Influence of anti-tumour necrosis factor therapy on cardiovascular risk factors in patients with active rheumatoid arthritis

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**Background:** Tumour necrosis factor (TNF) is known to increase the concentrations of interleukin (IL) 6 and C reactive protein (CRP) and to induce proatherogenic changes in the lipid profile and may increase the cardiovascular risk of patients with rheumatoid arthritis (RA) and other inflammatory disorders.

**Objective:** To assess whether anti-TNF therapy modifies the cardiovascular risk profile in patients with RA.

**Methods:** The lipoprotein spectrum and the inflammation markers CRP and IL6 were investigated in 33 patients with RA treated with human anti-TNF monoclonal antibodies (D2E7, adalimumab, Humira) and 13 patients with RA given placebo, before and after 2 weeks' treatment.

**Results:** In the anti-TNF treated group, the mean (SD) concentrations of HDL-cholesterol were significantly higher after 2 weeks' treatment (0.86 (0.30) mmol/l v 0.98 (0.33) mmol/l, p<0.01), whereas LDL and triglyceride levels were not significantly changed. Additionally, a significant decrease in CRP (86.1 (54.4) mg/l v 35.4 (35.0) mg/l, p<0.0001), and IL6 (88.3 (60.5) pg/ml v 42.3 (40.7) pg/ml, p<0.001) concentrations was seen in this group. No changes in lipid profile, IL6, or CRP levels were seen in the placebo group.

**Conclusions:** TNF neutralisation with monoclonal anti-TNF antibodies increased HDL-cholesterol levels and decreased CRP and IL6 levels after 2 weeks. Therefore this treatment may improve the cardiovascular risk profile of patients with RA.

Moreover, carotid artery intima media thickness, as measured by ultrasound, is increased in patients with RA, suggesting a greater prevalence of subclinical atherosclerosis. In addition, C reactive protein (CRP) and interleukin (IL) 6 levels are both raised in patients with active RA, and these acute phase reactants have recently been shown to be associated with cardiovascular risk. In patients with RA, treatment with antifolates was shown to raise homocysteine levels, which was also characterised as an independent risk factor for developing cardiovascular disease.

Therapeutic strategies, aimed at TNF neutralisation with monoclonal antibodies or TNF receptor fusion proteins, have been shown to reduce disease activity and structural damage and to improve the quality of life in patients with RA. Moreover, blocking TNF results in a rapid decrease of acute phase reactants. Given these facts, we asked the question whether anti-TNF treatment would also change the cardiovascular risk profile. To this aim, we assessed the lipid profile and acute reaction markers before and 2 weeks after the first dose of anti-TNF or placebo in patients with active RA enrolled in monotherapy trials with a fully human anti-TNF monoclonal antibody (D2E7, adalimumab, Humira; Abbott Laboratories) at our centre.

**PATIENTS AND METHODS**

**Patients**

Patients with active RA included in phase I, double blind clinical studies with adalimumab monotherapy at our centre were studied. Patients fulfilled the 1987 American College of Rheumatology criteria, had an active disease as defined by a Disease Activity Score (DAS) >3.2 at baseline, and underwent a washout period for disease modifying antirheumatic drugs of at least 3 weeks before the start of the study. Stable doses of non-steroidal anti-inflammatory drugs and prednisone (<10 mg/day) were allowed during the study. Measurements of the variables studied were made on blood samples collected before the administration of an anti-TNF dose, at baseline and 2 weeks after starting treatment.

**Methods**

Fasting blood samples were collected in vacutainer tubes (Beckton & Dickinson, Rutherford, NJ) containing K3-EDTA (1 mg/ml), centrifuged at 3600 rpm for 8 minutes at 4°C, supplemented with saccharose as a cryoprotectant (final concentration 6 mg/ml), and frozen at −80°C until assay. Cholesterol and triglyceride (TG) were determined by commercially available enzymatic reagents on the Hitachi.

**Abbreviations:** CRP, C reactive protein; DAS, Disease Activity Score; HDL, high density lipoprotein; IL, interleukin; LDL, low density lipoprotein; RA, rheumatoid arthritis; TG, triglyceride; TNF, tumour necrosis factor.

747 analyser (Boehringer Mannheim, Germany), while HDL-cholesterol was determined by the phosphotungstate/Mg\textsuperscript{2+} method.\textsuperscript{10} LDL-cholesterol was calculated with the Friedewald formula, which provides reliable values up to a TG concentration of 8.0 mmol/l. IL6 was determined by a commercial enzyme linked immunosorbent assay (ELISA; BioSource Etten-Leur, The Netherlands), according to the manufacturer’s instructions.

CRP was measured by immunoturbidimetry with the Hitachi 747 analyser using reagents from Roche (Nos 1776371 and 1776428) and the calibrator BCDI. Sensitivity level was 1 mg/l and the coefficient of variation was <2%.

**Statistical analysis**

Within group comparisons were made using the Wilcoxon signed rank test (for IL6 and TG) and the paired Student’s t test (for CRP, HDL, LDL, and total cholesterol). Significance was set at the 0.05 level. Values are expressed as mean (SD).

**RESULTS**

Within 2 weeks of anti-TNF administration a 6% increase in total cholesterol concentrations was observed (4.70 (1.08) mmol/l v 5.02 (1.16) mmol/l, p = 0.001). This was mainly explained by an increase in the HDL-cholesterol concentrations (mean of 15%, from 0.86 (0.30) mmol/l to 0.98 (0.33) mmol/l, p<0.0001) (fig 1A). In contrast, LDL-cholesterol and TG measurements were not significantly changed (3.26 (0.88) mmol/l v 3.37 (1.02) mmol/l, mean 3%; fig 1B, and 1.46 (0.60) mmol/l v 1.38 (0.73) mmol/l, respectively). The LDL:HDL ratio and the total cholesterol:HDL ratio, significantly decreased in the anti-TNF treated group compared with placebo treated patients (4.01 (1.85) v 4.36 (2.16), p = 0.017, and 5.76 (2.42) v 6.24 (2.69), p = 0.004, respectively). The concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, and TGs, as well as the LDL:HDL and total cholesterol:HDL ratios, did not change after placebo administration. Figure 2 shows that the levels of CRP and IL6 decreased significantly within 2 weeks of anti-TNF administration (p<0.0001 and p<0.001 for CRP and IL6, respectively), whereas no changes were observed after placebo.

Clinical improvement occurred rapidly after initiation of anti-TNF therapy but not after placebo. This was reflected by a decrease in the DAS in the treated group and a stable DAS in the placebo group (5.24 (1.05) to 4.06 (1.14) and 4.8 (1.13) to 4.96 (1.4), respectively).

**DISCUSSION**

In this study we show that TNF\(\alpha\) neutralisation with anti-TNF\(\alpha\) monoclonal antibodies in patients with active RA significantly increases the total cholesterol, mainly owing to enhanced HDL-cholesterol concentrations already within 2 weeks of treatment. In contrast, there were no significant changes in the LDL-cholesterol and triglyceride concentrations, and the LDL:HDL ratio significantly decreased. These changes are concomitant with a significant decrease in the disease activity and acute phase reactants CRP and IL6. We therefore suggest that if these rapid changes in lipid pattern are maintained, this may also result in a decreased cardiovascular risk in patients with chronic inflammatory disorders.

It is well known that TNF\(\alpha\) induces hypertriglyceridaemia in animals and humans.\textsuperscript{2} This is due to an increased de novo fatty acid synthesis in the liver and esterification to TGs, to induction of lipolysis in adipose tissue, and to decreased lipoprotein lipase activity.\textsuperscript{2} From this perspective, one would expect a decrease in TG concentrations after anti-TNF therapy, though this was not the case in our study. This might be explained by the short follow up period or, alternatively, by the lower but still persistent inflammation at week 2.

Besides TNF\(\alpha\), other cytokines, including IL1\(\beta\), IL6, and interferon \(\gamma\), can modulate the lipid metabolism.\textsuperscript{2} TNF is a major inducer of these cytokines and TNF neutralisation may
result in a decrease of the above-mentioned cytokines. Therefore, possibly, the positive effect of TNFα blockade on the lipid profile is also mediated by the inhibition of other cytokines.

Before our study two other small studies had assessed the effect of TNF neutralisation with the chimeric monoclonal antibody infliximab on lipoproteins in patients with rheumatic diseases. Hurlimann et al also found a slight increase in total cholesterol in 11 patients treated for 12 weeks, but no data were provided about HDL, LDL, and TGs.11 In another study, Cauza et al found an increase in TGs and a decrease in HDL concentrations in seven patients with RA after an average of 3 weeks’ treatment.12 The discrepancies between the latter study and ours may be explained by the small number of patients tested by Cauza.

In patients with active RA, IL6 and CRP concentrations are increased and this is most probably mediated by the action of TNFα. The effect of anti-TNF therapy on the levels of acute phase reactants observed in this study is similar to the effect previously described.13–15 IL6 and CRP, as markers of inflammation, have recently been shown to be positively associated with the cardiovascular risk.6 In the general population, CRP information, have recently been shown to be positively associated with the cardiovascular risk. In the general population, CRP levels much lower than those found in RA are already associated with an increased cardiovascular risk. It is tempting to speculate that the decreased CRP, as a mirror of inflammatory status, leads to a decreased risk for atherosclerosis and cardiovascular events in patients with RA treated with TNF blocking agents, as an association between anti-inflammatory treatment and cardiovascular comorbidity was also previously seen in these patients.14

In conclusion, we would like to propose that anti-TNF treatment through both improving the lipid pattern (higher HDL-cholesterol and decreased LDL:HDL ratio) and decreasing inflammation, improves the cardiovascular risk profile of patients with RA. This is strongly supported by a recent study in a large cohort of patients with RA, where anti-TNF therapy was associated with a decreased incidence of cardiovascular events.15 Moreover, we are currently testing this hypothesis in our clinic in larger cohorts of patients, with a longer follow up and using objective measurements of the atherosclerotic process.

ACKNOWLEDGEMENT

Dr Calin Popa was partly supported by a grant from EULAR.

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Accepted 1 June 2004

Published Online First 1 July 2004

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