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SHORT COMMUNICATION

Involvement of BDNF in knee osteoarthritis: the relationship with inflammation and clinical parameters

Adriano Prado Simão · Vanessa Amaral Mendonça · Tássio Málber de Oliveira Almeida · Sérgio Antunes Santos · Wellington Fabiano Gomes · Candido Celso Coimbra · Ana Cristina Rodrigues Lacerda

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Abstract The aim of this study was to analyze the levels of brain-derived neurotrophic factor (BDNF) in both the plasma and synovial fluid of patients with primary knee osteoarthritis compared with control individuals and to investigate the relationship between BDNF levels and self-reported pain. Twenty-seven patients with knee osteoarthritis (OA) and 19 healthy subjects were enrolled in the study. Anteroposterior knee radiographs were taken to determine the disease severity of the affected knee. Radiographic grading of OA in the knee was performed using the Kellgren–Lawrence criteria. The BDNF levels in the plasma and synovial fluid were measured by enzyme-linked immunosorbent assay. The mean plasma BDNF levels of the knee OA patients were significantly higher than that of the healthy controls (2,378 ± 1,067.2 vs. 1,756 ± 804.3 pg/mL, $p < 0.05$). BDNF levels in the synovial fluid of OA patients (358.9 ± 178.4 pg/mL) were sixfold lower than in corresponding blood samples ($p < 0.0001$) and fourfold lower than in the plasma of healthy controls ($p < 0.0001$). Subsequent analyses showed that the plasma BDNF levels significantly correlated with self-reported pain (Western Ontario and McMaster Universities Osteoarthritis Index) ($r_s = 0.39$, $p = 0.04$). Furthermore, no correlation was found between the plasma and synovial fluid BDNF concentrations and knee OA severity. The findings of this study suggest that systemic BDNF levels are most likely associated with the mechanism of joint pain in knee OA in the acute stage of joint inflammatory process. Further studies are necessary to address the functional role of BDNF in the modulation of pain to establish new therapeutic implications.

Keywords Brain-derived neurotrophic factor · Knee · Osteoarthritis · Synovial fluid

A. P. Simão · V. A. Mendonça · T. M. de Oliveira Almeida · W. F. Gomes · A. C. R. Lacerda
Integrated Center for Graduate Studies and Research Health (CIPq-Health), Federal University of Jequitinhonha and Mucuri Valleys (UFVJM), Diamantina, Minas Gerais, Brazil

A. P. Simão (✉)
Nursing School, Physiotherapy Course, Federal University of Alfenas (UNIFAL), Av. Jovino Fernandes Sales, 2600, Alfenas, Minas Gerais 37130-000, Brazil
e-mail: adrianopsimao@ig.com.br

S. A. Santos
Hospital Nossa Senhora da Saúde, Diamantina, Minas Gerais, Brazil

C. C. Coimbra
Institute of Biological Sciences (ICB), Federal University of Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil

Introduction

Osteoarthritis (OA) is a chronic progressive degenerative joint disease that involves not only articular cartilage but also synovium, subchondral bone, and the surrounding muscles and ligaments. OA is characterized by cartilage destruction, subchondral bone sclerosis, and osteophyte or cyst formation. Knee osteoarthritis is a major cause of severe pain, joint stiffness, limited motion, and disability [1, 2]. Previous studies have suggested that neurotrophins are involved in arthritic processes [3–6]. The neurotrophin brain-derived neurotrophic factor (BDNF) is reported to be involved in the joint inflammatory process, and its production is increased in response to pro-inflammatory cytokines [4].

The role of neurotrophins as important factors in arthritis has been discussed previously [4, 7]. High BDNF mRNA

expression levels as well as the expression of the receptors TrkB and p75NTR have been detected in the synovial fluid cells of OA, rheumatoid arthritis, and spondyloarthritis patients [8]. Furthermore, positive immunostaining for BDNF, TrkB, and p75NTR in synovial tissue sections of OA and RA patients has been reported [3]. TrkB was detected in nerve fibers in synovial tissue sections of knee OA and RA patients [3]. In addition, TrkB expression has been found in articular chondrocytes and inflammatory infiltrates in the knee joints of local injection-induced mice [4]. Previous studies have reported increased amounts of BDNF immunostaining in fibroblasts and macrophages from the synovial tissue of OA and RA patients compared with healthy controls [9]. Evidence that synovial fibroblasts actively secrete BDNF when exposed to increased levels of ATP, a signal of inflamed and damaged tissue, was a novel discovery [6]. BDNF is known as a crucial neuromodulator involved in nociceptive hypersensitivity in the central nervous system [6, 10], and BDNF levels are modified in some persistent pain states and during inflammation [11, 12]. Our group has previously investigated the plasma BDNF levels in elderly individuals with knee osteoarthritis and found a positive effect of aerobic exercise training on plasma BDNF levels and clinical parameters such as pain perception [13]. Moreover, the addition of whole body vibration to squat exercise training also increased BDNF plasma levels and improved lower limb muscle performance in elderly women with knee OA [unpublished data].

In this study, we postulated that increased BDNF levels in the plasma and synovial fluid might be associated with pain perception in knee OA patients. Therefore, the objective of this study was to analyze the concentrations of BDNF in both the plasma and synovial fluid of patients with primary knee osteoarthritis and compare the results with those of the control individuals, as well as to investigate the relationship between BDNF levels and self-reported pain.

Methods

Subjects

We enrolled 27 patients (17 females and 10 males) who met the American College of Rheumatology criteria for knee osteoarthritis (OA group) and 19 healthy individuals (11 females and 8 males) with no radiographic hip OA or knee OA, as indicated by Kellgren and Lawrence (KL) [14] grades of 0 for both hips and both knees (control group). OA and control groups were matched by age, sex, and BMI. Participants were excluded on the basis of having arthropathy due to gout, pseudogout, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, previous knee injury or previous joint infection, histories of corticosteroid medication, cancer. Patients with any systemic inflammatory or autoimmune disorders or any type of malignant or chronic illnesses were not included in this study. Ethical approval of this study was obtained from the Internal Review Board of the University Federal of Jequitinhonha and Mucuri Valleys (#029/11). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was received from the patients and healthy volunteers prior to their participation in the study.

Procedures

Initially, the volunteers received a detailed explanation about the objectives and procedures of the study. Then, the volunteers signed a consent form, and their demographic data as well as self-reported levels of pain, stiffness, and physical function were collected. Patients in the OA group were subjected to synovial fluid aspiration from the affected knee using sterile knee puncture by experienced orthopedic physicians. Immediately after collection of synovial fluid, blood sample was collected from the patients in the OA group, centrifuged to remove cells and debris, and stored at −80 °C until used. No synovial fluid was extracted from the controls due to ethical concerns, only venous blood samples were collected from the controls and were centrifuged and stored at −80 °C until used.

Radiographic evaluation

To ensure that the participants had knee OA and to reliably standardize the samples, radiological evaluations were performed on all volunteers. The severity of the disease was determined using weight-bearing anteroposterior radiographs of the affected knee. Knee radiographs were evaluated according to the KL classification [14]: grade 1, doubtful narrowing of the joint space and possible osteophytic lipping; grade 2, definite osteophytes and possible narrowing of the joint space; grade 3, moderate multiple osteophytes, definite narrowing of the joint space, some sclerosis, and possible deformity of the bone contour; grade 4, large osteophytes, marked narrowing of the joint space, severe sclerosis, and definite deformity of the bone contour. A grade 2 classification (definite osteophytes and possible narrowing of the joint space) was used as the cutoff to determine knee OA. The controls were defined as having no radiographic evidence of hip or knee OA, as indicated by KL grades of 0 for both hips and both knees. The grading of the more severely affected knee in each patient was used for data analysis.

Clinical and functional parameters

Self-reported levels of pain, stiffness, and physical function were assessed with the 24-item questionnaire from the

Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [15], which is a valid and reliable instrument for OA. This questionnaire was translated and adapted to Portuguese [16]. The WOMAC questionnaire aims to measure health status and assess a patient's self-perception of pain (5 questions), joint stiffness (2 questions), and functional performance (17 questions) during the previous 72 h. The WOMAC score was presented to patients on a Likert-type scale, where questions received a score of 0, 25, 50, 75, or 100 for no pain, a little pain, moderate pain, intense pain, and very intense pain, respectively [15]. A higher score on the WOMAC scale represents poorer function or greater pain.

Laboratory analyses

Double-blinded quantitative measurements of plasma and synovial fluid BDNF levels were performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine, R&D Systems, Minneapolis, MN, USA) following the protocol provided by the manufacturer. The detection limit for this assay was 5 pg/mL.

Statistical analyses

Demographic data of volunteer patients and controls were compared using unpaired Student's t tests where appropriate. Comparisons between the groups were performed using one-way analysis of variance (ANOVA) with Tukey's post hoc test if ANOVA showed significance. Correlations between plasma and synovial fluid BDNF levels and disease severity were assessed using Spearman's correlation coefficient (r_s). Data were expressed as the mean ± SE of the mean. p values <0.05 were considered statistically significant for differences and correlations.

Results

Twenty-seven knee OA patients, aged 56–88 years, and 19 healthy individuals, aged 59–86 years, were recruited for this study. There were no statistically significant differences between the groups for any parameters assessed (Table 1).

As shown in Fig. 1, plasma BDNF levels were lower in healthy participants than in OA patients [1,756 ± 804.3 pg/mL (95 % CI 1,369–2,144) vs. 2,378 ± 1,067.2 pg/mL (95 % CI 1,956–2,800), $p = 0.03$]. BDNF levels in the synovial fluid of the OA patients [358.9 ± 178.4 pg/mL (95 % CI 289.7–428.1)] were sixfold lower than in the corresponding blood samples ($p < 0.0001$) and fourfold lower than in the plasma of the healthy controls ($p < 0.0001$).

There was a moderate positive correlation between BDNF plasma concentration and self-reported pain levels (WOMAC) ($r_s = 0.39$, $p = 0.04$) (Fig. 2). However, there were no correlation between BDNF plasma concentration and others WOMAC domains (joint stiffness and physical function). The results of the clinical and functional parameters evaluated for WOMAC were: pain self-reported [199.1 ± 129.6 (95 % CI 147.8–250.4)], joint stiffness [80.56 ± 55.18 (95 % CI 58.73–102.4)], and physical function [666 ± 404.7 (95 % CI 502–822.1)].

With regard to the radiological KL classification, patients were categorized into two groups for OA grading. Fifteen patients were classified as grade 2, and 12 patients were classified as grade 3. In addition, the associations between plasma and synovial fluid BDNF levels and osteoarthritis disease severity were investigated. No correlation was found between plasma and synovial fluid BDNF concentrations and knee OA severity ($r_s = 0.007$, $p = 0.97$ and $r_s = -0.20$, $p = 0.31$, respectively). In addition, there was no difference between grade 2 and grade 3 patients regarding plasma [2,177 ± 322.4 pg/mL (95 % CI 1,709–3,092) vs. 2,242 ± 422.4 pg/mL (95 % CI 1,748–3,607), respectively] and synovial fluid BDNF [331 ± 49.67 pg/mL (95 % CI 308.8–521.8) vs. 237.4 ± 41.72 pg/mL (95 % CI 209.6–393.2), respectively].

Discussion

Previous studies have demonstrated that BDNF is present in the synovial fluid of inflamed joints in patients with spondyloarthritis [5], rheumatoid arthritis [5, 6], and OA [5, 6]. However, gaps remain in the literature with regard

Table 1 Sample characterization of the OA group and control group

Characteristics	OA group ($N = 27$)	Control group ($N = 19$)	Size effect	t value (95 % CI)	p value
Age (years)	68 (9)	72 (8)	−0.47	1.88 (−9.60 to 0.33)	0.06
Body mass (kg)	70 (10.5)	70 (12.3)	0.0	0.15 (−7.73 to 6.65)	0.87
Height (meters)	1.65 (0.08)	1.64 (0.09)	0.12	0.55 (−0.04 to 0.07)	0.58
BMI (kg/m^2)	25.62 (3.01)	26.15 (3.61)	−0.16	0.52 (−2.60 to 1.54)	0.60
Severity of knee OA*	2 3				
(%)	56 44		–	–	–

Data presented as means (SD)

OA osteoarthritis, *BMI* body mass index, *95 % CI* 95 % confidence interval

* p value ≤0.05

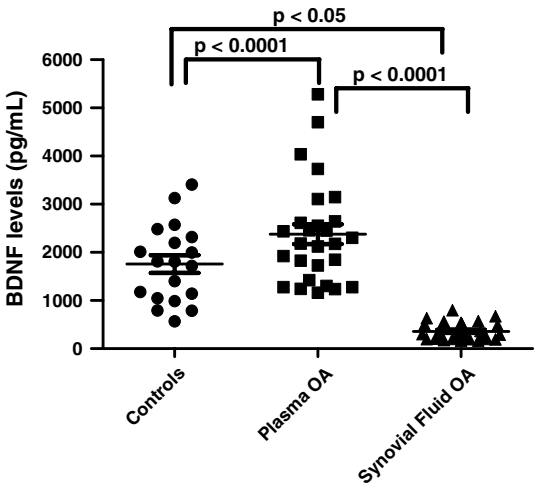

Fig. 1 BDNF levels in plasma and synovial fluid in patients with OA and healthy controls

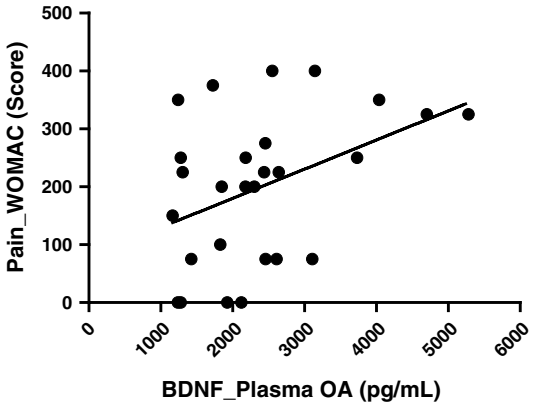

Fig. 2 Positive correlation between BDNF plasma levels in patients with knee osteoarthritis and pain self-related (WOMAC) ($r_s = 0.39$, $p = 0.04$)

to the differences in BDNF levels in the plasma and synovial fluid of elderly patients with knee OA compared with that of healthy controls. Furthermore, it remains unclear whether there is any relationship between BDNF levels and clinical parameters such as self-reported pain. This study is the first to demonstrate the presence of BDNF in both the plasma and synovial fluid of all patients with primary knee OA. The BDNF levels in the synovial fluid of OA patients were sixfold lower than in the corresponding blood samples and fourfold lower than in the plasma of healthy controls. Moreover, there was a positive correlation between BDNF levels and self-reported pain.

Although patients with knee osteoarthritis had markedly higher plasma BDNF levels than synovial fluid BDNF levels, there was no correlation between these concentrations. The study of Rihl et al. [5] also showed no significant correlation between BDNF levels in the serum and synovial fluid in patients with OA and spondylosis. These authors commented that the lack of correlation may be related to the fact that the BDNF found in the synovial fluid is produced locally by chondrocytes, with autocrine and paracrine roles in the joint cartilage, in response to the joint inflammation process [6, 17].

Another finding of this study was that the plasma concentration of BDNF in healthy elderly patients was lower than in elderly patients with knee OA. This finding is contrary to the study of Rihl et al. [5], which found a higher plasma concentration of BDNF in healthy subjects compared with patients with inflammatory arthritis and synovitis. Nevertheless, comparison of knee OA and inflammatory arthritis is difficult, and the results are not expected to be comparable once those authors did not provide adequate data for interpretation, i.e., subject's characteristics especially to healthy group. Given this, other factors may affect the serum BDNF levels, and it is unknown if all experimental and healthy groups were matched by age, sex, and BMI.

The BDNF levels in the plasma correlated positively with self-reported pain in patients with knee OA. This finding is supported by the literature, as Grimsholm et al. [3] found that the expression of BDNF in rheumatoid arthritis and OA patients has not only neuroprotective but also pain-mediating effects.

This study had some inherent shortcomings. First, the investigation was a single-center trial with a relatively small number of participants. Given this, factors such as synovitis level, associated factors of knee OA, characteristics of the study patients (age, weight, previous medications), as well as characteristics of the controls could have influenced the results of the present study. Additional studies conducted on random sets of patients from multiple centers with larger sample sizes are warranted to validate our results. Second, BDNF levels were only measured in the plasma and synovial fluid. Further studies analyzing BDNF expression in local tissues, in relation to the synovial and circulating BDNF levels, could provide a more valuable insight into the pathogenic role of BDNF in OA. Third, for ethical reasons, synovial fluid samples from healthy controls were not taken. Fourth, patients with knee OA were sampled in the acute articular inflammation stage. Our conclusions thus cannot be extended to patients in the remission stage of OA. Last, as this was a cross-sectional study, determining definite cause-and-effect relationships might not be possible. Prospective longitudinal investigations are needed to demonstrate disease progression and determine the exact role of BDNF in knee OA.

Conclusion

In conclusion, this study revealed that plasma BDNF was significantly increased in patients with knee OA compared

with healthy controls and that the BDNF concentration in the synovial fluid was remarkably lower than that in the paired plasma samples. Furthermore, synovial fluid BDNF levels were greatly reduced compared with the paired plasma BDNF levels. However, patients in this study were in the acute stage of joint inflammatory process; so, results in this study cannot be extended to patients in the remission stage of OA.

This study also investigated the relationship between plasma and synovial fluid BDNF levels and clinical parameters. A positive correlation was found between plasma BDNF levels and self-reported pain, suggesting a role of neurotrophins in the pain-mediating effects of knee OA patients in the acute stage of joint inflammatory process. Further studies are necessary to address the functional role of BDNF in the modulation of pain to establish new therapeutic implications.

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Conflict of interest The authors declare that they have no conflicts of interest.

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