Decrease in articular hypoxia and synovial blood flow at early time points following infliximab and etanercept treatment in rheumatoid arthritis

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Abstract

Objective

An important feature of rheumatoid arthritis (RA) is hypoxia-driven synovial angiogenesis, but the relationship between change in vascularity, as measured by power Doppler ultrasound (PDUS), and oxygen tensions is unaddressed.

Methods

Metacarpophalangeal (MCP) joint PDUS was assessed in 23 patients with RA, alongside arthroscopic synovitis and oxygen tension measurements, at baseline and 4 weeks after anti-tumour necrosis factor (TNF) inhibitors.

Results

Anti-TNF reduced PDUS scores, which were negatively correlated with rise in oxygen tensions. The latter was related to good EULAR response at week 52.

Conclusion

Anti-TNF results in rapid reduction in synovial blood flow, with a corresponding rise in oxygen tension most marked in EULAR good responders.

Key words
tumour necrosis factor inhibitors, rheumatoid arthritis, ultrasonography, synovium

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Introduction

Tumour necrosis factor (TNF) inhibitors have greatly advanced the management of RA. Available agents differ in pharmacokinetics and ability to bind lymphotoxin, crosslink membrane-bound TNF and induce apoptosis (1). Whether these differences have biological consequences in RA is an unresolved question.

An early feature of RA is synovial angiogenesis which is thought to play a key role in progression of disease (2, 3). Quantitative measures of synovial vascularity using power Doppler ultrasound (PDUS) correlate with radiological damage over the following year (4), and changes in synovial PDUS signal can be detected following treatment with steroids or anti-TNF inhibitors (5). An important driver of synovial angiogenesis in RA is hypoxia, resulting from high metabolic demands, synovial proliferation outstripping angiogenesis, raised intra-articular pressure and dysfunctional neovasculature (3, 6).

We report findings comparing the effects of infliximab and etanercept on intra-articular oxygen tension and PDUS measurements in relation to clinical outcomes.

Patients and methods

Eligible patients were aged ≥18, who fulfilled the 1987 ACR classification criteria for RA, were rheumatoid factor or anti-CCP positive, with a disease duration >6 months and DAS28 >4.0, who previously failed at least one DMARD and were on a stable dose of ≥7.5mg methotrexate weekly. No other DMARDs were allowed within the 4 weeks prior to commencing treatment. Participants were randomised to either infliximab 3 mg/kg at weeks 0, 2 and 6 and then every 8 weeks, or etanercept 25 mg twice weekly for 52 weeks. Therapy was kept stable for the first 3 months but then changed as required. Clinical data was collected up to week 52. The study was conducted in compliance with the Helsinki declaration with ethical approval from West Glasgow Ethics Committee. All subjects gave written informed consent. Clinical trial registration number ISRCTN44880063.

Ultrasound

At baseline and week 4, all metacarpophalangeal joints (MCPJs) were assessed in the dorsal transverse plane using an Esaote MPX Technos (Esaote Biomedica, Genova, Italy) and a linear probe with a frequency range of 8–14 MHz (LA424). PDUS machine settings were kept constant (Gain 133, PRF 750Hz, medium wall filter) to facilitate comparison. Vascularity and synovial thickness were assessed on semi-quantitative scales (range 0–5) and results for all 10 MCPJs were summed.

Arthroscopy

At baseline and 4 weeks, subjects underwent single portal knee joint arthroscopy using a Storz 4.7mm arthroscope. Macroscopic synovitis was graded according to the system of Lindblad and Hedfors (range 0–4) (7). Oxygen concentrations were measured by amperometry using a 125 μm silver disc working electrode formed from a teflon-coated wire embedded in epoxy resin in a 19G needle (8-10). A large-area Ag/AgCl electrode was attached to the skin of the patient's ipsilateral foot and current measurements were made using a purpose-made potentiostat equipped with an isolated head stage. The operating voltage was chosen to lie at the midpoint of the plateau of the current-voltage curve. The resulting diffusion-limited current is independent of the applied potential, and directly proportional to the oxygen concentration (10). Measurements were taken at week 0 and 4 from the same knee which underwent arthroscopy.

Statistical analysis

Differences between groups and change over time were assessed using the Mann Whitney U and Wilcoxon tests. Correlations were performed using the Spearman rank test, and the Fisher's exact test was used to test associations with week 52 EULAR outcomes. Results were analysed by intention-to-treat.

Results

Patients

Twenty-three subjects were recruited; 12 randomised to infliximab and 11 to etanercept. Baseline characteristics were similar in both groups (Table I).

Table I. Baseline characteristics of subjects with RA randomised to etanercept or infliximab. Values are median (IQR) or number (%). Ultrasound (US) and power Doppler US (PDUS) scores were obtained by summing the results for all 10 MCPJs (Maximum score 50). The two treatment groups were compared with the Mann Whitney U-test, or Fisher's exact test where the data is categorical.

	All (n=23)	Etanercept (n=11)	Infliximab (n=12)	*p*-value
Age (years)	50 (46, 64)	55 (46, 68)	48 (45, 60)	0.41
Female (%)	22 (96)	10 (91)	12 (100)	0.48
Disease duration (years)	10 (4, 15)	12 (7, 15)	7 (2.5, 15)	0.15
Currently smoking	2	1	1	1.0
Methotrexate dose (mg/week)	15 (12.5, 20)	12.5 (7.5, 20)	15 (15, 20)	0.24
DAS28	6.73 (5.36, 7.07)	6.73 (5.73, 6.84)	6.72 (5.09, 7.34)	1.00
HAQ	1.6 (1.2, 2.1)	1.4 (1.1, 2.2)	1.6 (1.2, 2.0)	0.89
Knee synovitis score	3 (2, 4)	3 (3, 4)	3 (2, 4)	0.84
Intra-articular O_2 tension (mmHg)	31.1 (24.6, 47.5)	27.9 (25.1, 40.5)	36.5 (18.9, 48.7)	0.60
US synovial thickness score	17 (12, 31)	17 (13, 34)	19.5 (11.5, 28)	0.64
PDUS score	14 (9, 22)	12 (9, 25)	14.5 (8.5, 20.5)	0.92

Two patients discontinued etanercept at weeks 29 and 33 following adverse reactions, one was lost to follow-up. Three patients switched from infliximab to etanercept due to inadequate response, at weeks 12, 34 and 44. One patient in the infliximab group was lost to follow up at week 26.

Clinical, ultrasound and O_2 measurements

Median change in DAS28 scores at 4 and 12 weeks were -1.28 (IQR -2.6, -0.65; $p<0.001$) and -1.58 (IQR -2.4, -0.67; $p<0.001$). HAQ scores changed by -0.38 (IQR -0.62, -0.12; $p<0.001$) and -0.65 (IQR -1.12, -0.25; $p<0.001$). PDUS scores reduced from a mean (SD) of 16 (11) to 9 (8; $p<0.001$). Conversely, there was a rise in oxygen tensions from a median at baseline of 31.1 mmHg (range 10.3-64.5) to 44.6 mmHg (range 19.3-64.5) at 4 weeks (n=19; $p=0.018$). No differences in these measures were detected between infliximab and etanercept. Contrastingly, there was no overall change in median synovial thickness, although a small reduction did occur in the etanercept group (median change -3.0 *vs*. 1.0; $p=0.009$). No change in median synovitis scores was seen at 4 weeks (Fig. 1).

There was a statistically significant correlation between macroscopic synovitis in the knee and oxygen tensions at baseline (r=-0.49; $p=0.03$) but not at 4 weeks (r=-0.25; $p=0.30$). Patients with knee swelling at baseline (n=17) had a lower median pO2 than those without (n=3), although this did not reach statistical significance (28 mmHg *vs*. 40 mmHg; $p=0.34$). Patients without knee swelling at 4 weeks (n=5) had a trend to greater median rise in oxygen tensions than those with residual swelling (n=14), (+24 mmHg *vs*. +4mmHg; $p=0.21$). Change in oxygen tension at week 4 was correlated with change in power Doppler signal (r= -0.57; $p=0.02$), but not ultrasonographic synovial thickness, DAS28, tender or swollen joint counts, global VAS, ESR or HAQ. The ultrasonographic synovial thickness score correlated with the number of swollen (r=0.52; $p=0.01$) but not tender (r=0.04; $p=0.87$) MCPJs.

Prediction of EULAR good response at week 52

At week 52, 7 patients had a EULAR

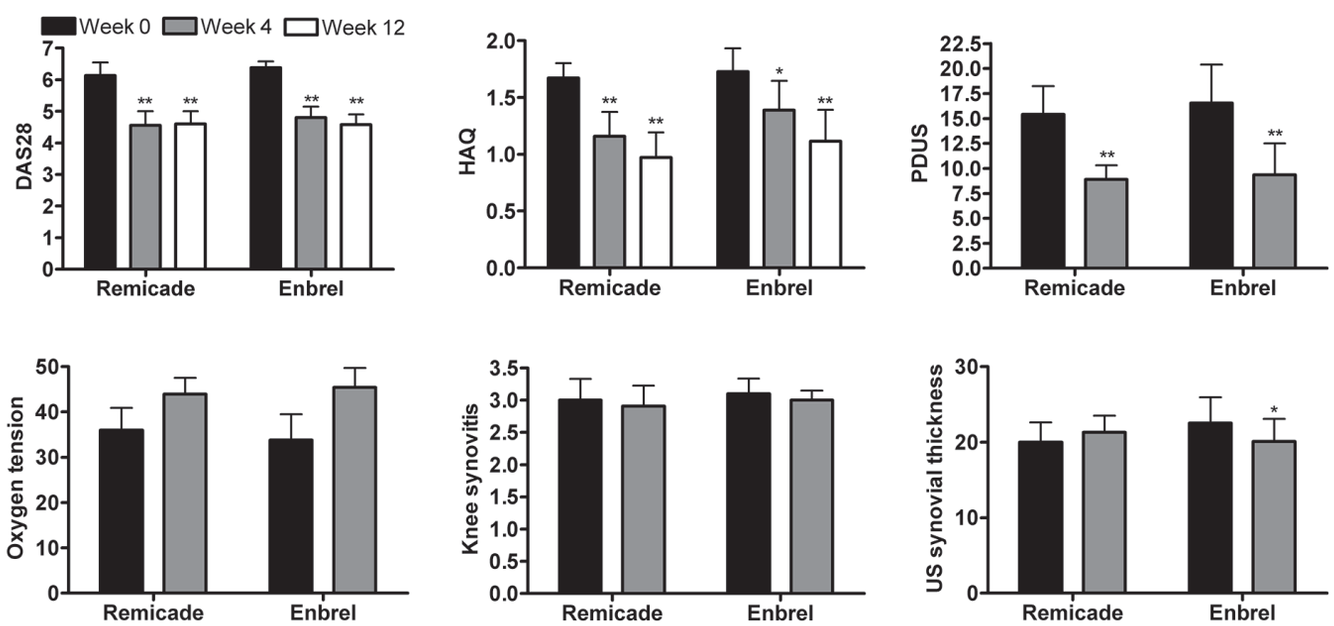

Fig. 1. Clinical, physiological and ultrasonographic outcomes at early time point in subjects with RA randomised to infliximab or etanercept. PDUS and US synovial thickness each measured on a semi-quantitative scale (0-5 for each MCPJ, summed so that total scores 0-50). Macroscopic synovitis in a knee joint, graded 0-4 at time of arthroscopy. Oxygen tension (mmHg) measured in a knee joint with a silver microelectrode. Results are mean (SE). DAS28, Disease Activity Score 28. HAQ, Health Assessment Questionnaire. IFX, infliximab. ETA, etanercept. $^{*}p<0.05$, $^{**}p<0.01$.

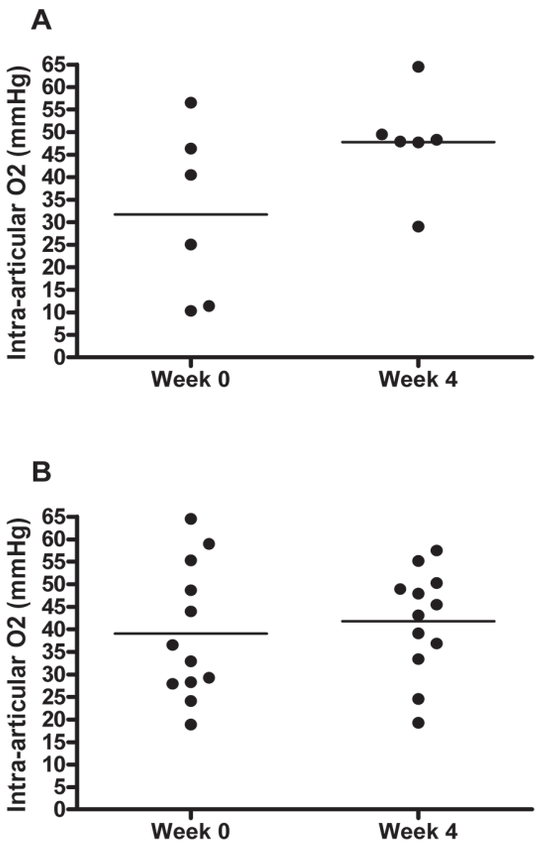

Fig. 2. Results of intra-articular oxygen tension measurements taken with a silver microelectrode from the knee joints of subjects before and 4 weeks after treatment with anti-TNF. Bars indicates mean.
A: Subjects who went on to have a EULAR good response at week 52 (n=6);
B: Subjects with non-response or moderate response at week 52 (n=12).

good response (5 and 2 in the etanercept and infliximab groups respectively; p=0.19), 12 a moderate response (7 infliximab and 5 etanercept), 2 no response (failed both infliximab and etanercept) and 2 were lost to follow-up. Clinical, ultrasonographic and oxygen tension measurements at 4 weeks were used in an attempt to predict good response at 1 year. No predictor was statistically significant but there was a trend for oxygen tension measurements. Three out of 4 patients (75%; the 4th being lost to follow-up) with a rise >20 mmHg at 4 weeks had a EULAR good response compared with 3/15 (20%) without (p=0.07). The median increases in oxygen tensions at 4 weeks were 2 mmHg and 14 mmHg for those with non-response (n=2) and good response (n=6) at week 52 respectively (Fig. 2).

Discussion

Angiogenesis is an early feature of synovial proliferation in RA (11, 12) and may be of importance in perpetuation of synovitis (2). It is promoted by hypoxia (13) independently of TNF, and previous studies in RA have demonstrated low oxygen tensions in both synovial fluid (3, 14) and synovium (9). More recent data has shown a correlation between the level of hypoxia and synovitis (13, 15, 16). Kennedy *et al*. recently reported a rise in synovial tissue pO2 in patients with RA and psoriatic arthritis 3 months post anti-TNF treatment.

In this study we confirmed the observations of intra-articular hypoxia in RA with values as low as 10.3 mmHg (median 31 mmHg). We also showed that baseline oxygen levels correlate with macroscopic synovitis. A demonstrable rise in oxygen tension was observed after 4 weeks of treatment with anti-TNF in the context of a randomised trial. At this early time point, synovial vascularity measured by PDUS was significantly reduced, no change was found in macroscopic synovitis in the knee, or in overall MCPJ synovial thickness measured in the transverse plane by ultrasound, notwithstanding a small reduction in the etanercept group, suggesting that the rise in oxygen tension at 4 weeks reflects a reduction in metabolic demand rather than synovial volume; the latter resolving more slowly. This is supported by the lack of correlation between macroscopic synovitis and oxygen tension at week 4. Contrastingly Kennedy *et al*. found that macroscopic synovitis correlated with synovial tissue pO2 before and after anti-TNF therapy. This may reflect the later post-therapy time point of 3 months used in their study, although there were differences in the methods of measuring oxygen tension and synovitis, and in the cohorts studied (*e.g*. anti-CCP prevalence 45% *vs*. 83% in the study reported here).

A novel finding is that change in oxygen tension was negatively correlated with change in PDUS signal, despite being measured in different joints, whereas no significant relationship was seen with clinical variables at this early time point. PDUS may provide a useful surrogate for pathophysiological data that is too subtle to be detected by routine clinical measures of local inflammatory burden in joints that are assessed in a binary manner (either swollen or not swollen). This further justifies the ongoing investigation of PDUS and its pathophysiological correlates as a biomarker in RA (17). The rapid fall in vascularity on TNF inhibition may partly be due to reduced angiogenesis and partly decreased vascular shunting. The resulting restoration of blood flow autoregulation would be accompanied by a trend to normalisation of tissue oxygen tension. In keeping with this hypothesis, our study also suggests that large rises in oxygen tension at 4 weeks might be predictive of a EULAR good response at 52 weeks

In conclusion, anti-TNF therapy is accompanied by a rapid reduction in synovial blood flow, assessed by PDUS, and a corresponding rise in oxygen tension that is most marked in EULAR good responders.

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