"Interleukin-6 Blockade Raises LDL Via Reduced Catabolism Rather Than Via Increased Synthesis – A Cytokine-Specific Mechanism For Cholesterol Changes In Rheumatoid Arthritis"

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Abstract

Objectives: Patients with rheumatoid arthritis (RA) have reduced serum LDL-cholesterol (LDL-c), which increases following therapeutic IL-6 blockade. We aimed to define the metabolic pathways underlying these lipid changes.

Methods: In the KALIBRA study, lipoprotein kinetic studies were performed on 11 patients with severe active RA at baseline and following three I.V. infusions of the IL-6R blocker Tocilizumab. The primary outcome measure was the fractional catabolic rate (FCR) of LDL.

Results: Serum total cholesterol (4.8 v 5.7mmol/L, p=0.003), LDL-c (2.9 v 3.4mmol/l, p=0.014) and HDL-c (1.23 v 1.52mmol/L, p=0.006) increased following Tocilizumab therapy. The LDL FCR fell from a state of hypercatabolism to a value approximating that of the normal population (0.53 v 0.27 pools/day, p=0.006). Changes in FCR correlated tightly with changes in serum LDL-c and CRP but not CDAI.

Conclusions: RA patients have low serum LDL-c due to hypercatabolism of LDL particles. IL-6 blockade normalises this catabolism in a manner associating with the acute phase response (and thus hepatic IL-6 signalling) but not with RA disease activity as measured clinically. We demonstrate that IL-6 is one of the key drivers of inflammation-driven dyslipidaemia.

Introduction

Patients with rheumatoid arthritis (RA) have serum low-density lipoprotein cholesterol (LDL-c) levels lower than those of age-and sex-matched controls (1-3), despite also having an approximately 50% greater risk of developing cardiovascular disease (CVD) (4, 5). Conversely, increases in LDL-c or LDL particle numbers have also been observed following treatment of RA with the interleukin-6 (IL-6) receptor blocker Tocilizumab (6-8) and the janus kinase (JAK) inhibitors (9). The mechanisms underlying this so-called "lipid-paradox", and the influence of therapy-driven LDL-c changes on cardiovascular risk, remain only partially understood(10).

Reduced serum LDL-c might be due to reduced LDL synthesis (predominantly from lipolysis of very low-density lipoprotein [VLDL] and intermediate-density lipoprotein [IDL]) or increased LDL turnover. Hypercatabolism of LDL has previously been identified in hypertrigliceridaemic patients, with catabolic ratesnormalising following reduction of serum TG with fibrates(11).

Tocilizumab is an established and effective treatment for RA which has been shown to increase LDL-c by up to 20% on average (7). We therefore hypothesised that Tocilizumab would reduce LDL catabolism and thus increase serum LDL-c in patients with active RA.

Methods

In the KALIBRA study (**K**inetics of the **A**poB-containing **L**ipoproteins in **I**L-6 **B**lockade for **RA**) we performed kinetic studies on patients with active RA before and after ten weeks' treatment with Tocilizumab. The primary outcome measure was the change in fractional catabolic rate (FCR) of LDL-associated ApoB.

Recruitment

Subjects were recruited from rheumatology clinics in Glasgow, UK. Patients were provided with information on the study for at least 48 hoursbefore a screening visit where written, informed consent was provided. All further assessments took place at the Clinical Research Facility (CRF) of the Western Infirmary Glasgow. Subjects met the following inclusion criteria: RA (2010 ACR criteria); DAS28≥5.1; failure of two conventional DMARDS, including methotrexate; and suitability for tocilizumab therapy. Exclusion criteria included: familial dyslipidaemia; ApoE 2/2 homozygosity; diabetes mellitus; use of lipid-lowering therapeutics; fasting TC ≥6.5mmol/L; fasting TG ≥3mmol/L; pregnancy; or untreated hypothyroidism. Use of oral steroid was permitted at a steady dose, but parenteral corticosteroid was prohibited.

Ethical approval for the KALIBRA study was granted locally by the West of Scotland Research Ethics Committee.

Kinetic studies

Each subject underwent a kinetic study at baseline, followed by at least 10 weeks (i.e. three infusions) of Tocilizumab 8mg/kg IV, and a subsequent "on-treatment" kinetic study.

In each kinetic study, a tracer in the form of the stable isotope d3-leucine at 10mg/kg body weight bolus was administered. Leucine is taken up by hepatocytes and incorporated into apoB. Fasting blood samples were obtained at 23 timepoints over 96 hours. Density-gradient ultracentrifugation, precipitation and gas chromatography mass spectrometry (GCMS) could then be used to determine the tracer/tracee ratio in all the apoB-containing lipoproteins at each timepoint. A 15-compartment mathematical model was used to analyse the the lipolytic pathway through VLDL1, VLDL2, IDL and LDL. SAAM software was then employed to calculate the production rate (PR) and fractional catabolic rate (FCR) of LDL.

Sample handling & processing

At each kinetic study, blood samples were obtained for beta-quantification of lipids; CRP and ESR; Lp(a); insulin; PCSK9 levels; apolipoproteins by immunoturbidimetry; and activity of CETP and heparin-inducible lipasesDAS28 and CDAI were assessed by a clinical research fellow in rheumatology.

Statistical analyses

Statistical analysis was performed at the Robertson Centre for Biostatistics, University of Glasgow. Pre- and post-treatment analyses were performed using paired t-test or Wilcoxon matched-pairs test. Correlations were performed using Spearman's r, due to the high prevalence of non-parametric data.

In normal subjects, the FCR of LDL-associated apoB is around 0.3 pools per day. In those with increased catabolism due to hypertriglyceridemia in a previous study, the FCR is around 0.5 pools per day; this corresponded to a large decrease in LDL-c of around 1.5mmol/l (12). In contrast, ciprofibrate has been shown to increase LDL FCRfrom 0.32 to 0.38 pools per day, with a 22% fall in LDL-c (13). This is similar LDL-c changes seen following tocilizumab therapy, and reflects a biologically significant change in FCR. Using these figures, we used a conservative change in FCR of around 0.05 pools per day to determine power. A sample size of 15 subjects allows us to detect a difference in fractional catabolic rate of 0.05 with SD 0.05 at 90% power and alpha error at 5%. This sample size is typical for kinetic trials of this type.

Reagent supply

D3-Leucine was prepared by Tayside Pharmaceuticals Ltd, Dundee, UK. Tocilizumab for this study was graciously provided by Roche Products Ltd

Results

Demographics

Twelve subjects were recruited (Table 1). One subject withdrew before their second kinetic study, leaving useable data for eleven subjects.

Clinical response

At baseline, mean DAS28-CRP and CDAI were 5.16 and 29.9 respectively. Changes in parameters of disease activity are summarised in the online supplementary figure S1. After treatment, seven subjects were in DAS28 remission, whilst one met the criteria for CDAI remission.

Serum cholesterol

Beta-quantification of serum lipids is shown in online supplementary table S1. Elevations were observed in TC, LDL-c and HDL-c. No change was seen in the TC/HDL-c ratio.

LDL kinetics

Production and catabolic rates for LDL are displayed in Figure 1. Median (IQR) FCR, the primary outcome measure for the study, fell from 0.53 (0.32-0.68) pools/day to 0.27 (0.19-0.37) pools/day (p=0.002), with median change from baseline of -30%. LDL PR also fell significantly, with reduced apoB transfer from VLDL-2 through IDL to LDL.

LDL FCR associated strongly with LDL-c both at baseline and in degree of change from baseline (Figure 2A). At baseline, FCR correlated with CRP (r=0.74, p=0.012) and showed a non-significant trend to association with ESR (r=0.54, p=0.091). However, FCR did not associate at all with clinical assessment of disease activity, as measured by CDAI (r=0.04, p=0.91) (Figure 2B). Similar, non-significant trends were seen in degree of change for FCR versus CRP (r=0.46, p=0.15), ESR (r=0.30, p=0.37) and CDAI (r=-0.37, p=0.26)

Secondary outcomes

Serum Lp(a) fell, whilst ApoAI, ApoAII, and ApoB increased. Activity levels of CETP, lipoprotein lipase, and hepatic lipase, and serum levels of insulin or PCSK9, did not change (online supplementary table S2).

Safety

Two serious adverse events were reported. One patient developed a subcutaneous abscess;; this was successfully treated, and the patient resumed therapy and completed the study. Another patient developed paronychia and withdrew from the study.

Discussion

KALIBRA is the first study to demonstrate increased catabolism of LDL particles in active RA. This hypercatabolism correlates tightly with serum LDL-c and acute phase reactants but not with clinical measures of RA activity, and normalises following IL-6 blockade with tocilizumab. Tocilizumab also

reduced LDL production, though this appears to be of negligible biochemical significance as LDL-c ultimately increased.

These findings allow us to draw some conclusions. Firstly, IL-6 appears to be a key driver of LDL change in RA. We surmise this from tocilizumab's mechanism of action, but also from the association of LDL kinetics ith the acute phase response (driven by IL-6) rather than clinical disease activity; hence, hepatic IL-6 signalling, rather than synovitic bulk, might lie behind LDL changes. Finally, these results are reassuring in terms of the safety of IL-6 blockade, as normalisation of pathological LDL hypercatabolism seems unlikely to drive new atheroma formation.

Charles-Schoeman et al previously performed lipid kinetic studies in 33 RA subjects before and after 6 weeks of treatment with tofacitinib, a Janus kinase (JAK) inhibitor, and 31 healthy volunteers (14). This cohort had increased catabolism of cholesterol ester (but not LDL-associated ApoB) at baseline, which was reduced by tofacitinib. The authors hypothesised decreasing process of reduced hepatic HDL clearance, with LDL then gaining cholesterol via CETP, reflecting a potentially different mechanism to our own observations.

We believe that KALIBRA holds some advantages over this earlier work. Firstly, using tocilizumab allows us to identify IL-6 as the key molecule behind our results. Tofacitinib, as an inhibitor of multiple JAKs (and thus many upstream cytokines), cannot provide such precise mechanistic information. Secondly, all our patients had severe active RA with DAS28≥5.1. This makes our results more relevant to clinical practice, and gives greater scope for detectable inflammation-driven pathology (and subsequent detectable reversal of that pathology). Charles-Schoeman did not provide values for DAS28, CDAI, ESR or CRP, though the inclusion criteria seem to indicate lower disease activity than our own cohort. Thirdly, our collection of clinical data pre- and post-treatment allowed us to ascertain clinical response to the drug, and correlate this with metabolic changes.

Our study has some important limitations. The small sample size, whilst in keeping with previous such kinetic studies, limited our ability to analyse secondary outcome data. Local technical limitations precluded a control group; however, the magnitude of kinetic changes observed are close to previously-described population values of hypercatabolism (at baseline) and normal metabolism (post-treatment).

In conclusion, we demonstrate for the first time that serum LDL-c levels are reduced in active RA due to abnormally elevated LDL catabolism. Additionally, we show that this hypercatabolic state is exquisitely linked to IL-6 signalling, and normalises following therapeutic IL-6 blockade regardless of any clinical reduction in disease activity.

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Competing Interests

IM, NS, DM, CP and DP have received honoraria from or provided consultancy services for Roche / Chugai. JR has received personal fees from Janssen outside the submitted work.MC report no relevant conflicts of interest.

Contributorship

NS and IM conceived the study. IM, NS and CP wrote the study protocol. DP acted as principle investigator for the study. JR drafted and revised the manuscript. All other authors reviewed the draft manuscript and provided feedback for the final version.

Selected results from this manuscript have been presented previously at the 2016 EULAR congress. Ref: "IL-6 Blockade raises LDL via reduced catabolism rather than via increased synthesis: a cytokine-specific mechanism for cholesterol changes in RA. Robertson J, Porter D, Sattar N, Packard C, Caslake M, McInnes I, McCarey D. Ann Rheum Dis 2016;**75**:Suppl 2 476-477

Ethical Approval Information

Ethical approval for the KALIBRA study was granted locally by the West of Scotland Research Ethics Committee.

Data sharing statement

Full kinetic data for apoB-containing lipoproteins (VLDL-1, VLDL-2, IDL and LDL) may be available on discussion with study authors.

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Tables

	Mean (%)
Age	49.9
Sex (F)	10 (83%)
RF / CCP +	10 (83%)
Methotrexate	4 (33%)
Other DMARD	8 (67%)
Prednisolone	2 (17%)
NSAID	8 (67%)
Previous Biologic therapy	3 (25%)

Table 1. Demographic data of KALIBRA subjects at baseline. N=12.

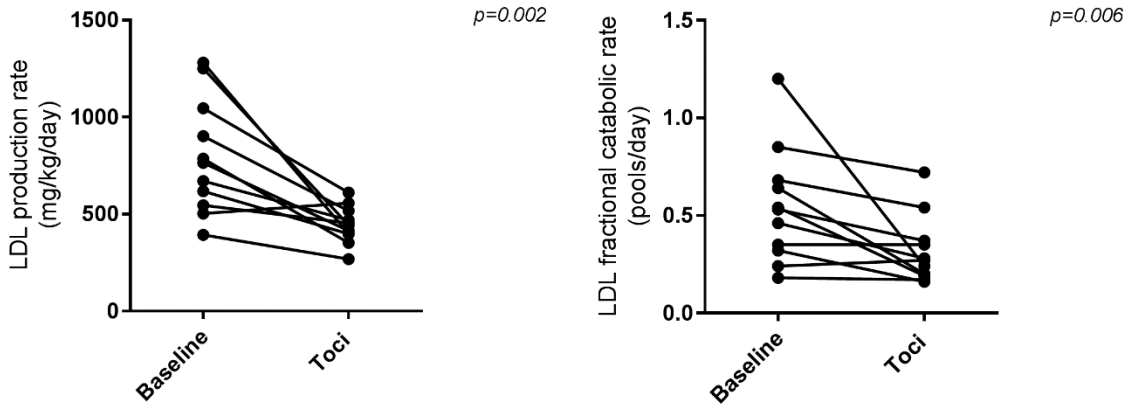

Figure 1. Changes in LDL production rate and FCR after treatment with tocilizumab (N=11). P value generated by Wilcoxon matched-pairs test. FCR,fractional catabolic rate; LDL, low-density lipoprotein.

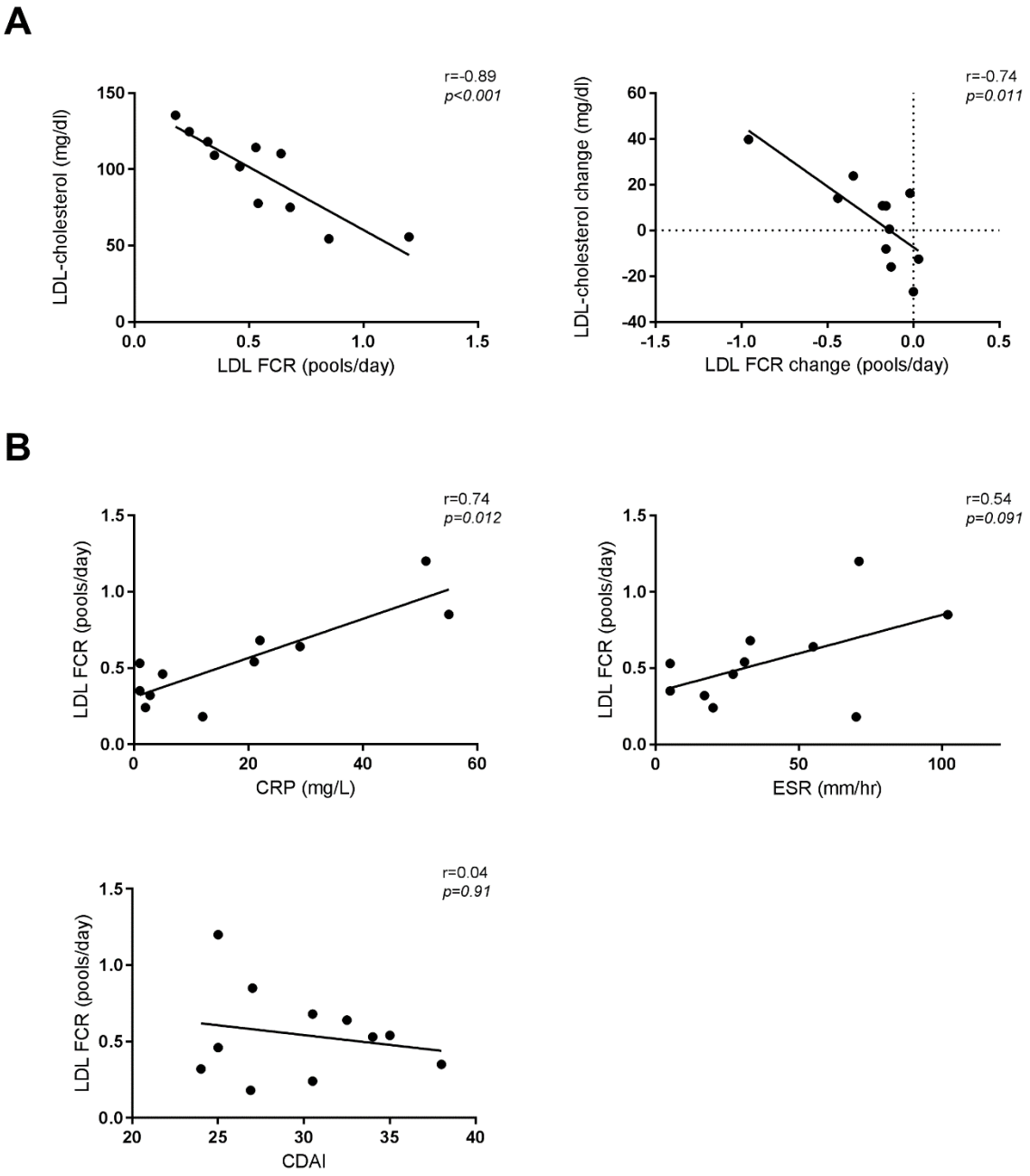

Figure 2. (A) Correlations of LDL FCR with LDL-c at baseline (left) and in degree of change (right). (B) Correlations of LDL FCR with markers of RA disease activity. N=11. R and p values calculated by Spearman's *r* test. CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FCR, fractional catabolic rate; LDL-c, low-density lipoprotein cholesterol; RA, rheumatoid arthritis.