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Treatment of Takayasu arteritis with the IL-6R antibody tocilizumab *vs.* cyclophosphamide

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ABSTRACT

Objective: To evaluate the treatment effects of the IL-6R antibody tocilizumab and cyclophosphamide (CTX) in patients with Takayasu arteritis (TA) and explore the mechanism by analyzing their effects on various cytokines.
Methods and materials: This study included 9 TA patients treated with tocilizumab, 15 TA patients treated with CTX and 24 healthy controls. Treatment effects were evaluated based on: (1) Kerr score and Indian Takayasu Clinical Activity Score (ITAS2010), (2) improvement of previous lesions and occurrence of new vascular lesions on magnetic resonance angiography (MRA), (3) changes in various cytokine levels, (4) glucocorticoid sparing, and (5) adverse effects. ELISA was used to analyze the cytokine levels.
Results: Before treatment, all the patients had active disease accompanied with elevated C-reactive protein (CRP), serum amyloid A (SAA), interleukin (IL)-6 and pentraxin-3 (PTX3). Moreover, an imbalance in the MMP-TIMP system was also observed in these patients. Among them, IL-6 and MMP-9 levels were significantly associated with vascular enhancement scores and stenosis scores, respectively. At 6 months after treatment, improved clinical manifestations and glucocorticoids sparing were observed in both groups without any severe side effects. Although no significant improvement occurred in the vascular stenosis, thickness and enhancement scores in both groups, a more degree of decrease of ESR, CRP level, significantly decreased MMP-9 level and increased MMP-2 level were found in the tocilizumab group than in the CTX group.
Conclusions: The IL-6R antibody may be more effective in mitigating vascular inflammation and remodeling than CTX *via* inhibition of IL-6 and MMP-9.

1. Introduction

Takayasu arteritis (TA) is a chronic vascular inflammatory disorder that is mainly characterized by chronic vascular inflammation and fibrosis [14]. It is also defined as a type of non-specific granulomatous large vessel vasculitis that involves the aorta and its main branches and shows a predilection for child-bearing women (<50 years) [1]. Although TA is more prevalent in Asian countries, it has been reported and been studied in many countries worldwide.

Due to the involvement of various arteries, patients with TA present with a range of manifestations that not only include systemic symptoms such as fever and weakness, but also artery-related conditions such as claudication and pulselessness. Due to the latent onset of TA, arterial involvement in TA is already in an advanced stage with thickened arteries at the time of diagnosis and treatment [2]. Glucocorticoids (GCs) and cyclophosphamide (CTX) are commonly used as the mainstay treatment regimen. They are effective in controlling systemic inflammation by inhibiting certain inflammation-related factors. However, it seems that they do not inhibit vascular fibrosis [3]. Based on our clinical practice, vascular wall thickness has been found to develop persistently in a great proportion of TA patients treated with CTX. Thus, strategic drugs that target vascular inflammation or remodeling would be more effective in TA treatment.

Multiple inflammatory proteins, such as pentraxin (PTX3), interleukin (IL)-6, IL-17, matrix metalloproteinase (MMPs) and tissue inhibitor of metalloproteinase (TIMPs), have been found to play important roles in TA development [4–7]. Among them, IL-6 is considered to be the most promising biomarker and treatment target as it can infiltrate local vascular tissue and promote the activation of various immune cells. Recently, several studies [8,9] have proved that the humanized IL-6 receptor (IL-6R) antibody tocilizumab was effective in treating

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refractory patients with TA. In addition, according to our previous study, IL-6/IL-6R can promote phenotypic transformation of aortic adventitial fibroblasts so that they secrete not only cytokines such as IL-6 and monocyte chemotactic protein-1 but also extracellular matrix, which promotes macrophage infiltration and vascular remodeling [10]. Therefore, IL-6R antibodies would be promising for TA treatment.

Previous studies on tocilizumab treatment in TA have only reported the clinical effect in several cases or a single cohort. No systemic research has been conducted to clarify the specific mechanism in terms of the effect of tocilizumab on vascular inflammation or TA-related fibrosis. Therefore, the present study aims to investigate the mechanism of action of tocilizumab based on cytokine changes in patients treated with the IL-6R antibody and routine drugs (GC and CTX).

2. Materials and methods

2.1. Patients

In our center (Department of Rheumatology, Zhongshan Hospital, Fudan University, Shanghai, China), there is a prospective observational cohort of 287 TA patients recruited since year 2010. In this cohort, all patients were diagnosed according to 1990 American College of Rheumatology (ACR) classification criteria [11] by three rheumatology experts. Based on their treatment regimen and completeness of clinical data and biological samples, 24 patients from this cohort were included in this study (Supplementary Fig. 1. The enrollment flow chart). Fifteen patients were treated with GC and CTX, and 9 patients were treated with tocilizumab [Roche] and GC. In addition, 25 healthy controls were enrolled.

In the tocilizumab treatment group, eight patients were treated with tocilizumab because they were diagnosed with severe TA accompanied by active disease (severe symptoms or signs such as severe dizziness, amaurosis, and vision loss, accompanied by high erythrocyte sedimentation rate (ESR)), while the remaining 1 patient was refractory to GC and more than two kinds of immunosuppressants before tocilizumab was used. In the CTX treatment group, all the patients were treated with GC and CTX from the time of disease diagnosis. All procedures will be conducted in accordance with the Declaration of Helsinki and its amendments. The study protocol was approved by the Ethics Committees of Zhongshan Hospital (B2016-168). All patients provided their written informed consent for inclusion in this study.

2.2. Clinical assessment

All general information and clinical characteristics (signs and symptoms, lab results, and imaging data) of the patients were collected at the baseline and every 3 months during the follow-up period. The lab tests included blood routine test, urine routine test, biochemistry indices, ESR, C-reactive protein (CRP), serum amyloid A protein (SAA), fibrinogen, and various cytokines. Moreover, whole-body contrast-enhanced magnetic resonance angiography (CE-MRA) data were collected at the time of diagnosis and 6 months after treatment.

The treatment effect of the two regimens was determined based on the following five parameters: (1) Patients' overall status (active/inactive): clinical manifestations, Kerr score [12] and Indian Takayasu Clinical Activity Score (ITAS2010); (2) Imaging assessment: improvement of previous lesions and development of new vascular lesions; (3) Laboratory evaluation: changes in the levels of various proteins; (4) GC sparing: reduction in GC usage; (5) Adverse effects.

2.3. Whole-body CE-MRA

This examination was conducted as previously described [13]. According to the MRA scoring system our group established [13], lumen stenosis, vascular wall thickness and enhancement of different arteries were scored, including both the common carotid arteries, subclavian arteries, brachiocephalic artery, ascending aorta, aortic arch, descending (thoracic) aorta, abdominal aorta and both renal arteries. The renal arteries were excluded from vascular thickness and enhancement measurement for technical reasons.

In the present study, all the patients had undergone the initial and follow-up MRA at 6 months. Their imaging values were analyzed to assess the treatment effect. In addition, new lesions and collateral circulation formation were also evaluated after treatment.

2.4. Treatment

Patients in the tocilizumab group were treated with tocilizumab (8 mg/kg) and followed up for 6 months with the same dosage, while patients in the CTX group were treated with cyclophosphamide (600−800 mg/mo, which accumulated to at least 3 g at month 6). Moreover, GC (0.8 mg/kg/d) was used in all the patients of both groups, with the exception of one patient in the tocilizumab group who was refractory to GC and other drugs and was treated with a GC dosage of 15 mg/d. In addition, two patients in the tocilizumab group were also treated with leflunomide (20 mg/d) and MTX (10 mg/w) respectively.

2.5. ELISA

At our center, peripheral blood samples are obtained when patients are recruited and every three months during the follow-up period. In addition, normal serum was obtained from 25 healthy controls. Serum was collected and stored at −80 °C until analysis. Commercial ELISA kits were used to measure the levels of serum IL-6, IL-17, PTX-3, MMP-2, MMP-3, MMP-9, TIMP-1 and TIMP-2 (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

The level of MMP2, MMP-9, TIMP-1 and TIMP-2 was also determined in the serum samples of the 25 healthy controls. In addition, normal levels of IL-6, IL-17, PTX3 and MMP3 were referenced from the healthy cohort used in our previous studies [4,6].

2.6. Assessment of adverse effects

Patients were asked about whether they experienced any side effects after each tocilizumab infusion or intravenous CTX administration. The short-term side effects reported were intense irritation (included rashes), gastrointestinal reaction, hair loss, oral ulceration, fever, respiratory infection and amenorrhea. In addition, routine blood, urine and biochemical tests were conducted at every visit to determine if there were any potential adverse effects.

2.7. Statistical analysis

Continuous data (such as age, blood indicators, and various cytokine levels) are expressed as the mean ± standard deviation (SD) values and analyzed using the Student's *t*-test. Enumeration data are presented as median values (25%−75% quartile). Correlation analysis was performed to determine the correlation between various serum indicators, or between serum proteins and imaging data. All statistical analyses were performed using SPSS version 22.0 (Chicago, IL, USA). The receiver operating characteristic curve was employed to assess the specificity and sensitivity of a particular protein for the assessment of imaging features by the MedCalc statistical software (MedCalc software, Belgium). Histograms were generated using GraphPad Prism 5 (GraphPad Software Inc., USA). p Values <0.05 were considered to indicate statistical significance.

3. Results

3.1. Baseline characteristics

3.1.1. Baseline general patient characteristics

Patients in the tocilizumab treatment group were 32.11 (SD = 11.76) years old with a female:male ratio of 8:1, while patients of the CTX treatment group were 43.00 (SD = 16.68) years with a female:male ratio of 10:5. The median delayed diagnosis time was 10 (25%−75% = 5−43) and 2 (25%−75% = 1−24) months in the tocilizumab group and CTX group, respectively. According to the Kerr activity assessment, all the recruited patients were in an active disease state at the baseline. No significant differences were observed in the general characteristics of the TA and healthy groups. No differences were observed between enrolled and excluded patients either (Supplementary Table 1).

3.1.2. Baseline clinical presentations

In the present study, the most common presentation was headache or dizziness (11, 45.83%); this was followed by fever (10, 41.67%), chest distress (9, 37.5%) and weakness (8, 33.33%) (Table 1). In addition, the common signs were hypertension (7, 29.17%), neck pain (5, 20.83%) and asymmetric blood pressure (5, 20.83%). No obvious difference was found with regard to the occurrence of these signs, symptoms and ITAS, ITAS-ESR, ITAS-CRP assessment scores between the tocilizumab and CTX group.

3.1.3. Baseline imaging features

The commonly involved arteries were the left subclavian artery (9, 52.94%), left common carotid artery (9, 52.94%), right common carotid artery (6, 35.29%), and abdominal aorta (6, 25%) in the whole patients. No significant differences were observed in the distribution of vascular involvement or the scores for vascular stenosis, wall thickness and vascular enhancement between the tocilizumab and CTX group. However, vascular enhancement was slightly higher in the tocilizumab group than in the CTX group (Table 2).

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