 months prior to the study in the area of the affected joint; (6) had any conditions that would adversely affect their ability, in the opinion of the investigator, to complete the study or its measures; or (7) had an alcohol intake of >2 standard drinks per day. The final review of each participant's eligibility was conducted by one member of the research team.

Once chosen, the study's participants were grouped into treatment arms, in groups of 10 via block randomization. Each participant was assigned a computer-

generated randomized identification, which corresponded to one of the study's 2 arms: (1) an intervention group receiving 2-Beta Coxatene (2BCT) or (2) a positive control group receiving a supplement with glucosamine and chondroitin. The patients, investigators, and research staff were unaware of which test compound a participant was assigned. The clinical research was carried out in accordance with the Belmont report and the Declaration of Helsinki. All research conducted was voluntary and based on informed consent, which was obtained from each participant.

Procedures

Osteoinduction Assay. The assay was conducted by WuXi Apptec (Philadelphia, PA, USA) using male, athymic, nude rats that were 6 weeks old. Each rat received 2 intramuscular pockets, one in the femoris of each bicep, using both blunt and sharp dissection techniques. Each pocket was filled with approximately 250 mg ± 25 mg of a single test material loaded into a gelatin capsule and implanted with a sterile forceps. The pocket and skin were closed with sutures, and the animals were observed daily for any abnormal general health status and sacrificed after 28 days.

Upon the sacrifice, the implants were removed, and the tissue was fixed with a 10% neutral, buffered formalin prior to routine decalcification and processing into paraffin blocks. Four sections were cut, mounted on slides, and stained with hematoxylin and eosin. De novo collagen and bone synthesis were evaluated by a pathologist using a semiquantitative method to verify histological changes.

2-Beta Coxatene. The supplement evaluated, 2BCT, is a bifunctional, nutritional supplement combining 2 active ingredients for the preservation of joint health, and is composed of (1) Cyplexinol, a patented, bioactive, osteoinductive, BMP complex; and (2) resin from the *B serrata* plant, chemically enriched to a minimum of 65% of AKBA.

The Cyplexinol component of the 2BCT was used for the osteoinduction assay. It consists of a unique network of type 2 collagen fibers and associated bioactive cytokines and proteins bound together. Those proteins include insulin-like growth factor, basic fibroblast growth factor, and members of the transforming growth factor-β superfamily, which includes various isoforms of BMPs. Cyplexinol is extracted through a proprietary process (ZyCal, Toms River, NJ, USA) from bovine bones in a Good Manufacturing Process–certified plant, under custom-designed standard operating procedures. All lots of Cyplexinol are tested by 3 independent labs for basic biochemistry and nutrition for heavy metals in accordance with California Proposition 65 Chemical List, as well as for bioactivity, using the indirect enzyme-linked immunosorbent assay.

The gum resin of *B serrata* (Indian frankincense) was produced from resin collected from the trunk of the *B serrata* tree and was extracted with and standardized to an AKBA content of 65%. The AKBA content and percentages were confirmed by HPLC analysis.

The AKBA component of the 2BCT is blended with the Cyplexinol to create 2BCT. For the current study, 135 mg of the 2BCT were encapsulated into a zero-size gelatin capsule by Atlantic Essential Products (Hauppauge, NY, USA), with the remaining volume occupied by a microcrystalline cellulose (MCC) excipient.

Controls. Commercially purchased, unhydrolyzed collagen type 2 was used as the negative control for the osteoinduction assay, because it lacks the native proteins necessary to stimulate tissue growth. As a positive control for the clinical trial, glucosamine and chondroitin were purchased commercially from Atlantic Essential Products, and 750 mg of glucosamine and 600 mg of chondroitin were combined at with the MCC excipient and pressed into a pill at Atlantic Essential Products (Hauppauge, NY, USA).

Washout Period. Before starting the trial, participants were asked to participate in a 2-week washout period, during which time they were not permitted to take any joint-related supplements or over-the-counter (OTC) pain killers.

Interventions

Upon completion of the washout period, each participant was provided with 3 bottles containing 60 pills of the treatment for his or her assigned group. Each pill contained either 67.5 mg of 2BCT or 750 mg of glucosamine and 600 mg of chondroitin. Participants were instructed to take 2 pills a day (ie, 135 mg in total for the intervention group and 1500 mg of glucosamine and 1200 mg of chondroitin for the positive control group). They were asked to provide pill counts during each scheduled evaluation to ensure compliance with the study's guidelines. Rescue medications were not permitted; however, lifestyle-based therapies, such as cryotherapy and thermotherapy, and stretching and exercise were permitted. Participants took the supplements for 3 months.

Outcome Measures

Participants in the clinical trial were evaluated for parameters of pain and joint function at baseline (day 0) and at days 7, 30, and 90 using the WOMAC OA index and a visual analogue scale (VAS).

WOMAC OA Index. The questionnaire has 24 questions that are used to evaluate 3 aspects of joint health on a scale of 1 to 10: (1) pain—5 questions, (2) stiffness—2 questions, and (3) issues related to functionality—17 questions. The 2 scoring methodologies are either by a nominal scale or visual analog format. Because of this formatting functionality changes that decrease are considered positive based on the degree of difficulty of each task decreasing.

The questionnaire was administered to participants over the phone. Phone interviews were chosen for ease of

Table 1. Histological Evaluation of Osteoinduction in Rat Model

	Assay Validity	Histological Results	New Bone Formation	Conclusion
Unhydrolyzed type 2 collagen	• No abnormal health symptoms; rat survived for the duration of the experiment • Surgical implantation successful	• Negative for chondrocytes, osteoblast, and de novo cartilage tissue	• No	Nonosteoinductive
Cyplexinol	• No abnormal health symptoms; rat survived for the duration of the experiment • Surgical implantation successful	• Positive for de novo chondrocytes and osteoblasts and for de novo cartilage and osteoid formation	• Yes • Positive for de novo chondrocytes and osteoblasts and for de novo cartilage and osteoid formation	Osteoinductive

reporting and increased adherence to follow-up. In addition, a direct correlation has been shown between the results of phone interviews and on-site testing results.[46] Thus, phone interviews for the WOMAC OA index are a valid method of measuring OA outcomes.

Visual Analogue Scale. The scale was used as second, validated questionnaire aimed specifically at evaluating pain intensity and pain frequency. The VAS is a widely accepted tool for assessment of clinical pain management.[47] In the current study, each line contained endpoint anchors and 3 additional descriptors evenly spaced along the VAS scale at 2.5-cm intervals to orient the participants. The descriptors identified the values for the 2 scales. For pain intensity, 0 = no pain, 2.5 = mild pain, 5 = moderate pain, 7.5 = severe pain, and 10 = worst possible pain. For pain frequency, 0 = occasional pain, 0% of the day; 2.5 = frequent pain, 25% of the day; 5 = intermediate pain, 50% of the day; 7.5 = frequent pain, 75% of the day; and 10 = constant pain, 100% of the day. The participants were provided with a paper copy of the VASs for each assessment period and were instructed to mark the scales in accordance with the pain intensity and pain frequency that had been experienced within the previous 24 hours.

Statistics

The reported WOMAC scores for each section were normalized to a value of 100 to calculate a mean score and a percentage change from baseline. The VAS scores were recorded by measuring the distance from the sides of the scale to a participant's mark and were normalized to 100. After the scores from each component were normalized, a 2-tailed, paired *t* test was used to test for statistical significance. Results are shown as means ± standard errors of the means (SEMs). An alpha level of 0.05 was set for the type 1 error and significance. All statistical analyses were done using Graphpad's PRISM statistical analysis software, version 5.0 (La Jolla, CA, USA).

Results

Osteoinduction

The in vivo, osteoinductive capabilities of the Cyplexinol component of the 2BCT and of the unhydrolyzed type 2 collagen were evaluated. Each ingredient was implanted into the femoris muscle of each bicep of an athymic male rat, and during the subsequent 28 days, no rat exhibited abnormal symptoms related to general health.

The results from the histological analysis of the intramuscular pocket and the surrounding tissues are presented in Table 1. Both tested samples were observed to be positive for assay validity, which indicated both successful implantation of the test material and survival of the animal for the duration of the study. However, only the intramuscular pocket containing the Cyplexinol tested positive for histological evidence of osteoinduction, as defined by the presence of bone and cartilage tissue, fragments, and cells. In addition, samples stained with hematoxylin and eosin were positive for the presence of chondroblasts, cartilage, and osteoids.

No histological evidence of osteoinduction was reported in tissues implanted with the unhydrolyzed type 2 collagen. Amphophilic regions indicating accumulations of collagen were noted in the pocket containing that collagen. However, that material was amorphous and globular in nature with evidence of degradation, with the remains of the implanted material indicating that the material was probably, at the most, not the product of de novo synthesis. The degradation had instigated a local immune response, resulting in the partial degradation of the material.

Table 2. Participant Demographics

Demographic	Intervention Group (n = 34)	Positive Control Group (n = 33)
Gender, n (%)		
Male	11 (32.4%)	16 (48.5%)
Female	23 (67.6%)	17 (51.5%)
Age, y, mean ± SEM	67.7 ± 7.0	66.0 ± 7.3
Height, cm, mean ± SEM	161.5 ± 10.1	167.2 ± 7.4
Weight, kg, mean ± SEM	70.7 ± 14.9	77.7 ± 12.4
BMI, mean ± SEM	25.1 ± 2.7	27.6 ± 3.3
Joint, n (%)		
Hip	20 (58.8%)	7 (21.2%)
Ankle	2 (5.9%)	3 (9.1%)
Knee	8 (23.5%)	16 (48.5%)
Spine	4 (11.8%)	7 (21.2%)
Metric, mean ± SEM		
Pain	6.7 ± 1.1	6.7 ± 1.4
Stiffness	6.6 ± 1.5	5.8 ± 1.7
Comorbidities, n		
High cholesterol	5	4
Cardiovascular disease	5	5
Diabetes	11	5
Osteoporosis	4	2

Abbreviations: SEM, standard error of the mean; BMI, body mass index.

Table 3. Participant Compliance

	Intervention Group (n = 40)	Positive Control Group (n = 40)
Self-withdrawal	3	5
Medical withdrawal	1	0
Noncompliance	2	2

Compliance and Adverse Reactions

The demographics of the participants who finished the clinical trial are listed in Table 2.

For those subjects that failed to finish the study a summary of the participants' compliance and dropout rates is presented in Table 3. Of the 40 participants enrolled in both arms, 15% (6 of 40) were withdrawn from the intervention group, and 17.5% (7 of 40) were withdrawn from the positive control group. Of the participants who did not complete the trial, 3 participants in the intervention group and 5 participants in the control group self-withdrew. Follow-up interviews reported that the withdrawals were due to the lower than expected efficacy of the tested compounds. Two participants were removed from the trial in both groups due to noncompliance with the study's protocol. Those participants missed scheduled evaluations and/or violated the study's protocol by taking rescue medication. One medical withdrawal was reported in the intervention group.

The main adverse events reported in the control group were nausea (n = 1) and headache (n = 2). Throughout the clinical trial, 3 minor adverse events among all participants were reported for the intervention group, which also included nausea (n = 1) and headaches (n = 2). A fourth adverse event occurred for a participant enrolled in the intervention group, and she was withdrawn at the behest of her primary care practitioner due to the onset of heart palpitations. That adverse event was reported on day 5 of the trial, and the participant discontinued therapy. The participant was removed from the trial and sought medical attention. Follow-up interviews reported that the participant had a familial predisposition to heart palpitations that persisted after removal from the trial, which potentially indicated a lack of connection between the supplement and the adverse event.

Metric Assessment

Data calculations were done utilizing the results of participants who completed the study in its entirety. A summary of the mean, normalized WOMAC scores for each group is presented in Table 4. For the participants in the intervention group, the baseline scores were (1) for joint pain, 48.4 points—95% confidence interval (CI), 42.1 to 54.7; $P < .0001$; (2) for stiffness, 64.6 points—95% CI, 57.0 to 72.1; $P < .0001$; and (3) for functionality, 34.3 points—95% CI, 27.3 to 41.3; $P < .0001$. Baseline scores for the control group were for (1) joint pain, 54.3 points—95% CI, 48.5 to 60.1; $P < .0001$; (2) stiffness, 73.6 points—95% CI 67.7 to 79.5; $P < .0001$; and

(3) functionality, 43.0 points—95% CI, 38.9 to 47.2; $P < .0001$. In the course of the study, both groups reported an improvement in all evaluated metrics.

At 7 days, participants in the intervention group reported a significant decrease for (1) pain, of 10.8 points—95% CI, −15.3 to −6.3; $P < .05$; (2) for stiffness, of 13.9 points—95% −19.7 to −8.1; $P < .05$; and (3) for functionality, of 7.8 points—95% CI, −9.6 to −6.0; $P < .05$. Participants continued to report significant improvements in their WOMAC evaluations, culminating in a decrease at day 90 for (1) pain, of 27.8 points—95% CI, −34.2 to −21.4; $P < .05$; (2) stiffness, of 34.0 points—95% −42.1 to −25.9, $P < .05$; and (3) functionality, of 19.9 points—95% CI, −25.2 to −14.6; $P < .05$.

Participants assigned to the positive control group also reported decreases in the WOMAC scores. However, significant changes were not observed in the WOMAC scales until day 30 for stiffness and day 90 for pain and functionality. A significant decrease was reported for stiffness at day 30 of 13.9 points—95% CI, −12.3 to −0.8; $P < .05$. A significant decrease was reported at day 90 in pain of 9.5 points—95% CI, −12.5 to −2.5; $P < .05$ and in functionality of 10.2 points (95% CI, −9.3 to −1.3; $P < .05$).

Although both groups reported improvements in the WOMAC scores, participants in the intervention group reported a higher percentage of improvement in the WOMAC scores compared with the control group (Figure 1). At day 7, the WOMAC scores of the intervention participants decreased 22.3%, 21.5%, and 22.7% for pain, stiffness, and joint functionality, respectively, compared with decreases of 11.2%, 12.5%, and 13.0% for pain, stiffness, and joint functionality, respectively, for participants in the control group. By the end of the 90-day study, the WOMAC scores for pain, stiffness, and joint functionality improved by 57.4%, 52.5%, and 58.0%, respectively, for the intervention group, whereas participants given

Table 4. Summary of Participant Data Postintervention for the WOMAC Osteoarthritis Index

	Intervention Group (n = 34)		Positive Control Group (n = 33)	
	Mean ± SEM	**95% CI**	**Mean ± SEM**	**95% CI**
Pain				
Baseline	48.4 ± 3.2	42.1 to 54.7	54.3 ± 3.0	48.5 to 60.1
Δ 7 d	-10.8 ± 2.3[a]	-15.3 to -6.3	-6.1 ± 1.8	-5.9 to -1.1
Δ 30 d	-22.5 ± 3.4[a]	-29.2 to -15.8	-8.4 ± 2.2	-8.7 to 0.0
Δ 90 d	-27.8 ± 3.2[a]	-34.2 to -21.4	-9.5 ± 2.5[a]	-12.5 to -2.5
Stiffness				
Baseline	64.6 ± 3.9	57.0 to 72.1	73.6 ± 3.1	67.7 to 79.5
Δ 7 d	-13.9 ± 3.0[a]	-19.7 to -8.1	-9.2 ± 2.5	-9.7 to 0.0
Δ 30 d	-31.9 ± 4.4[a]	-40.5 to -23.3	-13.9 ± 2.9[a]	-12.3 to -0.8
Δ 90 d	-34.0 ± 4.1[a]	-42.1 to -25.9	-13.3 ± 3.4[a]	-15.6 to -2.2
Functionality				
Baseline	34.3 ± 3.6	27.3 to 41.3	43.0 ± 2.1	38.9 to 47.2
Δ 7	-7.8 ± 0.9[a]	-9.6 to -6.0	-5.6 ± 1.5	-5.2 to -0.1
Δ 30 d	-15.1 ± 4.1[a]	-23.2 to -7.0	-9.8 ± 2.0	-8.1 to -0.1
Δ 90 d	-19.9 ± 2.7[a]	-25.2 to -14.6	-10.2 ± 2.1[a]	-9.3 to -1.3

[a] $P < .05$.

Abbreviations: WOMAC, Western Ontario and McMaster University; SEM, standard error of the mean; CI, confidence interval; Δ, change.

Table 5. Summary of Participants' Data Postintervention for the VAS

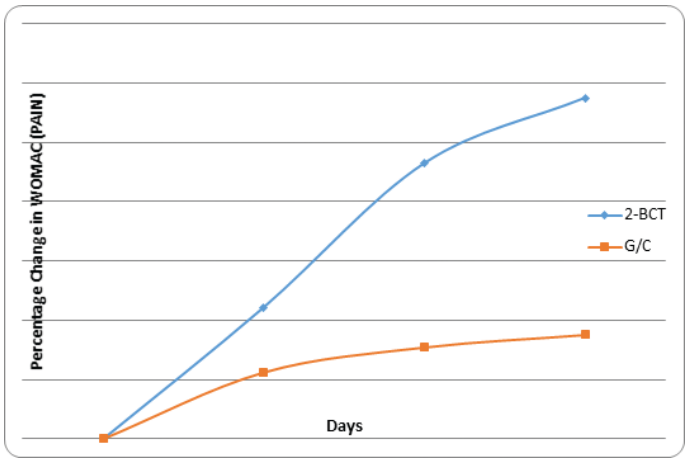
	Intervention Group (n = 34)		Positive Control Group (n = 33)	
	Mean ± SEM	**95% CI**	**Mean ± SEM**	**95% CI**
Pain Intensity				
Baseline	5.6 ± 0.3	5.0 to 6.3	6.1 ± 0.4	6.8 to 5.4
Δ 7 d	-1.6 ± 0.3 [a]	-0.9 to -2.1	-0.6 ± 0.2	0.0 to -0.7
Δ 30 d	-2.6 ± 0.3 [a]	-2.0 to -3.2	-0.9 ± 0.1	-0.0 to -0.6
Δ 90 d	-3.2 ± 0.3 [a]	-2.6 to -3.8	-1.1 ± 0.2 [a]	-0.2 to -1.0
Pain Frequency				
Baseline	4.8 ± 0.3	4.1 to 5.4	5.4 ± 0.4	4.7 to 6.1
Δ 7 d	-1.1 ± 0.3 [a]	-0.6 to -1.6	-0.5 ± 0.3	0.0 to -1.0
Δ 30 d	-2.4 ± 0.3 [a]	-1.8 to -3.2	-0.6 ± 0.3 [a]	-0.1 to -1.5
Δ 90 d	-2.7 ± 0.4 [a]	-2.0 to -3.4	-0.8 ± 0.4 [a]	-0.4 to -1.9

[a] $P < .05$.

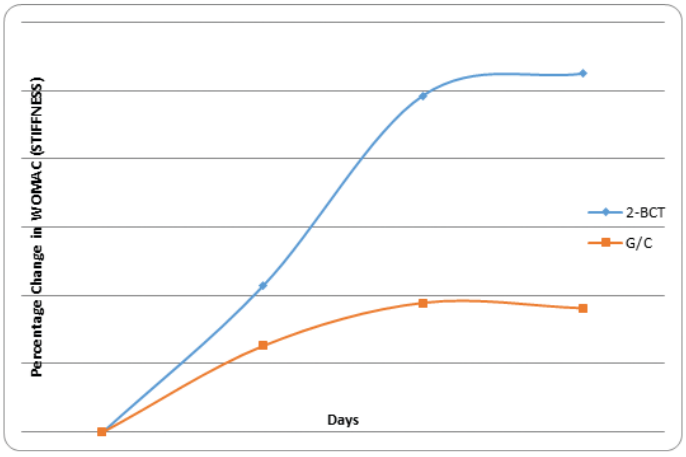
Abbreviations: SEM, standard error of the mean; CI, confidence interval; Δ, change.

Figure 1. Mean percentage change in pain (A), stiffness (B), and functionality (C) as measured by the WOMAC osteoarthritis index for participants given either orally administered 2BCT, the intervention; or G/C, the positive control.

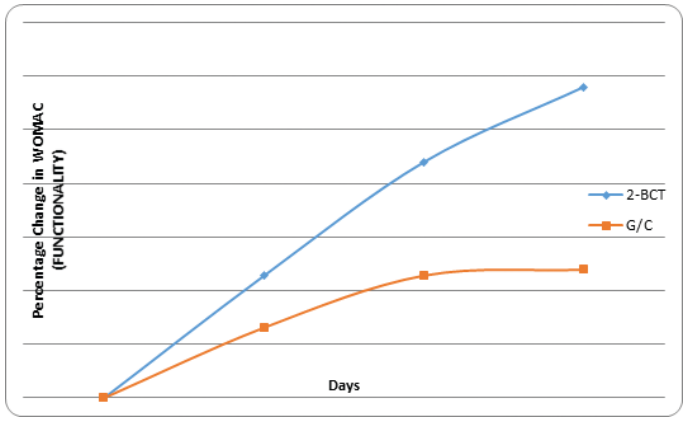
A.



B.



C.



Abbreviations: WOMAC, Western Ontario and McMaster Universities osteoarthritis index; 2BCT, 2-Beta Coxatene; G/C, glucosamine and chondroitin.

glucosamine and chondroitin reported improvements of 17.5%, 18.1%, and 23.7% for the same metrics.

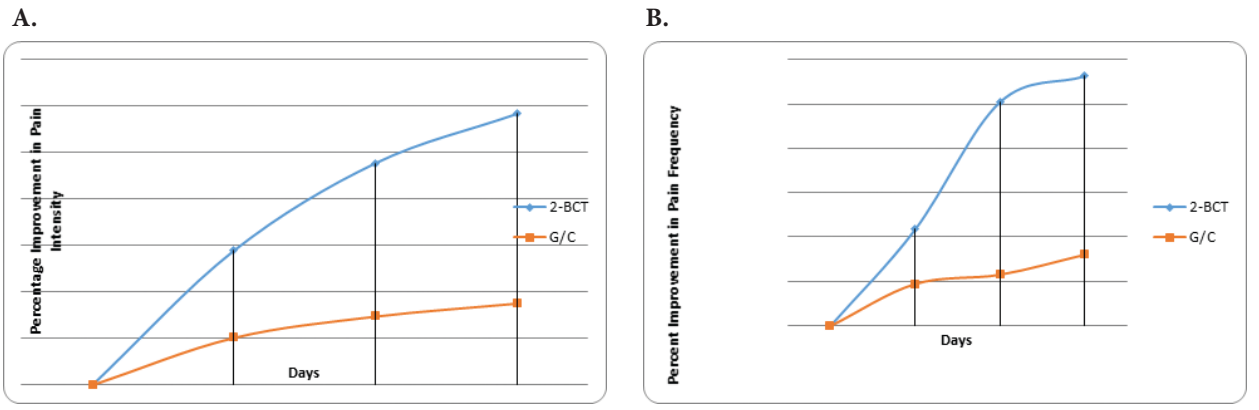
A summary of pain intensity and pain frequency using two 10-cm VASs is presented in Table 5. For the participants in the intervention group, the baseline mean VAS scores were 5.6 points—95% CI, 5.0 to 6.3, for pain intensity and 4.8 points—95% CI, 4.1 to 5.4, for the pain frequency. For the positive control group, the scores were 6.1 points—95% CI, 6.8 to 5.4, for pain intensity and 5.4 points, 95% CI, 4.7 to 6.1, for pain frequency. Although the mean baseline scores were higher for participants in the control group, that difference was not statistically significant.

At day 7, intervention participants showed a statistically significant decrease in pain intensity of 1.6 points (95% CI, −2.1 to −0.9) and in pain frequency of 1.1 points (95% CI, −1.6 to −0.6). At the same point, participants in the control group showed a decrease in pain intensity of 0.6 points (95% CI, −0.7 to 0.0) and of and pain frequency of 0.5 points (95% CI, −1.0 to 0.0) that were determined not to be statistically significant during the same period. Participants in both groups continued to report improvements in both pain intensity and pain frequency for the duration of the trial.

At day 90, participants in the control group reported a statistically significant decrease in pain intensity of 1.1 points (95% CI, −1.0 to -0.2). For pain frequency, that group showed a statistically significant decrease of 0.6 (95% CI −1.5 to −0.5) at day 30 and of 0.8 at day 90. However, at day 90, participants in the intervention group reported a greater decrease in mean pain intensity, 3.2 points (95% CI, −3.8 to −2.6); and in mean pain frequency, 2.7 (95% CI, −3.4 to −2.0).

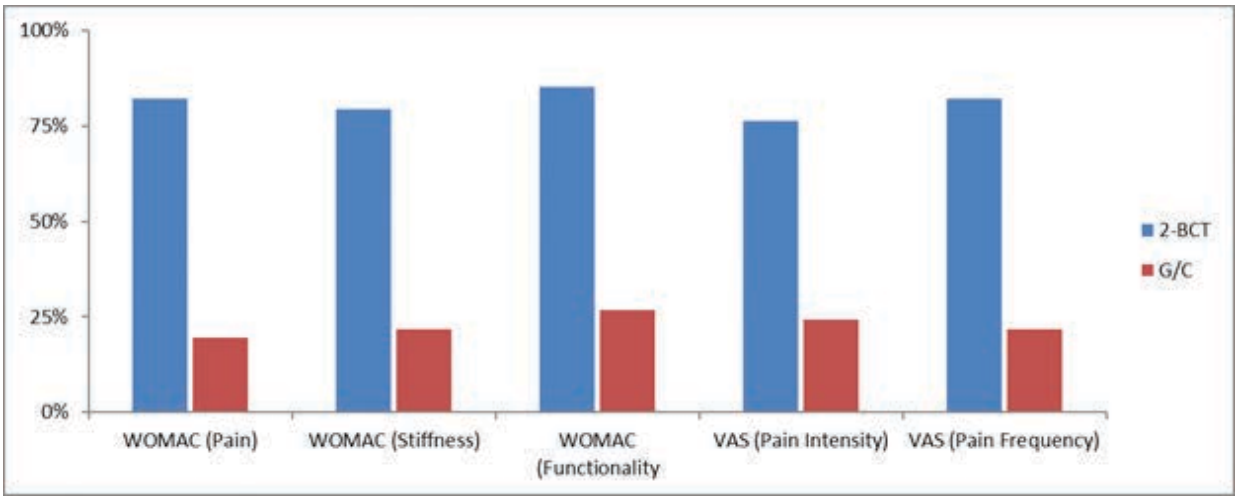
The mean percentage changes in the VAS scores from baseline confirmed the improvements in both groups with a greater change observed in the intervention group. By day 7, participants in the intervention group reported a change in VAS pain intensity and pain frequency at 28.6% and 22.9%, compared with an improvement of 9.8% and 9.3% for participants in the control group. By day 90, pain intensity and pain frequency decreased by 57.1% and 56.3% for participants given the intervention compared with a mean improvement from baseline of 18.0% and 14.8% for participants in the control group (Figure 2).

Figure 2. Mean percentage changes in pain intensity (A) and pain frequency (B) as measured by VAS for participants given either the orally administered intervention (2BCT) or G/C (the positive control).



Abbreviations: VAS, visual analog scale; 2BCT, 2-Beta Coxatene; G/C, glucosamine and chondroitin.

Figure 3. Percentage of participants in the intervention (2BCT) and positive control (G/C) groups who reported a significant improvement in the VAS, defined as percentage of responders >30% decrease in given metric from baseline to postintervention.



Abbreviations: 2BCT, 2-Beta Coxatene; G/C, glucosamine and chondroitin; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities osteoarthritis index.

Efficacy

Efficacy was calculated as a percentage of participants reporting a greater than 30% decrease from baseline. In the intervention group, 82.4%, 79.4%, and 85.3% of participants reported a statistically significant decrease after 90 days in pain, stiffness, and physical function, respectively, as measured on the WOMAC OA index. Only 19.5%, 21.9%, and 26.8% of participants given glucosamine and chondroitin reported significant changes in their WOMAC evaluations of pain, stiffness, and functionality, respectively, after 90 days.

Similar trends for efficacy were observed in the VAS analysis. Of the participants given the intervention, 76% and 82.4% reported significant changes in pain intensity and frequency, respectively, at the end of 90 days. In comparison, 24.3% and 21.9% of participants in the control group reported a significant decrease in pain intensity and frequency after 90 days (Figure 3).

In total, an average of 81.2% of participants give the intervention reported positive changes in joint comfort compared with only 22.9% of participants in the control group, when averaging the 90-day efficacy for the WOMAC and VAS indices together.

Discussion

In 2008, it was estimated that nearly 27 million people in the United States were affected by OA. For the last 7 years, that estimate has most likely increased due to a

shift in population demographics toward older individuals and an elevation in comorbidities such as obesity. Of the multiple therapies available for OA, which include lifestyle changes, NSAIDs, supplements, and surgical interventions, none in the past have provided the ability both to alleviate symptoms and to stimulate the synthesis of de novo cartilage, better known as osteoinduction.

Furthermore, clear limitations exist in those treatment modalities. Lifestyle changes provide only long-term symptomatic relief. The benefit of prolonged NSAID use needs to be weighed against the elevated risks of adverse reactions.[11-13] Supplement use has also been of questionable effectiveness. Finally, surgical intervention is not appropriate for all sufferers of OA due to comorbidities.

Alternative therapies are currently being investigated to address the shortcomings in current therapeutic approaches. The supplement in the current study, 2BCT presents a novel ingredient that can both alleviate the symptoms of OA and regrow bone and cartilage tissue. It has been well documented in the literature that the host of BMPs that are present in the Cyplexinol component are required for the maintenance of bone and joint tissue.

In an open-label preliminary study, the Cyplexinol was shown to have significant effects in OA of the hip, knee, and ankle in the subjective parameters of pain and joint function for 44 individuals.[48] That study also showed that the complex, when given orally, could increase the metrics tracking quality of life.[48]

In the current study, the osteoinductive capability of the Cyplexinol component was assessed through histological evaluation. The histological evidence clearly demonstrated that Cyplexinol initiates de novo bone growth in vivo. Those findings are further supported by both human case studies and animal models that have shown that Cyplexinol can increase tissue regeneration when administered orally.[49]

Its osteoinductive ability is coupled with an immunoprotective effect from the BMPs within the Cyplexinol component. Through activation of the SMAD and MAPK pathways, those BMPs can downregulate key markers of inflammation, specifically IL-1 and IL-6. It is the immunologic effect of Cyplexinol that accounts for 2BCT's rapid onset of action and joint comfort (ie, within 7 days). In addition, that mechanism of action helps promote long-term joint health through the downregulation of MMPs, which further degrade the joint as a part of the inflammatory response.

The second component of 2BCT is the enriched *B serrata* extract. *B serrata*, a potent natural ingredient for anti-inflammatory support, has been known and used by a variety of ancient cultures and continues to be used today. Modern science has identified the key components within the resin that provides the anti-inflammatory support. Of the 6 Boswellic acids present within the resin, AKBA has been identified as the most potent. In the past, scientists have had the ability to concentrate the AKBA to 20% or 30% for a standard enriched *B serrata*.

A high-potency enriched *B serrata*, concentrating the AKBA to 65%, has been developed and combined with the Cyplexinol by ZyCal to produce 2BCT. That enriched *B serrata* binds with the human X receptor, activating anti-inflammatory pathways and resulting in a rapid decrease in the inflammatory response.

The current study has evaluated the 2BCT in a clinical setting in people with moderate to severe joint discomfort. When the 2BCT was administered, participants reported a statistically significant decrease in both the WOMAC and VAS scores in as little as 7 days. Improvements continued for the duration of the current trial, with an average of 81.2% for the WOMAC OA index and the VAS combined, in participants reporting a significant response to the treatment, defined as a 30% or greater change in physometrics. When compared against glucosamine and chondroitin, the most common ingredient for joint conditioning, the 2BCT demonstrated both a faster and more effective response in participants. One rationale for that finding was the fact that the study's design did not limit participants to those having a diagnosis of OA. Although the majority of patients within the trial had a diagnosis of OA from the primary care setting, this did not account for every individual with in the trial. The robustness of response may show that 2BCT can be effective in many conditions that change the underlying joint architecture, not only for OA.

One limitation for the current clinical trial was population size. Including larger sample populations through additional studies is warranted, especially with increased treatment time to evaluate the long-term clinical outcomes and safety parameters further. Given the comparative nature of the current study, further comparisons between the active nutraceutical ingredients would benefit from a placebo control to anchor the study's findings. Because effect size can vary greatly with trials assessing pain and joint functionality, having those comparisons would further validate the use of 2BCT as a potent treatment in OA. However, although the current study did not have a placebo arm, many clinical trials are conducted comparing new agents against a known, control agent.

Earlier in the current year, chondroitin sulfate and glucosamine were compared against celecoxib.[19] That trial looked at 606 participants in a randomized, noninferiority study and found glucosamine and chondroitin (G/C) to be as effective as celecoxib. Given that the results linked that study's test agent with that of the current study, the evidence of the added benefit provided by 2BCT versus G/C is compelling still regardless of placebo control.

Conclusions

In the current study, the in vivo testing clearly demonstrated that the Cyplexinol component of the 2BCT stimulated de novo production of bone and cartilage tissue. When enhanced with AKBA as an oral supplement, decreases in pain intensity and frequency were evident in

the VAS scores. Significant improvements in the scores for pain, stiffness, and functionality were also found for the intervention group using the WOMAC OA index. Adverse event reports were similar between the positive control and the intervention groups. The current study has highlighted the effectiveness of the 2BCT and suggests a new therapeutic option for suffers of joint conditions, both those associated with and those not associated with OA.

Author Disclosure Statement

There were no conflicts of interest to disclose for the research. At the time of the study, the primary author was an unpaid independent consultant with her own separate private practice.

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