Clinica Chimica Acta 453 (2016) 164–169

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Methotrexate regulates Th-1 response by suppressing caspase-1 and cytokines in psoriasis patients

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ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 15 December 2015
Accepted 15 December 2015
Available online 18 December 2015

Keywords:
Psoriasis
Methotrexate
Interleukin-18
Caspase-1
Th-1 immune cells

ABSTRACT

Background: Caspase-1 induces the proinflammatory cytokines which appears to be a promising target in Th1-type inflammatory diseases, like psoriasis. We determined the effect of MTX on caspase-1, TNF-α and IL-18 in psoriasis patients.
Methods: We recruited 45 control subjects and 58 psoriasis patients for this study. The patients were treated with 7.5 mg of MTX per week for 12 weeks. Folic acid was given at 5 mg once daily except on the day of MTX for 12 weeks. Blood samples and lesional skin biopsy were taken. Histological examination has been done. IL-18 and TNF-α levels were analyzed by using ELISA. Caspase-1 expression was analyzed by western blot and Real Time PCR.
Results: Histological examinations showed MTX decreased acanthosis in psoriasis skin. Plasma IL-18 level and serum TNF-α were increased in psoriasis and deduced significantly ($P < 0.001$) after MTX treatment. Protein and mRNA expression of caspase-1 in skin biopsy were higher in psoriasis and reduced significantly ($P < 0.001$) after MTX treatment.
Conclusion: Decreasing inflammatory caspase and proinflammatory cytokines by MTX, inhibits the Th1 response in psoriasis. This shows the therapeutic effect of MTX in controlling the immunopathogenesis of psoriasis.

1. Introduction

Psoriasis is a common, immune-mediated inflammatory skin disorder which affects approximately 2% of the general population [1]. Despite numerous studies, the pathogenesis of psoriasis has not been fully elucidated [2]. First, the disease was regarded as a biochemical disturbance, then as a keratinocyte-mediated condition [3]. Nowadays, the concept of immunological genetics-related disturbances is widely accepted [3,4].

The innate immune system is the first line of defense against infection and plays a crucial role in the initiation of the adaptive immune response. The presence of innate immune cells and their products in psoriatic skin plaques suggests a role for innate immunity in this disease. In addition, the innate immune system can direct the development of pathogenic T cells in psoriasis. T helper(h)1 and Th17 lymphocytes contribute to the pathogenesis of psoriasis through the release of inflammatory cytokines that promote further recruitment of immune cells, keratinocyte proliferation and sustained inflammation [5]. T-cell mediated inflammation plays an important role in the etiology of psoriasis. Activated T lymphocytes secrete various cytokines and growth factors in psoriasis [6]. Tumor necrosis factor-α is a pivotal pro-inflammatory cytokine of the innate immune response and a key for skin inflammation [7]. TNFα induce the expression of ICAM-1 on KC through the mediation of p55 and ICAM-1 induces the infiltration of MNCs in the dermis, which promotes the development and progression of psoriasis vulgaris [8]. Thereby it causes hyperproliferation in psoriasis.

Caspases play important roles not only in the induction of apoptosis but also in inflammatory processes [9]. Inflammasomes are multiprotein complexes that contain NALPs (NACHT/LRR/PYD-containing proteins) and inflammatory caspases like caspase-1, 5. Together they form functional units that carry out two major tasks: (i) sensing cytosolic pathogen associated molecular patterns and (ii) reacting to these patterns through activation of proinflammatory cytokines and thereby initiating inflammation [10]. Caspase-1 belongs to the group of inflammatory caspases and is the activating enzyme for the proinflammatory cytokine interleukin-1β, interleukin 18 (IL18), a cytokine known to play an important role in the pathogenesis of psoriasis [9].

Interleukin 18 was first identified as an interferon γ-inducing factor [11,12]. It is closely related to IL1α and IL1β. Two IL18 receptors, IL18Rα and IL18Rβ, have been identified and cloned, and both are important for

Abbreviations: MTX, methotrexate; IL-18, interleukin-18; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon gamma.

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http://dx.doi.org/10.1016/j.cca.2015.12.022
0009-8981/

IL18 signaling. IL18 is a pleiotropic cytokine that has costimulatory functions on Th1 cytokines. IL18 mRNA is widely expressed in human tissues. IL18 binding protein (IL18BP) belongs to a new family of secreted proteins which act as inhibitors of IL18 signaling [13]. IL18BP effectively blocks IL18 by forming a high affinity complex that exhibits a very low dissociation rate. It possesses the capacity to stimulate innate immunity as well as Th1- and Th2-mediated responses [14] and exerts its activity on human defense system, especially in inflammatory, infectious and autoimmune diseases [15].

Keratinocytes, traditionally thought to produce but not process IL-18 [16], have now been shown to secrete biologically active IL-18 when treated with dinitrochlorobenzene and proinflammatory mediators such as LPS. In addition to keratinocytes, Langerhans cells (LC) also produce IL-18, which in turn contributes to the regulation of LC migration [17]. IL-18 expression and effector function in inflammatory diseases has been studied across a broad range of tissues.

But, the role of IL-18 in psoriasis has not been fully elucidated. It is speculated that IL-18 produced by human keratinocytes enhances IFN-γ production in inflammation and thus IL-18 seems to be a promising target in Th1-type inflammatory diseases, like psoriasis [18,19].

Methotrexate has been considered as a gold standard therapy for moderate and severe psoriasis. It exerts immunomodulatory effects in psoriasis. It increases T cell apoptosis [20], reduces cell proliferation [21], altered cellular adhesion molecules like ICAM-1, E-selectin [22]. MTX alters T-cell production of several cytokines, including IL-1, IL-2, IL-4, IL-8, INF-γ and TNF-α [23–26]. Methotrexate acts by slowing skin turnover in psoriasis remains pervasive in lay literature. Methotrexate has good control over psoriasis but few evidence based literature available.

2. Materials and methods

2.1. Patient details

The prevalence of psoriasis in India is 0.44 to 2.8% in 2010 [27]. Based on this data, sample size of psoriasis patients was calculated using the formula.

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

Where,

t confidence level at 95% [standard value 1.96].
P prevalence value (in decimals).
m constant value (0.05).

Psoriasis vulgaris patients ($n = 58$) who visited Dermatology Department, SRM Hospital, Kattankulathur were recruited for this study. The age of all patients ranged from 18 to 70 years (mean ± SD, 46.4 ± 14.1 years), including 27 men and 31 women. Healthy volunteers ($n = 45$) consisting of 21 men and 24 women, aged from 21 to 70 years (mean ± SD, 44.6 ± 15.5 years) served as control.

Patients with 18 years of age or older, who had more than 20% body surface area involvement and who had not received any topical or systemic therapy for at least a month were included in this study. Exclusion criteria were as follows: children (<18 years old), pregnant and lactating women, patients with unstable psoriasis, liver and renal impairment, infertility, anemia, excessive alcohol intake or any other systemic diseases such as diabetes, hypertension, rheumatoid arthritis, cardiovascular diseases and respiratory syndrome. Detailed history regarding the age of disease onset and family history was recorded. Physical examination and the presence of any other dermatological condition were noted. The study protocol was approved by the institutional ethical committee. Informed consent documents were signed by all the patients.

2.2. Treatment regimen

Patients with psoriasis were treated with 7.5 mg of MTX per week for 12 weeks. Folic acid was given at 5 mg once daily except on the day of MTX for 12 weeks. During systemic treatment, no concomitant antipsoriatic therapy was permitted, with the exception of emollients. Clinical evaluations were performed by the same dermatologist at 4 intervals (i.e., day 0, 2, 6 and 12 weeks) until completion of the study. Scoring was based on the Psoriasis Area Severity Index (PASI) scoring system.

2.3. Collection of blood samples

Blood samples of psoriatic patients were collected and analyzed before (day 0) and after (at the end of 6 weeks and 12 weeks) treatment with MTX. In control subjects, blood samples were collected only once.

2.4. Collection of tissue samples

In all patients, 10 mm of lesional and nonlesional skin biopsies were taken after local anesthesia, lidocaine hydrochloride and adrenaline bitartrate IP were given intradermally. Lesional biopsies were taken before (day 0) and after (6 and 12 weeks) treatment with MTX. Nonlesional skin biopsy served as control, which was collected only once. Biopsies of psoriatic lesional skin were taken within a lesion, 1 cm from the edge of the plaque border. Biopsies of nonlesional skin were taken 2 cm beyond the plaque border. Skin biopsies were immediately immersed in protease inhibitor cocktail and RNAlater solution (Qiagen) and finally stored at −20 °C until further use.

2.5. Histological examination

Formalin-fixed skin biopsy were embedded in paraffin and processed routinely. Hematoxylin eosin staining was used to examine the histological changes in psoriatic skin before and after MTX treatment.

2.6. Measurement of IL-18 and TNF-α by ELISA

Freshly isolated heparinized peripheral blood samples were immediately centrifuged for 10 min at 500 *g*, and the plasma was separated. The concentration of IL-18 in plasma was measured using MBL Human IL-18 ELISA Kit according to manufacturer's instructions. Blood was collected in blood collection tube, allowed to clot for 30 min, and centrifuged at 2500 rpm for 10 min and the serum was separated. TNF-α concentrations in serum were measured by using Bender Med System kits according to manufacturer's instructions.

2.7. Western blot analysis

Total cell extracts were prepared from skin biopsy as described earlier [28]. Caspase-1 expression was determined by Western blot analysis [29] using the primary antibody caspase-1 [caspase-1 p10 (M-20)] purchased from Santa Cruz and β-actin-peroxidase clone Ac-15; Sigma-Aldrich). Band intensity was analyzed using ImageJ software (http://rsb.info.nih.gov/ij/).

2.8. Real-time quantitative PCR

Total RNA was isolated from lesional (before, during and after MTX treatments) and non-lesional skin biopsies from psoriasis patients (AxyPrep Multisource Total RNA Miniprep Kit, Applied Biosystems). High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) was used for reverse transcriptase reactions. Primer sequence for caspase-1 and the housekeeping gene β-actin as depicted in Table 1. cDNA was PCR-amplified under the following conditions: at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C, 2 min at