Arch Rheumatol 2021;36(x):296-301
doi: 10.46497/ArchRheumatol.2021.7719

ORIGINAL ARTICLE

A probiotic intervention on pain hypersensitivity and microbiota composition in patients with osteoarthritis pain: Study protocol for a randomized controlled trial

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ABSTRACT

Objectives: This study aims to examine the effects of probiotics on pain hypersensitivity at the end of a six-week intervention program in patients with osteoarthritis (OA)-related pain.

Patients and methods: This double-blind randomized controlled clinical trial with two parallel arms will be conducted between January 2021 and July 2022. At least 30 participants (age range, 50 to 90 years) of both sexes with a diagnosis of symptomatic hip or knee (Kellgren-Lawrence scale ≥3) will be recruited in each arm (total n=60) to achieve adequate statistical power in the analyses. The intervention will be administered for six weeks followed by a four-week follow-up period. The experimental group will receive a probiotic product plus the usual medical care. The control group will receive a probiotical sham plus the usual medical care. Assessment points will be measured at baseline, end of intervention, and one-month post-intervention. The outcomes of this intervention will be a change in visual analog scale pain and the gut microbiota composition. Group by time effects will be compared using mixed-model analysis of variance.

Conclusion: A reduction in pain hypersensitivity in patients with knee OA-related pain could suggest an involvement of microbiota, or part of it, in chronic pain state mechanisms.

Keywords: Microbiota, osteoarthritis, pain, probiotics.

Osteoarthritis (OA) is the most common form of chronic pain condition (34%) reported and entails a high economic and social burden for society.[1] OA-related pain is considered a complex integration of sensory and neural processes involving peripheral and central levels of the nervous system. Recent theories support that OA involves complex mechanisms of altered pain transmission.[2,3] Interestingly, emerging evidence leads to the hypothesis that alterations in the gut microbiome could also be considered as possible triggering factors in the onset of inflammatory arthropathies such as OA.[4]

Theories of the microbiota involvement in clinical manifestations of inflammatory diseases are becoming increasingly widespread. Therefore, microbiome could be a crucial factor in OA management, since it is considered an inflammatory condition.[5,6] The "brain-gut axis" may undergo an alteration due to stress and pain, which may develop a dysbiosis and increased rate in bacterial translocation in the intestine

Received: July 17, 2019 ***Accepted:*** January 15, 2020 ***Published online:*** January 27, 2021

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Citation:
Pedersini P, Savoldi M, Berjano P, Villafañe JH. A probiotic intervention on pain hypersensitivity and microbiota composition in patients with osteoarthritis pain: Study protocol for a randomized controlled trial. Arch Rheumatol 2021;36(x):296-301.

and inflammatory products.[7] Recent studies in pharmaco-microbiome show that gut microbiota may also affect the efficiency of the drug.[8]

The microbiota profile changes with aging and the loss of microbiota diversity and the alterations in the optimal composition and quantity of beneficial microbes are believed to increase the risk of many diseases. It has been hypothesized that patients with OA-related pain will demonstrate an alteration of the gut microbiota associated with pain intensity. It has been hypothesized that modifying the microbial environment by means of probiotics, for instance, may help to improve symptoms in patients with OA-related pain.[9] This hypothesis is supported by studies using animal models that found an improvement by reducing pain, inflammatory responses, and articular cartilage degradation in rats after probiotic supplementation.[10] Therefore, in this study, we aimed to examine the effects of probiotics on pain hypersensitivity at the end of a six-week intervention program in patients with OA-related pain.

PATIENTS AND METHODS

This double-blind randomized controlled trial will take place at IRCCS Fondazione Don Carlo Gnocchi between January 2021 and July 2022 and include 60 patients (age range, 50 to 90 years) of both sexes with a diagnosis of hip or knee OA. A physiatrist (physician) will establish the diagnosis of OA. Each subject will undergo subjective and physical examination performed by a physical therapist experienced in musculoskeletal physiotherapy to evaluate inclusion and exclusion criteria. Subjects who meet the following criteria will be included to ensure the precision of the results of this trial: all subjects will have hip or knee OA according to radiographic findings (Kellgren-Lawrence scale ≥3) and will require and be eligible for chronic, daily therapy with non-steroidal anti-inflammatory drugs (NSAIDs) to control OA signs and symptoms. Exclusion criteria were as follows: *(i)* inflammatory rheumatic conditions (i.e., spondyloarthritis and rheumatoid arthritis), *(ii)* psychiatric or neurological disorders, *(iii)* celiac disease, *(iv)* lactose intolerance, or *(v)* allergies or other ongoing illnesses (i.e., irritable bowel syndrome, diabetes, ulcerative colitis) or *(vi)* recent antibiotic treatment (i.e., <3 months before the beginning of the study); *(vii)* participants with known hypersensitivity to celecoxib, ibuprofen, naproxen, aspirin or esomeprazole; *(viii)* participants who smoked more than 10 cigarettes per day. Patients will also be excluded if they score more than 6 points on the Beck Depression Inventory or more than 30 points in the State Trait Anxiety Inventory[11] or if they suffer from dementia. Due to the high level of language skills required for questionnaires and quantitative sensory testing, non-Italian speakers will also be excluded from the trial. The study protocol will be approved by the IRCCS Fondazione Don Carlo Gnocchi (Italy) Ethics Committee for Clinical Trials. A written informed consent will be obtained from all subjects before enrollment. The study was registered at ClinicalTrials.gov under registration number NCT03968770 and conducted in accordance with the principles of the Declaration of Helsinki.

Those considered eligible for the study will be invited for a first interview, at which point the inclusion and exclusion criteria will be confirmed and medical history and physical examination will be completed. The present document was prepared according to the editorial format of medical publishing and Consolidated Standards of Reporting Trials publishing rules (Figure 1).[12]

The main outcome measures will be pain and gut microbiota composition. The outcome measure will be a change in pain intensity of hip or knee, which will be assessed with a visual analog scale (VAS; 0: no pain, 100: maximum pain). This will be used to assess two separate pain status levels: (a) average level of pain over the last 24 hours and (b) average level of pain over the last week.[13]

Pressure pain threshold (PPT) has been validated to evaluate knee or hip pain with excellent reliability both in healthy people and in knee or hip OA patients.[14] PPT will be assessed bilaterally in the center of the anterior aspect of the patella (knee),[15] the trochanter site (hip),[16] the C5-C6 zygapophyseal joint, and the tibialis anterior muscle by an assessor blinded to the subject's condition.[17]

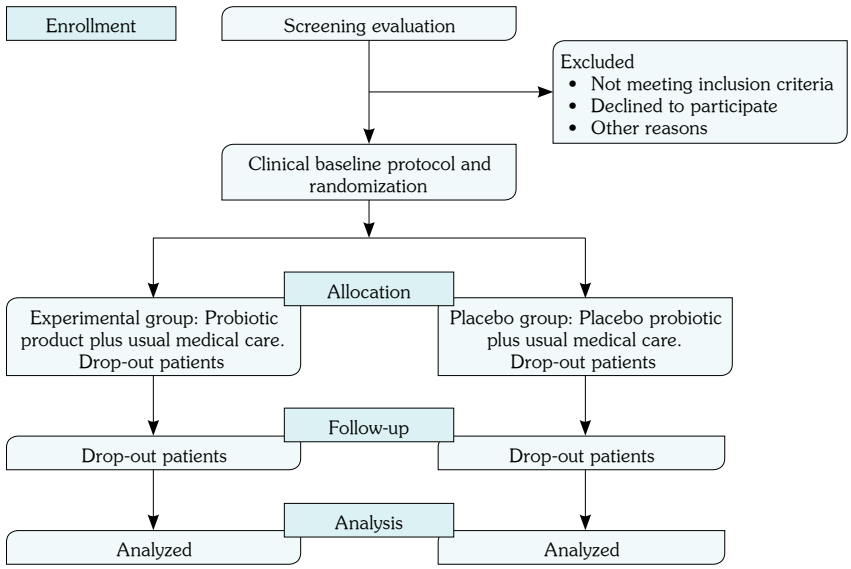

Figure 1. Standard protocol items: diagram for enrollment, allocation, and follow-up.

As part of secondary outcomes, lower limb function limitations for hip and knee (self-reported data will be assessed using the Western Ontario and McMaster Universities Osteoarthritis Index questionnaire[18] and the Knee injury and Osteoarthritis Outcome Score or Hip Disability and Osteoarthritis Outcome Score)[19,20] will be detected.

For inflammatory cytokines, fasted serum sample will be collected. Serum concentrations of interleukin (IL)-6, tumor necrosis factor-alpha, soluble IL-6 receptor, soluble IL-1 receptor, and C-reactive protein will be measured by enzyme-linked immunosorbent assays.[21] The detection procedure will be performed according to the manufacturer's instructions.

Microbiota composition will be identified through fecal samples for total genomic deoxyribonucleic acid (DNA) extraction, using the QIAamp DNA Stool Mini Kit (Qiagen Ltd., Crawley, West Sussex, UK), according to the manufacturer's instructions. Two polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis analyses will be performed to investigate total eubacteria and yeasts populations.[22] The bacteria belonging to *Clostridium (C.) sensu stricto (C. baratii, C. hystoliticum, C. butyricum, C. perfringens, C. botulinum,* and *C. tetani), Enterobacteriaceae, Escherichia coli, Bifidobacterium, Lactobacillus,* and yeast were dosed using quantitative PCR approach targeted on 16S ribosomal ribonucleic acid gene.

All outcomes will be measured at baseline, end of intervention, and one month postintervention.

Participants will be assigned to an experimental group (probiotics+usual medical care) or a control group (probiotical sham+usual medical care) by means of a stratified randomization method. The intervention will be administered for six weeks, followed by a four-week follow-up period for a total duration of 10 weeks.

The experimental group will receive 42 sachets of the commercial probiotic formulation of *Lactobacillus (L.) casei* (Probiotical S.p.A., Novara, Italy)[23] (one for each day). The amount of substance per kg body weight is as follows: *L. casei* (2×1010 cfu/kg, 500 mg/kg). The probiotic product will be kindly provided by Probiotical S.p.A., Novara, Italy.

The sham product will be administered for six weeks followed by a four-week follow-up period for a total duration of 10 weeks. The sham will be prepared with the same excipients without probiotic strains using an identical form of

package. The control group will receive 42 sachets of the sham product (one for each day).

Subjects in both groups will receive the same standardized usual daily care treatment for six weeks. This treatment consists of NSAID (diclofenac sodium)[24] use and the advice of a healthy lifestyle, as prescribed by their general practitioner (GP).[25]

A usual medical care consisting of education, lifestyle advice, exercise therapy, dietary therapy, and analgesics will be adopted. OA management will be tailored to the individual and include the following core intervention: self-management and OA education and weight control.[26] Subjects in both groups will receive the same standardized protocol exercise (aim: strength, aerobic, flexibility) by a physical therapist experienced in musculoskeletal physiotherapy. Every patient will complete a diet diary to elucidate the nutritional habits. In the diary, the caloric intake and the weight variations will be reported weekly. The researchers will not intervene in relation to drug prescription or lifestyle indications.

Statistical analysis

Data will be analyzed using the IBM SPSS version 25.0 (IBM Corp., Armonk, NY, USA), conducted following an intention-to-treat analysis using the last value forward method. After the completion of all baseline measurements, using a computer program (http://www.graphpad.com/quickcalcs/randomize1.cfm), subjects will be randomly assigned by an external assistant into either the experimental or the control group. Group data will be summarized using means and standard deviations. Baseline characteristics of the groups will be compared. For the outcomes of the study, a two-by-four mixed-model analysis of variance will be used to examine the effects of treatment on each outcome, with group (experimental and control) as the between-subject variable and time (baseline, postintervention, and one-month and three-month postintervention) as the within-subject variable. The sample size and power calculations will be performed with the ENE 3.0 software (GlaxoSmithKline©, Universidad Autónoma, Barcelona, Spain). In a previous study, patients with OA improved on average by 3.3 points in the VAS (range, 1 to 10) at four weeks after treatment.[17] To our knowledge, no previous data regarding change in VAS after use of probiotics are available. A score improvement of 2 cm (VAS) corresponds to minimal clinically important difference (i.e., from severe to moderate, moderate to mild, or mild to no painful). With 80% power, 5% significance level, two-sided tests, and mean changes (baseline to two months) in the VAS score of 2.0 in the experimental group (standard deviation of 0.4) and <2.0 in the control group, a sample of 25 patients per group will be needed. To account for possible dropouts, we plan to recruit 60 patients.

DISCUSSION

This protocol was designed to outline the design of a randomized clinical trial to test the probiotic treatment and determine the microbiota composition in subjects with OA-related pain. We hypothesize that patients with OA-related pain will demonstrate an alteration of the gut microbiota associated with the change in VAS pain. Most factors such as sedentary life, use of medicines, smoking, stress, travelling, incorrect dietary habits (Western diet), and life-sharing conditions are associated with drastic changes in the intestinal microbiota. For these reasons, the association between OA and gut microbiota is emerging as one of the considerable factors in the disease.[27]

The characterization and understanding of gut microbiota have recently increased and represent a wide research field. Gut microbiota is the major source of microbes which might exert beneficial as well as pathogenic effects on human health. The role of intestinal microbiome as a mediator of inflammation has only recently emerged. We expect to find, as previously in other populations,[22,23] that the intestinal microbiota will be strictly associated with this pain population. Reliable results will allow the professional to reach conclusions that have been minimally affected by external factors, thereby reducing the chances of error. This study protocol will open avenues for future research in the modulation of pain pathways, perhaps offering targets to optimize gut microbiota involvement in the pathophysiology for pain management in OA.

Osteoarthritis management is considered a multimodal process involving education, lifestyle advice, exercise therapy, dietary therapy, and

analgesics. Education regarding the disease is the basis for chronic pain self-management. Recent evidence suggests that a combination of education and supervised exercise on physical activity resulted in decreased pain, increased quality of life, and increased self-efficacy.[28,29] Appropriate exercise prescription has the greatest impact on the burden of OA, which reduces the risk of progression and negative consequences of the disease.[30] Current exercise programs consist of strength, aerobic, and flexibility sessions.[31] Dietary therapy is considered in the treatment of inflammatory rheumatic disease because it acts in reducing inflammation via intake of anti-inflammatory and pro-inflammatory components, reducing risk of co-morbidities (e.g. osteoporosis, hypercholesterolemia), controlling body weight via balancing intake with needs, and improving overall well being and quality of life.[32,33] Although several studies in different medical fields have tried to find treatments targeted to OA-related pain, there are still discrepancies between the symptoms of OA-related pain and the presence of the disease (radiographic findings).

Although this is a double-blind randomized sham-controlled study, it presents some limitations. The health responses related to gut microbiota show considerable individual variation, affected by absorption, metabolism, and genetic variations of subjects. A second limitation is that patients are often discharged before they complete the six-week course of therapy. The final limitation is that each individual is allowed to perform the OA self-management.

In conclusion, OA and gut dysbiosis share numerous triggering risk factors such as aging, sex, obesity, and nutrition.[23] This is the first randomized controlled trial to compare the effects of probiotics on pain hypersensitivity and microbiota composition in patients with OA-related pain. The results of this study can be implemented in clinical practice to help clinicians deal with this challenging patient population. Probiotics combined with conservative treatment is a cost-effective intervention that may improve OA-related pain as well as patients' quality of life and function. In the future, it is foreseeable that the gut microbiome profile may be used as a tool to predict performance and detect potential disorders. Moreover, manipulation of the gut microbiome could be a potentially novel intervention to tackle or prevent OA-related pain. However, this field needs to be further investigated. The present study is expected to provide evidence for the effects of probiotic products on well-established OA risk markers as well as on the human gut microbiota in patients with OA-related pain.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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