Atherosclerosis 274 (2018) 77–85

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Vascular brain-derived neurotrophic factor pathway in rats with adjuvant-induced arthritis: Effect of anti-rheumatic drugs

Martin Pedard [a, b], Aurore Quirié [a], Perle Totoson [c], Frank Verhoeven [c], Philippe Garnier [a], Anne Tessier [a], Céline Demougeot [c], Christine Marie [a, *]

[a] INSERM UMR1093-CAPS, Université Bourgogne Franche-Comté, UFR des Sciences de Santé, F-21000, Dijon, France
[b] Service de Neurologie, CHRU, Dijon, France
[c] EA4267 PEPITE, FHU INCREASE, Univ. Bourgogne Franche-Comté, F-25030, Besançon, France

ARTICLE INFO

Article history:
Received 27 November 2017
Received in revised form
11 April 2018
Accepted 1 May 2018
Available online 2 May 2018

Keywords:
Vascular BDNF
Adjuvant-induced arthritis
Anti-rheumatic drugs

ABSTRACT

Background and aims: In rheumatoid arthritis, the control of both disease activity and standard cardiovascular (CV) risk factors is expected to attenuate the increased CV risk. Evidence that brain-derived neurotrophic factor (BDNF) plays a role in vascular biology led us to investigate the vascular BDNF pathway in arthritis rats as well as the interaction between endothelial nitric oxide (NO) and BDNF production.

Methods: The aortic BDNF pathway was studied in rats with adjuvant-induced arthritis, (AIA) using Western blot and immunohistochemical analysis. Control of arthritis score was achieved by administration (for 3 weeks) of an equipotent dosage of etanercept, prednisolone, methotrexate, celecoxib or diclofenac. Aortas were exposed to an NO donor or an NO synthase inhibitor and vasoreactivity experiments were performed using LM22A-4 as a TrkB agonist.

Results: Vascular BDNF and full length tropomyosin-related kinase B receptor (TrkB-FL) were higher in AIA than in control rats. These changes coincided with decreased endothelial immunoreactivity in BDNF and $pTrkB^{tyr816}$ and were disconnected from arthritis score. Among anti-rheumatic drugs, only prednisolone and methotrexate prevented AIA-induced vascular BDNF loss. The effect of AIA on aortic BDNF levels was reversed by an NO donor and reproduced by an NOS inhibitor. Finally, LM22A-4 induced both NO-dependent vasodilation and phosphorylation of endothelial NO synthase at serine 1177.

Conclusions: Our study identified changes in the BDNF/TrkB pathway as a disease activity-independent component of AIA-associated changes in endothelial phenotype. It provides new perspectives in the understanding and management of the high CV risk reported in rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a frequent autoimmune chronic inflammatory joint disease. Beyond the destruction of joint cartilage and the resulting pain, disability and decreased quality of life of patients, RA is associated with high cardiovascular (CV) risk. The relative increase in the CV risk has been related to an acceleration of atherosclerosis [1,2] consecutive to endothelial dysfunction (ED). However, the mechanisms involved in ED remain uncertain. They may comprise a higher prevalence of standard CV risk factors, in addition to interactions between systemic inflammation and genetic and immune factors [3]. Accordingly, the EULAR's recommendations for the management of CV risk in RA include the optimal control of disease activity and standard CV factors by lifestyle changes [4].

The role of brain BDNF in neuroplasticity and neurogenesis through activation of its cognate full length (FL) tropomyosin-related kinase B receptors (TrkB) is well documented [5–7]. However, there is an increasing interest for the BDNF/TrkB pathway in vessels. BDNF and TrkB are expressed by the vascular wall [8,9]. In addition, there is some evidence that endothelium-derived BDNF binds to TrkB-FL expressed by endothelial cells. First, cultured endothelial cells secrete bioactive BDNF [10]. Second, the exposure of vessels to exogenous BDNF was reported to change vascular biology/function [10–13]. Third, endothelial TrkB receptors were

* Corresponding author. INSERM U1093, FR des Sciences de Santé, 7 boulevard Jeanne d'Arc, 21000, Dijon, France.
E-mail address: chmarie@u-bourgogne.fr (C. Marie).

https://doi.org/10.1016/j.atherosclerosis.2018.05.004
0021-9150/

recently reported to confer atheroprotection and play a protective role in the development of coronary artery diseases [9]. Finally, endothelial nitric oxide (NO) might stimulate endothelial BDNF synthesis [14] while cultured endothelial cells were reported to overproduce NO in response to exogenous BDNF [11]. Collectively, these data on vascular BDNF/TrkB support the exciting hypothesis that the increased CV risk in RA patients might be connected to changes in the endothelial BDNF/TrkB pathway.

The objective of the present study was to explore the effect of RA on the vascular BDNF/TrkB pathway and the link between vascular BDNF levels and disease activity and endothelial nitric oxide (NO) production. Experiments were performed on aortas collected in rats subjected to adjuvant-induced arthritis (AIA) at a time at which inflammatory symptoms and macrovascular function endothelial are fully developed. An efficient control of disease activity was achieved by administration (for 3 weeks from the onset of inflammatory symptoms) of diclofenac, celecoxib, prednisolone, methotrexate or etanercept. The connection between NO and BDNF production was assessed from *ex vivo* experiments on aorta.

2. Materials and methods

2.1. Animals

Experiments were carried out on 6-week-old male Lewis rats (n = 98) purchased from Janvier (Le Genest Saint Isle, France). They were conducted according to the French Department of Agriculture guidelines (license 21 CAE-102) and approved by the local ethics committee. The experimental procedures were performed in order to comply with ARRIVE guidelines. Animals were housed under a 12 h/12 h light/dark cycle and allowed free access to food and water. Anesthesia was induced by isoflurane 4% (Virbac, Carros, France) for arthritis induction. Aortas were harvested after chloral hydrate (400 mg/kg, i.p.; Sigma-Aldrich, Saint-Quentin-Fallavier, France) and pentobarbital anesthesia (60 mg/kg, i.p., CEVA Santé Animale, Libourne, France) for biochemical and vasoreactivity experiments, respectively.

2.2. Induction of arthritis and clinical evaluation of arthritis

Arthritis was induced by a single intradermal injection (120 μL) at the base of the tail of a suspension (10 mg/ml) of heat-killed *Mycobacterium butyricum* (Difco, Detroit, MI) suspended in mineral oil (Freund's incomplete adjuvant (Difco, Detroit, MI)). Non-arthritis, age-matched Lewis rats that were used as controls received an equivalent volume of saline. Arthritis was evaluated using the following arthritis scoring system [15]: inflammation (erythema and swelling) of one finger scores 0.1, weak and moderate arthritis of one big joint (ankle or wrist) scores 0.5 and intense arthritis of one big joint scores 1. The tarsus and ankle were considered the same joint. The arthritis score for a given limb ranged from 0 to 1.5 and the global arthritis score (four limbs) ranged from 0 to 6. The arthritis score was regularly determined from the first signs of inflammation (~at day 12 post-immunization) until sacrifice (at day 32 ± 3 post-immunization). At this time, inflammatory symptoms are maximal [16,17] and ED occurs at aortic level [17].

2.3. Collection of aortas

Aortas were collected from AIA rats and aged-matched controls and cut into rings (~2–7 mm-length). The rings were immediately frozen at −80 °C for western blot analysis, or embedded in OCT (Tissue-Tek, Sakura, USA) for immunohistochemical analysis, or incubated in an appropriate culture medium or used for vasoreactivity experiments.

2.4. Western blot analysis

Western blot analysis were performed to measure aortic levels of different proteins including mature BDNF (BDNF, 14 kDa), endothelial nitric oxide synthase phosphorylated at serine 1177 (peNOS^{ser1177}, 130 kDa, an enzyme that produces NO in large amount as compared to the unphosphorylated form [18]) as well as the two TrkB receptors that bind BDNF with the same affinity: the full-length TrkB isoform (TrkB-FL, 145 kDa), which is linked to a tyrosine kinase domain and the truncated TrkB isoform (TrkB-T, 95 kDa), which is devoid of this domain [19–21]. Of note, while binding of BDNF to TrkB-FL activates the ras/ERK1/2, PI3 kinase/Akt STAT and phospholipase Cγ pathways [5] as a result of receptor phosphorylation at tyrosine 816, its binding to TrkB-T receptors (a dominant negative receptor) inhibits TrkB-FL-dependent signaling [21].

Frozen rings were homogenized with five volumes of ice-cold lysis buffer [100 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EGTA, 1% triton X-100, 1% protease inhibitor cocktail (P8340, Sigma-Aldrich, Saint-Quentin-Fallavier, France)]. After centrifugation of the homogenates, an aliquot of the supernatant was kept for protein measurement by using the Lowry method. Equal amounts of protein were resolved by SDS-PAGE and electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (0.2 μm or 0.45 μm) for Western blotting. After blocking non-specific binding sites with a 5% solution of non-fat dry milk in Tris-buffered saline (20 mM Tris/HCl, 137 mM NaCl, pH 7.4) containing 0.1% Tween 20, membranes were probed with an anti-BDNF rabbit monoclonal antibody (1/3000 with 5% non-fat dry milk, ab108319, Abcam, Cambridge, United Kingdom) to detect BDNF, an anti-TrkB rabbit monoclonal antibody (dilution 1/1000, 610102, BD Transduction, San Jose, USA) to detect both TrkB-FL and TrkB-T or a mouse monoclonal antibody directed against peNOS^{ser1177} (dilution 1/1000 with 7.5% BSA, 612383, BD Biosciences, San Jose, USA). β-actin (dilution 1/10000, A5441, Sigma-Aldrich, Saint Quentin-Fallavier, France) was used as internal control. The membranes were then incubated with secondary antibodies conjugated with horseradish peroxidase [Jackson ImmunoResearch Laboratories, 111-035-144 (anti-rabbit, dilution 1/20000) and 115-035-166 (anti-mouse, dilution 1/50000), Interchim, Montluçon, France]. Gels were run in duplicate. Protein-antibody complexes were visualized using the enhanced chemiluminescence Western blot detection system (ECL2, 1151–7371, Fisher Scientific, Illkirch, France). The band densities were determined by scanning densitometry (GS-800, BIO-RAD Laboratories, Ivry sur Seine, France) and the results were expressed as percentages of control values.

2.5. RNA extraction and quantitative reverse-transcription PCR analysis

Total RNA was extracted from frozen aortic rings using the RNeasy Fibrous Tissue Mini Kit (74704, Qiagen, Hilder, Germany). After quantification, 500 ng of RNA was reverse transcribed using iScript Reverse Transcription Supermix (BIO-RAD Laboratories). Sequences of BDNF and housekeeping HPRT (hypoxanthine phosphoribosyl transferase) genes were obtained from the National Center for Biotechnology Information (NCBI) database, and the primers sequences (Eurogentec), described in supplemental data (Table 1), were designed using the Primer-Basic Local Alignment Search Tool (Primer-BLAST). Real-time PCR and quantification of gene expression were performed as described previously [14] with the relative transcription level of the bdnf gene calculated using the $2^{-\Delta\Delta Ct}$ method. Results were expressed as relative mRNA levels as