Joint Bone Spine 84 (2017) 175–181

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com/en

Original article

Expression of the calcium-sensing receptor in monocytes from synovial fluid is increased in osteoarthritis

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ARTICLE INFO

Article history:
Accepted 20 March 2016
Available online 23 June 2016

Keywords:
Calcium-sensing receptor
Monocytes
Osteoarthritis
Rheumatoid arthritis
Synovial fluid
Flow cytometry

ABSTRACT

Objectives: We assessed calcium-sensing receptor (CaSR) expression in monocytes isolated from synovial fluid of patients with different types of rheumatisms and explored whether CaSR expression was related to the inflammatory nature of synovial fluid.
Methods: Forty-one patients were included: osteoarthritis ($n = 10$), microcristallin rheumatisms ($n = 10$), rheumatoid arthritis ($n = 12$) and other inflammatory rheumatisms ($n = 9$). Surface and total CaSR expressions in monocytes isolated from synovial fluid and blood were assessed by flow cytometry analysis. U937 cells were cultured during 24 hours in presence of cell-free synovial fluids.
Results: Every monocyte population tested express the CaSR intra- and extracellularly. Whereas similar pattern of CaSR expression exist in monocyte isolated from blood or synovial fluids, our results indicate that higher CaSR expression levels can be observed in monocytes from synovial fluids than in circulating monocytes. In both populations of monocytes, surface and total CaSR expressions were found to be significantly increased in patients with osteoarthritis compared to rheumatoid arthritis. Similar data were obtained when U937 cells were incubated with cell-free synovial fluids from osteoarthritis patients. Still present, this effect was significantly lowered when "inflammatory" synovial fluids were introduced in culture.
Conclusions: Our results indicate that CaSR expression in synovial derived monocytes is higher in osteoarthritis than in inflammatory rheumatisms and that CaSR expression is modulated by the nature of the synovial fluid. Given the role played by monocytes in the pathogenesis of chronic rheumatisms, monocytes could be interesting therapeutic targets via the CaSR.

1. Introduction

The calcium-sensing receptor (CaSR) is a G-protein coupled receptor (GPCR) characterized by a very large extracellular domain [1]. As other GPCR, CaSR activity and signalling are regulated through its intracellular expression as well as its membrane insertion [2,3]. The primary physiological function of CaSR is the maintenance of systemic calcium homeostasis as it is able to sense very small variations of extracellular calcium in blood flow [4,5]. Highly expressed in tissues involved in calcium homeostasis [5], the CaSR is also expressed in a wide range of tissues not directly involved in calcium homeostasis, suggesting that the CaSR has several other physiological functions [6–8]. Indeed, the CaSR has been shown to have pleiotropic actions on cells at multiple levels such as regulating hormone secretion, gene expression and cellular fate [7,8]. In the past years, it has also been demonstrated that the CaSR expression depends on cell type, development stages and extracellular conditions [7].

Concerning monocytes, expression of the CaSR has previously been demonstrated in human circulating monocytes (CM) [9]. Furthermore, our team has recently demonstrated that more than 95% of CM express both intracellular and surface CaSR and that CM are the only cells among peripheral blood mononuclear cells to express the CaSR at the cell surface given the sensitivity of our measurement method [10]. Formerly considered as precursors for macrophages and dendritic cells, it has been now demonstrated

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http://dx.doi.org/10.1016/j.jbspin.2016.03.012
1297-319X/

that monocytes are made of numerous subpopulations with distinct functions according to molecules expressed at their cell surface and their ability to differentiate depending upon the stimuli they received [11,12]. Considering the roles played by monocytes in chronic inflammatory rheumatisms, an increasing number of studies emphasized that monocytes could be interesting therapeutic targets [13,14]. Hence, monocyte recruitment contributes to the pathogenesis of various aseptic inflammatory diseases such as chronic inflammatory rheumatisms and especially in rheumatoid arthritis (RA) in which CM recruitment in the joint could trigger the inflammatory cascade [14,15]. Moreover, CM can detect variations of extracellular calcium through the CaSR activation. Such process is supposed to be responsible for a chemotactic response of CM to gradients in concentrations of extracellular calcium which can be found in sites of bone resorption as well as at inflammatory sites [16–18]. Extracellular calcium can also stimulate the production of cytokines such as interleukin 6 by CM and can modulate their proliferation [19]. Noteworthy, it has been shown in different cellular models that CaSR expression can be altered by extracellular conditions like inflammation and particularly by pro-inflammatory cytokines [10,20–22]. However, the physiological significance of CaSR expression in CM is still widely unknown. Therefore, in the present study we investigated the CaSR expression in monocytes in different rheumatic situations.

We hypothesized that in case of articular effusion, the CaSR expression in monocytes present in the synovial fluid could reflect the inflammatory nature of the synovial fluid and thus differ depending on the type of rheumatism responsible for the articular effusion. Therefore, we investigated the CaSR expression in monocytes isolated from the synovial fluid of patients with different type of rheumatisms and explored whether CaSR expression in monocytes isolated from synovial fluid could differ depending on the type of rheumatism and whether the nature of synovial fluid could influence the CaSR expression in monocyte cells.

2. Methods

2.1. Study design

The present study is a pilot, cross-sectional, monocentric study. Patients presented with an articular effusion were prospectively enrolled from the Rheumatology Department of Amiens University Hospital, France. According to the French law, 41 patients gave their written informed consent and the study was approved by the University Hospital ethics committee (Comité de Protection des Personnes Nord-Ouest 2) (nº 2013-A00925-40).

2.2. Inclusion and exclusion criteria

Inclusion criteria included age $\geq$ 18 years old, presence of an articular effusion that could be punctured and capacity to understand the goals of the study. Patients were divided into 4 groups according to the disease responsible for articular effusion: osteoarthritis, microcristallin rheumatism, RA (according to the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA) and other inflammatory rheumatism. OA patients were assessed by X-ray of the involved articulation, according to the Kellgren and Lawrence score [23]. Exclusion criteria were as follows: pregnancy, ongoing infectious diseases, articular infiltration of corticosteroid or hyaluronic acid less than 6 months ago, blood coagulation diseases contra-indicating articular puncture, anti-osteoporotic treatment and septic arthritis.

2.3. Study protocol

Each patient underwent an articular puncture to collect synovial fluid at the inclusion in the study. A fraction of the synovial fluid was sent for bacteriologic and histopathologic analyses which are classically done in current practice. Total synovial calcium concentration was determined using the CA-c detection kit on ADVIA 2400 Siemens analyser. Information was obtained by means of a structured interview and laboratory tests. Medical records of all patients were reviewed. Demographic and clinical characteristics were collected. Blood samples were collected at the same time that the articular puncture. Serum calcium, albumin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and white blood cell count were analysed in an on-site biochemistry laboratory using standard auto-analyser techniques. Rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibody (ACPA) were only assessed in RA patients.

2.4. Isolation of mononuclear cells

Cells from synovial fluid and peripheral blood mononuclear cells were simultaneously isolated during the first 24 hours following the articular puncture in order to perform concomitantly flow cytometry analysis (FACS) to measure total and surface CaSR expression in monocytes from synovial fluid and in circulating monocytes. For each synovial fluid sample, cells were quantified using a Malassez chamber and then were isolated from synovial fluid by centrifugation (5 minutes at room temperature at 2200 *g*). Mononuclear cells were isolated by density gradient centrifugation. For each patient, 5 mL of blood were mixed with 15 mL of PBS-0.5% BSA. Then the 20 mL of diluted blood were carefully added to 10 mL of Histopaque™-1077 and the tube was centrifuged for 20 minutes at room temperature at 2200 *g*. After centrifugation, the interphase containing mononuclear cells was aspirated. The cells were washed before being centrifuged.

2.5. Cell culture and stimulation

U937 monocyte cells were maintained in Roswell Park Memorial Institute-1640 medium (RPMI) supplemented with 10% FBS, 2.0 mM GlutaMAX™ and 100 U/mL penicillin/streptomycin. Cells were cultured at 37 °C and 5% CO_2. Before each experiment, cells were serum starved and preincubated for 24 hours in 6 well plates at the rate of 1 million cells per well with 1 mL of RPMI-0.4% BSA and 1 mL of synovial fluid (which had been centrifuged at 16,000 *g* to remove cells and joint debris). Stimulation was stopped by aspirating the medium and washing the cells with 1 mL ice-cold PBS-0.1% BSA. Total CaSR expression was measured by FACS, as described below. The results were expressed according to the control condition in which the cells were incubated only with medium (RPMI-0.4% BSA).

2.6. Flow cytometry analysis

Mononuclear cells were thawed in water-bath at 37 °C prior to being washed with 0.5 mL of PBS-0.5% BSA. Cells were incubated with primary anti-calcium sensing receptor monoclonal antibody (ab19347, Abcam) or with negative control mouse IgG2a (X0943, DakoCytomation) for 30 minutes on ice. All other steps of the protocol were made as previously described [10]. Surface and total CaSR expressions in mononuclear cells were analysed by FACS (Aria cytometer).

During U937 cells immunostaining, we used a secondary IgG mouse antibody conjugated to FITC (Polyclonal Goat Anti-Mouse Immunoglobulin/FITC, F0479, DakoCytomation).