Induction of hyperadiponectinemia following long-term treatment of patients with rheumatoid arthritis with infliximab (IFX), an anti-TNF-alpha antibody

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Abstract. Tumor necrosis factor-alpha (TNF-alpha) plays an important role in forming atherosclerosis based on chronic inflammatory condition in vivo and animal models. In human system, it is not clear the involvement of TNF-alpha to atherosclerosis. To clarify the relevance of TNF-alpha to atherosclerotic factors in human, We performed a prospective cohort study to investigate the inhibition of TNF-alpha with anti-TNF-alpha antibody infliximab may contribute to increase serum adiponectin levels, adipocyte-derived hormone with antiatherogenic properties, in patients with RA. 97 patients with active RA had been treated every 8 weeks for 1 year (13 men and 84 women, 54.2 ± 12.6 years, disease duration; 8.5 ± 1.5 years). They received a fixed dose of infliximab of 3 mg/kg every 8 weeks for 52 weeks. We evaluated changes of inflammatory markers, high molecular weight form of adiponectin levels and blood lipid levels. We also studied the association between increment rate of serum adiponectin and improvement of disease activity and inflammatory markers. Infliximab were strikingly dropped inflammatory markers (p<0.01), increased total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) (p<0.05). Besides, serum adiponectin significantly increased, independent of RA activity and clinical backgrounds, suggesting that TNF-alpha and adiponectin exhibit opposite effects in human body. TNF-alpha blockade may interfere in the atherosclerosis directly or indirectly, by increasing serum adiponectin levels, therefore TNF-alpha blockade may improve cardiovascular morbidity and mortality in chronic inflammatory disease such as RA.

Key words: Atherosclerosis, Inflammation, Adiponectin, TNF-alpha

(RECENT studies showed that vascular endothelial and smooth muscle cells express tumor necrosis factor-alpha (TNF-α) and that such expression is upregulated in proatherogenic and pathophysiological conditions. TNF-α plays an important role in chronic inflammatory states and accelerated atherosclerosis. Chronic inflammatory states are associated with higher mortality rate in association with atherosclerosis. Adiponectin, an adipocyte-derived hormone, plays a protective role against the development of atherosclerosis and insulin resistance by inhibiting TNF-α in vitro, and overexpression of adiponectin has been shown to reduce atherosclerotic lesions in animal models of atherosclerosis [1]. In human, low serum adiponectin levels are found in patients with coronary artery disease (CAD), suggesting that serum adiponectin levels may be helpful in assessment of CAD risk [2–5].

Recently, pro-inflammatory cytokines have been the targets of biological therapy in some chronic inflammatory diseases. Infliximab (IFX) is a novel human/murine chimeric monoclonal antibody preparation, raised against TNF-α, specifically binds to human TNF-α and markedly neutralizes soluble TNF-α. IFX is cytotoxic to cells that express membrane-bound TNF-α. However, there is little information on the ef-
effects of anti-TNF-α on atherosclerosis, especially adiponectin levels in human. The aim of this prospective cohort study was to assess the effects of inhibition of TNF-α with anti-TNF-α on serum adiponectin concentrations in patients with RA.

**Materials and Methods**

97 patients with active RA had been treated every 8 weeks for 1 year (13 men and 84 women, 54.2 ± 12.6 years, ±SD, 14 patients with RA stage I, 32 with stage II, 22 with stage III, and 29 with stage IV, disease duration; 8.5 ± 1.5 years). All subjects had received and were maintained on standard methotrexate (MTX) therapy for more than 3 months. They were allowed to continue taking low-dose oral corticosteroids (3.3 ± 0.6 mg/day). Patients received a fixed dose of anti-TNF-antibody IFX of 3 mg/kg in a single 2-h infusion.

**Biochemical measurements**

Blood samples were drawn in 2003–2005, every month and evaluated every 6 months. Serum high molecular weight form of adiponectin levels was determined by enzyme-linked immunosorbent assay (ELISA). We also measured the levels of high-sensitive serum C-reactive protein (hs-CRP), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), representing markers of atherosclerosis. The disease activity score (DAS)-28, assessed using 28-joint counts for swelling and tenderness, tender joint count, and patients assessment of disease activity. The steroid dose was gradually reduced with reductions in the value of DAS-28. Informed consent was obtained from each subject, and the study was approved by national and local research ethics committees.

**Statistical Analysis**

Data are expressed as mean ± SEM. A paired t-test was used to assess at baseline and 52-week values of adiponectin, markers of atherosclerosis, and DAS-28. Changes in adiponectin were evaluated based on age, body mass index, systolic blood pressure were assessed unpaired t-test. The percent changes in adiponectin were correlated with improvement in disease activity, inflammatory markers, and atherosclerosis-related parameters by using the correlation analysis. All statistical tests were conducted using StatView software version 5. A levels of \( p < 0.05 \) was accepted as statistically significant.

**Results**

**IFX reduces serum CRP levels**

IFX treatment for 52 weeks resulted in significant suppression of inflammatory process as evidenced by reduction in serum CRP levels from 2.9 to 0.8 mg/dl \( (P<0.01) \). Most patients responded to the treatment and showed a stable level of relatively low disease activity assessed by DAS-28 (from 5.4 to 3.1, \( p<0.01 \)).

**IFX increases serum adiponectin and lipid levels**

Serum adiponectin levels significantly increased from 6.2 to 8.3 \( \mu \text{g/ml} \) \( (p<0.01) \), IFX also increased serum lipid levels, including HDL-C (from 59.9 to 65.3 mg/dl, \( p<0.01 \)), and total cholesterol (from 192.6 to 203.8 mg/dl, \( p<0.05 \)) (Fig. 1). Furthermore, such increased serum adiponectin levels were independent of percent changes in disease activity, dose of steroid intake (Table 1), patient background. BMI (21.1 ± 2.8 kg/m\(^2\)) and SBP (124 ± 4.3 mmHg), which are known risk factors for atherosclerosis, were normal and did not change during the treatment.

**Discussion**

In the present study, we demonstrated that anti-TNF-α blockade significantly increased serum adiponectin and HDL-C levels in patients with RA. TNF-α is key mediator of inflammation and progression of atherosclerotic vascular disease, a process linked to the induction of cytokines, chemokines and adhesion molecules. TNF-α plays a role in the development of insulin resistance and is linked to the metabolism of fatty acids. Moreover, TNF-α can modulate many neuroendocrine immune functions, leading to unfavorable changes in the direction of an overall pro-inflammatory state. Anti-TNF therapy does not only result in immunosuppression by inhibiting TNF-α, but it also restores hormonal balance [6]. RA is a representative chronic systemic inflammatory disease and there are striking similarities between the inflammatory mechanisms op-
INDUCTION OF HYPERADIPONECTINEMIA BY IFX

...erating in atherosclerotic plaque and in rheumatoid synovitis. In fact, the cardiovascular events in RA patients are related to atherosclerosis caused by vascular endothelial dysfunction [7]. Therefore, the number of those events in RA patients is reported to be four times higher than in age- and sex-matched individuals free of RA. In RA, the primary site of inflammation is the synovium from which cytokines can be released into systemic circulation. High-grade inflammation increases insulin resistance and also lowers HDL-C, which contribute to atherogenesis in RA [8]. Moreover, the magnitude and chronicity of systemic inflammation in RA is particularly deleterious, such that even during quiescent phases of disease, systemic levels of cytokines remain high relative to non-RA subjects and thus may continue to promote vascular risk [9]. Thus, the degree of endothelial dysfunction in RA may primarily depend on the control of inflammatory activity rather than specific treatment. IFX is reported to induce monocytopenia and rapid downregulation of variety of important pro-inflammatory mediators such as IL-1, IL-6, IL-8, cytokine-inhibitors, and acute-phase proteins. In this regard, Hurlimann et al. [10] reported that short-term TNF-α blockade improved endothelial function in RA patients. Furthermore, Gonzalez et al. [11] assessed the effects of at least 1-year anti-TNF-α therapy in RA patients and showed improvement of endothelial function through endothelial-dependent vasodilatation, however, such improvement was only maintained over a period not exceeding 4 weeks. They suggested the involvement of genetic factors in the development of atherosclerosis in RA [11]. Kiechl et al. [12] also emphasized the role of inflammation-related genetic factors in determining vascular risk, since genetic polymorphism in the toll-like receptor 4 (TLR4) gene, which is implicated in innate immunity, is also associated with coronary atherosclerosis and predicts the risk of cardiovascular events.

Adiponectin has substantial anti-inflammatory properties both in vitro and in vivo. Our results suggested that the high levels of adiponectin associated with IFX treatment were not related to age, changes in arterial blood pressure, BMI, disease activity, or dose of prednisolone. In our study, some patients were on short-term low-dose oral corticosteroid therapy (3.3 ± 0.6 mg/day). In comparison, previous studies showed that

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**Table 1.** Treatment with IFX increased serum adiponectin level in an independent manner on disease activity score (DAS28) and dose of prednisolone

<table>
<thead>
<tr>
<th></th>
<th>%change of serum adiponectin</th>
<th>p</th>
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<tbody>
<tr>
<td>PSL&lt;5 (mg/day)</td>
<td>32.5 ± 5.6</td>
<td>N.S.</td>
</tr>
<tr>
<td>5 (mg/day)≤PSL</td>
<td>38.1 ± 6.8</td>
<td></td>
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<tr>
<td>DAS28≤5.1</td>
<td>31.1 ± 6.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>DAS28&gt;5.1</td>
<td>40.5 ± 6.3</td>
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**Fig. 1.** Treatment with IFX increased serum adiponectin, total cholesterol, high-density lipoprotein levels in patients with RA. Changes of (A) serum adiponectin levels, (B) cholesterol levels, (C) high-density lypoprotein levels before and after IFX treatment. Vertical lines represent the mean ± SEM values of each group. *p<0.05; **P<0.01, compared with the before IFX treatment.
short-term course of steroids or use of a dosage tailored to disease activity, does not adversely affect insulin sensitivity, and hence atherosclerosis [13]. In our study, despite no change in DAS-28, IFX significantly increased serum adiponectin levels at least in some patients. It is possible that IFX has direct or indirect effects; increasing serum adiponectin levels, probably through induction of transcription in adipose tissue of proliferators-activated receptor-γ (PPAR-γ) and liver receptor homolog-1 (LRH-1) [14]. Alternatively, IFX could bind to membrane-bound TNF-α, leading to activation of certain signal transmission pathways in adipocytes, that consequently results in increased adiponectin production by adipocytes and hyperadiponectinemia. In patients who do not show adequate suppression of synovitis, the dose of IFX may be insufficient to control disease activity, or the primary cause of synovitis is cytokines or chemokines other than TNF-α.

In conclusion, we demonstrated in RA patients that long-term anti-TNF-α therapy increased serum adiponectin levels, independent of disease activity, age, and prednisolone treatment. TNF-alpha and adiponectin exhibit opposite effects in human body. We propose that TNF-α blockade may protect against atherosclerosis directly or indirectly, by increasing serum adiponectin levels. TNF-α blockade may improve cardiovascular and cerebrovascular morbidity and mortality in patients with RA or other chronic inflammatory diseases.

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References