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The Efficacy and Safety of Beef Tallow Extract Including Cis-9-cetylmyristoleate in Patients with Osteoarthritis - As an Adjuvant Pharmacological Treatment -

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Abstract

In this double-blind, placebo-controlled trial, we assessed the efficacy and safety of beef tallow extract (BTE) including Cis-9-cetylmyristoleate in patients with arthritis. Between May and December 2003, we selected 80 patients (n=80) who showed/manifested arthritic symptoms and whose radiological findings were suggestive of arthritis, and randomly assigned them to placebo-controlled (n=40) and treatment group (n=40). The placebo (corn starch 350 mg) and BTE (208 mg) were orally administered to placebo-controlled and treatment group three times a day, respectively. We assessed the efficacy and safety based on the visual analogue scale (VAS) and modified knee society knee scores (MKSKS) at baseline and endpoint, respectively. To assess the safety, we monitored the adverse effects noted in liver, kidney, cardiovascular and gastrointestinal system for 3 weeks. Then, we performed not only a questionnaire study but also laboratory tests (e.g., liver function test, kidney function test, urinalysis, electrocardiography [EKG], complete blood cell counts [CBC] and chest X-ray). For statistical analysis, Student *t*-test and paired *t*-test were done using SPSS® Version 11.0. Statistical significance was set at $p < 0.05$. The scores between VAS and MKSKS showed statistical significance ($p < 0.05$) with an improvement of 69.2% (27/39) and 3.8% (21/39) of treatment-group patients, respectively. Abnormal laboratory findings were noted in neither placebo-controlled nor treatment group. In conclusion, our results indicate that the administration of BTE was a safe and effective treatment regimen for patients with arthritis. In addition, the efficacy of BTE was more remarkable in alleviating the symptoms rather than improving the function.

Key words: beef tallow extract, Cis-9-cetylmyristoleate, osteoarthritis

INTRODUCTION

In aging society, the prevalence of degenerative arthritis has been gradually increasing. Patients with osteoarthritis account for more than 80% of people aged 75 years or older. In more than 80% of those aged 50 years or older, the radiologic findings are suggestive of osteoarthritis. Accordingly, people have been increasingly burdened with the medical expenses for arthritis. Moreover, social issues have been raised regarding the treatment of patients with arthritis.

To date, the chemotherapy using non-steroidal anti-inflammatory drugs (NSAIDs) has been commonly regarded as a mainstream, non-surgical treatment regimen for patients with osteoarthritis. However, the long-term administration of NSAIDs revealed some serious adverse effects. Among these adverse effects, gastrointestinal complications are the most prevalent, in which the mortality of treatment group has been reported to be 4.2 times higher than that of non-treatment group (3,4,6). Besides, gastrointestinal complications are known as the 15th most common death causes. Other side effects have also been noted, which include neurologic adverse effects seen in 4~5%, aggravated hypertension, hypersensitivity reaction and acute myocardial infarction (2,5). Therefore, the administration of NSAIDs is no longer a safe treatment regimen.

Under the background described above, many studies have been conducted not only to reduce the adverse effects of conventional anti-inflammatory drugs but also to develop the new anti-arthritic drugs. In recent years, selective cyclooxygenase-2 (COX-2) inhibitor has been developed to reduce the adverse effects of NSAIDs. Al-

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though still commercially available, selective COX-2 inhibitor has not overcome the limitations of NSAIDs. Moreover, the long-term administration of selective COX-2 inhibitor poses the issues of cardiovascular safety. Furthermore, it remains unclear whether the hypertensive or renal effects of selective COX-2 inhibitor are milder than those of the conventional NSAIDs (1,5). On the other hand, ongoing studies have been conducted to develop the new anti-arthritic drugs or health supplements. Recent animal studies have shown that beef tallow extract (BTE) including Cis-9-cetylmyristoleate is effective in preventing and alleviating the symptoms of arthritis. Experimentally, Cis-9-cetylmyristoleate was first extracted from NIH Swiss albino mice which shows the resistance to arthritis (7). In a murine model of induced arthritis, Cis-9-cetylmyristoleate was effective in reducing the symptoms of arthritis compared to normal controls. Some types of health supplement including Cis-9-cetylmyristoleate have become commercially available, although no clinical trials have demonstrated their efficacy and safety.

We conducted this study to assess the efficacy and safety of BTE including Cis-9-cetylmyristoleate in alleviating the symptoms and improving the joint function in patients with arthritis.

MATERIALS AND METHODS

Method of preparation

In the preparation of Cis-9-cetylmyristoleate, beef tallow was extracted and then purified for edible use. Then, the preparation was reduced to a powder by both cooling and heating. The manufacturing procedure can be described as follows: Before processing, raw beef tallow was frozen in wax state. To remove the heat produced while beef tallow was being pulverize, a cooling jacket was installed in a pulverizer. After dry ice was added, the pulverization was initiated. The pulverized extract was frozen again and then placed with the excipients into a vertical, high-speed mixer. To make the pulverized extract coarse powder, the mixer was set to rotate fast within the shortest time. The excipients absorbed to raw beef tallow in a wax state, and make it into powder. The coarse extract was heated again to be liquefied and then placed with the excipients into a ribbon mixer. Then, after the low-speed mixing, the powder with large-sized particles was made. The powder was heated again and then placed with the excipients into a vertical high-speed mixer. This finally made BTE with small-sized particles. After BTE was grinded using a grinder and then passed through a filter, BTE with fine particles was obtained. Finally, these BTEs were encapsulated. Each encapsulated BTE (208 mg) contained 41.6 mg of Cis-9-cetylmyristoleate.

Clinical trial

Patients : In this trial, we reviewed the medical records of patients who visited us with a chief complaint of arthralgia between May and December 2003. Inclusion criteria were (1) cases in which the typical findings of osteoarthritis were noted on physical examination (e.g., joint space swelling, tenderness or decreased mobility of the joint); and (2) those in which the clear evidences of osteoarthritis were seen on plain radiography (e.g., joint space swelling, subchondral sclerosis or osteophyte formation). Exclusion criteria were (1) cases in which the symptoms were alleviated using the conventional NSAIDs; and (2) those in which the further investigation and treatment were indicated with the presence of serious abnormal findings on baseline physical examination and laboratory test. We finally selected 80 patients (n=80), and randomly assigned them to placebo-controlled (n=40) and treatment group (n=40). To monitor the clinical course, we performed an outpatient follow-up at a 1-month interval for 3 months.

Methods : Throughout the trial, all treatment regimens were double-blinded, i.e., both investigators and patients were blinded to study the treatment. Each patient was to take two capsules at a time, three times a day in total. The placebo was the encapsulated corn starch 350 mg. The test product was the encapsulated BTE 208 mg. Treatment group patients were to take two capsules of BTE at a time, three times a day in total. To monitor the clinical course, we performed an outpatient follow-up at a 1-month interval. During the 3-month period, we monitored the clinical course and then assessed its changes between baseline and endpoint. In the statistical analysis, we did not consider any cases in which the administration was discontinued with the occurrence of discomfort associated with the study medication or gastrointestinal disturbances.

We assessed the efficacy and safety based on the visual analogue scale (VAS) and modified knee society knee scores (MKSKS) at baseline and endpoint, respectively. Based on the VAS system, each patient was to express his or her symptomatic profile on a 10 cm-ruler denoting from asymptomatic=0 to the-most-severe=100 (8). Formerly designed to evaluate the joint function, the MKSKS system comprises four parameters including the range of motion, instability, pain and function (9). However, we considered two parameters only, the pain and function because we assumed that the short-term administration would not improve the range of motion and instability. The pain was assessed based on its cause and prevalence noted during ambulation, whose score was as follows: the absence of pain=50 points, the presence of pain only

during the upward and downward movement=40 points, the presence of pain during the ambulatory movement= 30 points, the frequent presence of pain=20 points, the persistent presence of pain=10 points and the presence of severe pain=0 point. The function was assessed based on the ambulatory distance and the use of ambulatory aid, whose score ranged between 0 and 50 points. A questionnaire study was done by the interview between investigator and patient.

For statistical analysis, Student *t*-test and paired *t*-test were done using SPSS® Version 11.0. Statistical significance was set at $p<0.05$.

To assess the safety, we monitored the adverse effects noted in liver, kidney, cardiovascular and gastrointestinal system. Then, we performed not only a questionnaire study but also laboratory tests (e.g., liver function test, kidney function test, urinalysis, electrocardiography [EKG], complete blood cell counts [CBC] and chest X-ray). During the 3-month period, we monitored the clinical course, and assessed its changes between baseline and endpoint.

RESULTS

In our series, the mean age was 60.2 years (range 46~77 years) and a male-to-female ratio was 5:75. Mean height was 158.6 cm and mean weight was 64.3 kg. There were no significant differences in the age, sex, height and weight between the two groups. Moreover, between the two groups, there were no significant differences in the mean values of VAS scores and MKSKS at baseline. During the trial, three patients (two from placebo-controlled and one from treatment group) who presented with gastrointestinal symptoms were excluded from the statistical analysis.

The VAS scores

In the placebo-controlled group, except for two cases of gastrointestinal disturbances, the mean value of VAS scores was 56.7 at baseline and 57.6 at endpoint. The VAS scores improved in 18.4% (7/38). Paired *t*-test showed that there were no significant difference in the VAS scores between at baseline and endpoint.

In the treatment group, except for one case, the mean value of VAS scores was 63.1 at baseline and 35.9 at endpoint. There were significant difference in the VAS scores between at baseline and endpoint ($p<0.05$). Moreover, the VAS scores improved in 69.2% (27/39) (Fig. 1).

The MKSKS scores

In the placebo-controlled group, except for two cases of gastrointestinal disturbances, the mean value of MKSKS scores was 104.2 at baseline and 105.9 at endpoint. The MKSKS scores improved in 18.4% (7/38). Paired *t*-test showed that there was no significant difference in the MKSKS scores between the baseline and endpoint.

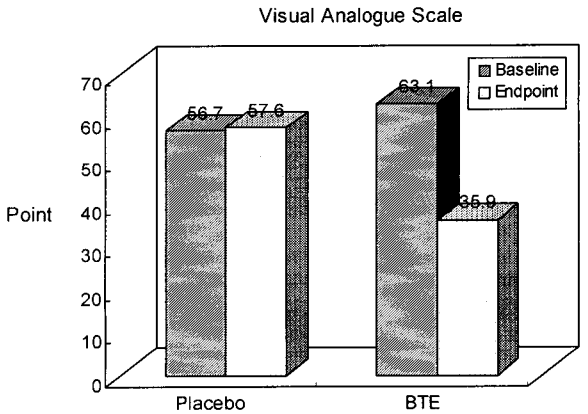

Fig. 1. The VAS Scores. In the treatment group, there were significant difference in the VAS scores between at baseline and endpoint ($p<0.05$).

In the treatment group, except for one case, the mean value of MKSKS scores was 102.8 at baseline and 113.3 at endpoint. Paired *t*-test showed that there were significant differences in the MKSKS scores between the baseline and endpoint ($p<0.05$). Moreover, the MKSKS scores improved in 53.8% (21/39) (Fig. 2).

Adverse effects

Patients whose laboratory test results were outside the normal limit were noted in neither placebo-controlled nor treatment group. At a 1-month follow-up, one case of heartburn was noted in placebo-controlled and treatment group each. In the placebo-controlled group, one case of abdominal distention was noted. The corresponding patient was dropped out of the present trial.

DISCUSSION

The pathophysiology of degenerative arthritis is complex,

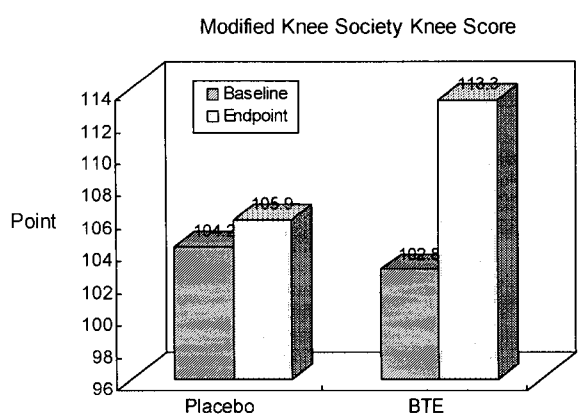

Fig. 2. The MKSKS Scores. In the treatment group, there were significant difference in the MKSKS scores between at baseline and endpoint ($p<0.05$).

in which physical, biochemical and genetic factors are involved (10). These factors render the most cases of degenerative arthritis characterized by the destruction of articular cartilage and the resulting presence of symptoms such as the pain, joint space swelling, articular deformity and decreased mobility of the joint.

From the perspective of physics, the permeability and elasticity of articular cartilage is decreased with aging. Presumably, this might be due to the overweight and excessive use of joint. According to recent biochemical studies, some factors are involved in deranging the equilibrium between anabolism and catabolism; among these factors, interleukin-1 (IL-1) and tumor necrosis factor (TNF) might be the key players. By stimulating the chondrocytes to secrete matrix metalloproteinases (MMPs), IL-1 not only prompts the destruction of cartilage matrix but also facilitates the synthesis of prostaglandin E_2 (PGE_2). Based on the concept that PGE_2 is closely related to the symptoms of arthritis, NSAIDs were used to inhibit the synthesis of prostaglandin, thus regarded as the mainstream, non-surgical treatment regimen for patients with arthritis. However, the conventional NSAIDs block the synthetic pathway of prostaglandin as well as thromboxane A_2. It is noteworthy that prostaglandin protects kidney and gastric mucosa and thromboxane A_2 is closely associated with the coagulatory activity of platelets. Therefore, the long-term administration of NSAIDs will trigger the adverse effects such as gastrointestinal (e.g., gastric ulcer), renal and thrombocytic disturbances (e.g., bleeding tendency) (1-6). To resolve these adverse effects, the development of new anti-arthritic agents is mandatory.

In 1994, Diehl and May first extracted Cis-9-cetylmyristoleate from NIH Swiss albino mice that showed a resistance to arthritis. In a model of induced arthritis, these authors demonstrated that Cis-9-cetylmyristoleate was effective in reducing the symptoms of arthritis (7). In the present trial, we used BTE extracted from raw beef tallow, and eventually noted that BTE including Cis-9-cetylmyristoleate was effective in alleviating the symptoms and improving the function in patients with arthritis. It deserves special attention that BTE was more effective in alleviating the symptoms rather than improving the function. The VAS score improved in 69.2% (27/39), but the MKSKS score did only in 53.8% (21/39) of treatment-group patients. These results might originate from the fact that degenerative arthritis is a chronic disease. In most cases, the skeletal deformity of joint substantially progressed with the presence of chronic arthritis; the movement was impaired; and the atrophy of peri-articular muscles was accompanied. These findings might contribute to our results that the improvement of function was unremarkable despite the clear evidence of pain alleviation.

The exact mechanism by which BTE including Cis-9-cetylmyristoleate showed a favorable effect on the symptoms of arthritis is not clearly understood yet. It can also be speculated that the efficacy of BTE originates from its innate anti-arthritic effect. Once produced *in vivo* during the hydrolysis, however, myristoleic acid will become pharmacologically effective (9). Despite the absence of clear evidences that myristoleic acid is effective for patients with arthritis, the anti-arthritic effect of oleic acid has been well documented (12). Furthermore, some studies have shown that linoleic acid was effective for patients with rheumatoid arthritis (13,14). Myristoleic acid could also be extracted from *Serenoa repens*, i.e., a sort of coconuts. Recent studies have disclosed that myristoleic acid induces the apoptosis of prostate tumor cells. Presumably, this might be due to the inhibition of 5-lipoxygenase (15). Produced in the biochemical pathway mediated by 5-lipoxygenase, the metabolites are involved in the inflammatory or allergic reactions (16,17). The anti-arthritic effect of cetyl myristoleate might be due to the inhibitory effect of myristoleic acid on the activity of 5-lipoxygenase.

In conclusion, our results indicate that the administration of BTE was a safe, effective treatment regimen for patients with arthritis. In addition, the efficacy of BTE was more remarkable in alleviating the symptoms rather than improving the function.

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