Cytokine 81 (2016) 117–126

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

The effect of interleukins 27 and 35 and their role on mediating the action of insulin Like Growth Factor -1 on the inflammation and blood flow of chronically inflamed rat knee joint

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ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form 16 February 2016
Accepted 11 March 2016
Available online 17 March 2016

Keywords:
Chronic joint inflammation
Histopathology
IGF-1
IL-27
IL-35
Synovial blood flow

ABSTRACT

Introduction: Previous studies have shown that some cytokines mediate the effect of IGF-1 on inflammation and also association between IGF-1 and vascular endothelial dysfunction. Due to the discrepancies in the inflammatory and anti-inflammatory roles of IL-27 and IL-35, the effects of these cytokines and their IGF-1-mediating role were investigated regarding chronic joint inflammation and synovial blood flow.
Method: Male rats were divided into two main groups of histopathology (n = 80) and blood flow (n = 72). These were further divided into ten subgroups of control, vehicle, IGF-1, IL-27, IL-35, their antagonists, IGF-1 + IL-27 antagonist, and IGF-1 + IL-35 antagonist. Inflammation was induced by intra-articular injection of complete Freund adjuvant. Two weeks later (in order to induce chronic inflammation), vehicle or drugs were injected into the joint space every other day until day 28, on which inflammatory indices were assessed histopathologically. In the second subgroups, vehicle or drugs were administered by super-fusion on day 28 and their effects on the joint blood flow (JBF, laser Doppler perfusion method) and the systemic blood pressure were assessed.
Results: Endogenous IL-27 and IL-35 had inflammatory roles and IGF-1 had no effect. IL-27 and IL-35 antagonists had the highest anti-inflammatory and anti-angiogenesis effects and these effects were inhibited by IGF-1. Total inflammation score was 4.5 ± 0.42, 3.50 ± 0.5, 2.25 ± 0.45 and 1.50 ± 0.42 for vehicle, IGF-1 antagonist, IL-27 antagonist and IL-35 antagonist respectively. A significant increase was induced in JBF by IGF-1 antagonist and combination of IGF-1 + IL-35 antagonist.
Conclusion: IL-27 and IL-35 antagonists may be suitable goals for the treatment of chronic joint inflammation while their anti-inflammatory effects are not exerted via the changes in JBF.

1. Introduction

Rheumatoid arthritis (RA) is the most prevalent inflammatory arthritis which has affected 0.5–1 percent of people worldwide [1]. Its' highest prevalence is between 40 and 70 years old that increases age dependently [2].

Considering the fact that Iran's population is aging and since the prevalence of RA increases with age, and also that disease causes large economic costs and morbidity in the affected population, it is important to assess the pathophysiological aspects of the disease and its mechanisms.

IGF-1 is a growth factor of mostly anti-inflammatory effect which decreases signaling of pro-inflammatory cytokines through producing IL-10 [3]. This is proposed to be exerted through NF-KB and alleviation of response to TNF-α [3–5]. IGF-1 plays a role in proliferation of fibroblasts and synthesis of cartilage and extracellular matrix proteins in the bones. In RA disease, IGF-1 decreases or remains normal in some cases, while it seems that its binding proteins (IGFBPs) increase [6] reducing its bioavailability.

IL-27 and IL-35, which have been discovered recently, are members of IL-12 family and play a vital role in differentiation of inflammatory cells [7,8]. IL-27 inhibits differentiation of Th-17 cells and may inhibit auto-immune inflammatory diseases such as RA [8–10]. Conversely, Cao et al. have assessed proteoglycans-induced arthritis and have verified the inflammatory effects of

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http://dx.doi.org/10.1016/j.cyto.2016.03.010
1043-4666/

IL-27 [11]. Fearson has claimed that mice which lackIL-27 receptors, are protected against progression of arthritis [12]-suggesting the inflammatory activity of IL-27.

The inflammatory and anti-inflammatory activities of IL-35 are still debated and it seems that its diverse effects are strongly dependent on the experimental model of inflammation [13]. Several studies have demonstrated the anti-inflammatory properties of IL-35, for instance in the inflammatory bowel disease, collagen-induced arthritis (CIA), or other autoimmune diseases [7,14,15]. In contrast, some recent findings have revealed pro-inflammatory properties of this cytokine in Lyme arthritis and CIA [16,17]. Furthermore, in a study conducted recently, up-regulation of IL-35 was observed in synovial tissue of RA patients, which implies pro-inflammatory activities of IL-35 in human and its potential role in pathogenesis of RA [13].

On the other hand a close relationship has been found between articular blood flow and joint inflammation [18] with lowest PO_2 values observed close to the articular cartilage [19]. In view of the low synovial fluid PO_2, avascular structures such as cartilage could be subject to injury and this could contribute to, or perhaps initiate processes such as cartilage destruction, angiogenesis and fibroblast proliferation which are known to occur in conditions such as RA. Therefore, the factors affecting articular blood flow are important in maintaining the health and integrity of articular tissues. It has been shown that vascular endothelial cells contain receptors with high affinity for IGF-1; and the injection of growth hormone into the brachial artery leads to vascular dilation. These effects are probably due to the effect of IGF-1 on vascular endothelium [20,21]. As the inhibition of IGF-1 production leads to endothelial dysfunction and damages to dilation properties of resistant arteries [22], it is probable that IGF-1 plays a role in the regulation of articular blood flow. Due to the existing controversies regarding the pro- or anti-inflammatory functions of IL-27 and IL-35, and since no study has so far assessed the mediating role of these cytokines in IGF-1 function in chronic joint inflammation, this study assessed (1) the effects of IGF-1, IL-27, and IL-35 on joint inflammation, and evaluated the mediating role of IL-27 and IL-35 in IGF-1 function in a chronic joint inflammation model which highly resembles the characteristics of RA [23]. (2) Due to close association of blood flow with inflammation and synovial fluid PO_2, the effect of IGF-1 and these cytokines on the blood flow of the inflamed joint was assessed as a probable mechanism for the effects of these agents on chronic joint inflammation. (3) Due to the role of angiogenesis in joint blood flow regulation and in joint damage in RA, the angiogenesis status of the synovial tissues of studied groups was also assessed.

2. Methods and materials

2.1. Materials

The following materials were used in this study: complete Freunds adjuvant (CFA, Sigma, UK), IGF-1 (SRP4121, Sigma, UK), IL-27 (SRP4187, Sigma, UK), IL-35 (10705-H02H, Sinobiological, USA), IGF-1 antagonist (SC-204008, Santacruz, USA), IL-35 antagonist (SC-7925, Santacruz, USA), IL-27 antagonist (MAB 2109, R&D, USA), Anti CD31 primary antibody (Clone1/A4, QBend 10, DAKO, GLostrup, Denmark) Chromogendiamino-banzidine tetra hydrochloride (DAB) (Sigma, USA), Halothane (ICI, India) and Sodium thiopental (Rotex Medica, Germany).

2.2. Animals

Experiments conformed to the national guidelines for conducting animal studies (Ethic committee permission no 92/451KA, Kerman University of Medical Sciences, Kerman, Iran) and was performed on 152 male rats within the weight range of 200–300 g. The animals were kept at a temperature of 23 ± 2 °C, 12-h dark/-light cycle, with free access to water and food in the animal room of Kerman University of Medical Sciences. The animals were randomly divided into two main groups of histopathology (n = 80) and blood flow assessment (n = 72). These two main groups were further divided into 10 and 9 subgroups respectively as follows: (1) CTL (non-inflamed), (2) Vehicle (inflamed, saline treated), and the other 8 groups were inflamed treated with: (3) IGF-1, (4) IL-27, (5) IL-35, (6) IGF-1 antagonist, (7) IL-27 antagonist, (8) IL-35 antagonist, (9) IGF-1 + IL-27 antagonist, and (10) IGF-1 + IL-35 antagonist. In the blood flow assessment group, all histopathology subgroups were present except the first one (non-inflamed subgroup).

In the histopathology and blood flow groups chronic inflammation was induced by the injection of 0.2 ml CFA into the right joint space by inserting a 28G needle through mid-patellar tendon on day 0 [(0.1 ml in the posterior space (higher depth) and 0.1 ml in anterior space (lower depth)] [24,25]. This is an antigen-induced arthritis model, which is an experimental model histologically mimicking human RA [23]. In the histopathology group, from day 14–28, 2 μg of agonists or 4 μg of antagonists or a combination of these were injected every other day into the joint spaces in the total volume of 0.2 ml. These doses were selected based on similar studies using IGF-1 and IGF-1 antagonist [26], IL-27 and IL-27 antagonist [10,27–29], and IL-35 and IL-35 antagonist [7]. In the blood flow assessment group, inflammation was induced similar to the histopathology group but, sterile normal saline was injected into the joint instead of drugs from day 14–28. The responses to the drugs (agonists/antagonists) were then investigated on day 28 (see below under "blood flow assessment" for details).

2.3. Measurement of the joint diameter and weight of the animals

The knee joint diameter was regularly measured during the 28-day period, during which the chronic inflammation was developed [30], as an inflammation indicator. For this, the medio-lateral diameter of the joint was measured, in the maximum diameter point, using a caliper (abd, China). For the first week, the frequency of measurement was daily and for the next three weeks it was performed every other day. The weight of the animals was measured every other day in the first week and then every three days for the next three weeks.

2.4. Histopathology assessment

At the end of the experiment on day 28, the animals were anesthetized by Sodium thiopental (50 mg/kg, ip) and were then killed with the injection of potassium chloride 3 M into their hearts. Their right joints were removed from 1 cm above and 1 cm below the knee joint and fixed in 10% formalin solution after removing the muscles around the bones. In the Pathology lab the joints were positioned in 10% nitric acid for at least 24 h, so that the bones became decalcified and softened. After routine processing of the tissues they were positioned in paraffin blocks. Five slices with the thickness of 5 μm were prepared by microtome from each block and, after dehydration by alcohol; they were stained by hematoxylen and eosin dyes before they were assessed by a Pathologist. The severity of inflammation was assessed by using Kapila method [31] based on following the scoring system:

(A) **Synovial hyperplasia**: 0 = one to three cell layers; 1 = 4–6 cell layers; 2 = 7 cell layers or more.