

## Inflammation and Atherothrombosis: The Beginning of the End of a Hypothesis

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### Abstract

The inflammatory hypothesis for atherothrombosis posits that inflammation drives the development of atherosclerotic plaque and its progression to thrombosis. Empirical data have suggested the relation of inflammation to the Virchow's triad: endothelial injury, blood stasis, and hypercoagulable state. Evidence from clinical studies also pointed to a potential benefit in thrombotic risk reduction from inhibiting inflammation. Until recently, interleukin-1 $\beta$  blockade with canakinumab has been investigated for preventing recurrent cardiovascular events among patients with prior myocardial infarction and demonstrated compelling outcomes. Furthermore, the results ascertain that a downstream inflammatory biomarker, high-sensitivity C-reactive protein, could be utilized to identify at-risk patient subsets associated with significant benefits from anti-inflammatory therapy. As research efforts are closing the knowledge gap between inflammation and thrombosis, the potential role of inflammation in other cardiovascular diseases also presents new challenges for future studies.

**Keywords:** Thrombosis; Atherothrombosis; Atherosclerosis; Acute Coronary Syndrome; Anti-Inflammatory Agents; Interleukin 1 $\beta$ ; C-Reactive Protein; Canakinumab

### Abbreviations

ACS: Acute Coronary Syndrome; hsCRP: High-Sensitivity C-Reactive Protein; IL: Interleukin; LDL-C: Low-Density Lipoprotein Cholesterol; MI: Myocardial Infarction; NSTEMI: Non-ST-Elevation Myocardial Infarction; STEMI: ST-Elevation Myocardial Infarction

### Introduction

Inflammation has been postulated to mediate the initiation and development of atherosclerotic plaque as well as its progression to thrombus formation and myocardial infarction [1]. Preclinical data have suggested an active role of proinflammatory cytokines in atherogenesis [2-5]. The inflammatory hypothesis was tested by subsequent clinical studies on agents with anti-inflammatory properties such as statins and colchicine [6-8]. It is noteworthy that the reduction in inflammatory biomarkers such as high-sensitivity C-reactive protein (hsCRP) was found to be predictive of treatment effect on the cardiovascular outcomes [9-11]. Recently, it was demonstrated that canakinumab, an interleukin-1 $\beta$  (IL-1 $\beta$ ) neutralizing antibody, reduced the risk of recurrent major adverse cardiac events in patients with a history of myocardial infarction and elevated hsCRP levels [12]. These results strongly support that inflammation is not only a pivotal mediator but also a malleable therapeutic target in atherothrombosis. This article aims to summarize the completed and ongoing studies that have advanced the knowledge concerning the association of atherothrombosis to inflammation, inflammatory biomarker, and anti-inflammatory therapy.

### Completed Studies

#### CARE Trial

In CARE trial (Cholesterol And Recurrent Events) [6], pravastatin 40 mg daily demonstrated an inhibitory effect on C-reactive protein (CRP) concentration in post-myocardial infarction (MI) patients without affecting plasma levels of low density lipoprotein cholesterol (LDL-C). It is noteworthy that CRP was significantly correlated with cardiovascular risks among post-MI patients. Therefore, it was concluded that pravastatin therapy may improve the atherosclerosis and atherothrombosis in post-infarct settings, to some extent based on its anti-inflammatory effect.

### AFCAPS/TexCAPS Trial

In AFCAPS/TexCAPS trial [13], a total of 6,605 cardiovascular disease patients without hyperlipidemia underwent therapy with lovastatin 20 mg daily. Elevated baseline CRP levels were found to be associated with a greater risk of cardiovascular events. Administration of lovastatin was associated with a significant reduction of CRP concentration, an effect independent of LDL-C concentration. Lovastatin was therefore considered to be a potential atheroprotective agent for patients with a normal lipid profile and high CRP through its anti-inflammatory effect [9].

### PROVE IT Trial

In the PROVE IT (Pravastatin or Atorvastatin Evaluation and Infection Therapy) trial (ClinicalTrials.gov identifier: NCT00382460) [10], an improvement in long-term cardiovascular outcomes with a significant decrease in CRP levels was observed in post-ACS patients after treatment with atorvastatin 80 mg or pravastatin 40 mg daily, which was completely independent of LDL-C plasma levels. At the end of the study it was suggested that CRP monitoring should to be included in cardiovascular events modifications secondary to statins.

### REVERSAL Trial

In the REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) trial that included patients with chronic ischemic cardiovascular disease [14], high-intensity statin therapy with atorvastatin 80 mg daily reduced both CRP and LDL-C, and led to subsequent atherothrombosis regression when compared to moderate-intensity statin therapy with pravastatin 40 mg daily. It appeared that a more profound cardiovascular benefit was related to a more prominent decline in CRP secondary to statin.

### A to Z Trial

In the A to Z (Aggrastat-to-Zocor) trial (ClinicalTrials.gov identifier: NCT00251576) [15], hsCRP was measured in non-ST-elevation MI (NSTEMI) and ST-elevation MI (STEMI) patients under statin therapy with simvastatin 40 mg daily for 1<sup>st</sup> month followed by 80 mg daily until 4<sup>th</sup> month. It is found that statin improved the long-term outcome and decreased the hsCRP level in post-ACS patients, independently of other potential confounding factors. The decline in cardiovascular risks was moderate in the first month with modest-dose statin, which later became amplified until the fourth month with high-dose statin regimen.

### JUPITER Trial

The highest attention attracted in this field was through the JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) (ClinicalTrials.gov identifier: NCT00239681) [7], which investigated the effect of rosuvastatin 20 mg daily in 17,802 healthy people without an indication for statin therapy (LDL-C < 130 mg/dL). It was demonstrated that rosuvastatin protected against major cardiovascular events in patients with higher levels of hsCRP, a non-specific inflammatory marker. However, a decline in the LDL-C levels secondary to statins has cast some doubt on the net conclusion of correlation between anti-inflammation and clinical benefit of atherosclerosis [16].

### ASCOT Substudy

A substudy of the ASCOT (Anglo-Scandinavian Cardiac Outcome Trial) trial [17] seemed to suggest that CRP concentration was not predictive of cardiovascular events in hypertensive patients. Decreased CRP levels secondary to atorvastatin 10 mg daily was not significantly related to cardiovascular risk reduction [18]. A plausible explanation for this controversy was the very low dosage of statin. Other possible reasons for the seemingly weak predictability for CRP levels have been reviewed in detail elsewhere [19].

### ENTRACTE Trial

The ENTRACTE trial (ClinicalTrials.gov identifier: NCT01331837) was designed to compare the cardiovascular risk in 3,000 patients with rheumatoid arthritis allocated to tocilizumab 8 mg/kg monthly or etanercept 50 mg weekly [20]. Tocilizumab and etanercept were associated with < 5% of various cardiovascular events, and there was no significant relationship between these anti-inflammatory therapies and increased cardiovascular risks.

### FRANCIS-ACS Trial

In the FRANCIS-ACS (Fewer Recurrent Acute coronary events with Near-term Cardiovascular Inflammation Suppression) trial (ClinicalTrials.gov identifier: NCT00743925), biomarkers such as LDL-C, hsCRP, and PLA2 along with major adverse cardiac events were compared in acute coronary syndrome (ACS) patients who received atorvastatin 80 mg daily plus either placebo or varespladib, a non-specific secretory PLA2 inhibitor [21]. There was a significant decrease in biomarkers but modest effect on long-term cardiovascular events after treatment with varespladib 500 mg daily.

### PLASMA I and II Trials

In the PLASMA (Phospholipase Levels And Serological Markers of Atherosclerosis) I and II trials (ClinicalTrials.gov identifier: NCT00525954) [22,23], varespladib 500 mg daily demonstrated a significant improvement in lipid profile; however, it was not associated with a significant effect on cardiovascular risks and atherothrombosis.

### VISTA-16 Trial

The VISTA-16 (Vascular Inflammation Suppression to Treat ACS for 16 weeks) (ClinicalTrials.gov identifier: NCT01130246) trial aimed to evaluate the effect of varespladib 500 mg daily in 5,000 ACS patients [24]. The study was terminated prematurely due to futility and possible harms. Data from the VISTA-16 trial showed no significant improvement in cardiovascular outcome related to treatment with varespladib.

### SOLID-TIMI 52 Trial

The SOLID-TIMI 52 trial (Stabilization of pLaques usIng Darapladib – Thrombolysis in Myocardial Infarction) (ClinicalTrials.gov identifier: NCT01000727) [25] included 1,813 subjects treated with darapladib 160 mg daily or placebo plus their routine therapies. After a follow-up duration of 2.5 years, no significant improvement in major adverse cardiac event was observed when comparing darapladib versus placebo.

### STABILITY Trial

In the STABILITY trial (STabilization of Atherosclerotic plaque By Initiation of darapLadib Therapy) (ClinicalTrials.gov identifier: NCT00799903) [26], a total 15,828 ACS patients were randomized to darapladib 160 mg daily or placebo and followed for a period of 3.7 years. There was no significant effect from darapladib on the composite endpoint of cardiovascular death, MI, or stroke.

### ARISE Trial

The ARISE (Aggressive Reduction in Inflammation Stops Events) trial (ClinicalTrials.gov identifier: NCT00066898) evaluated the effect of succinobucol 280 mg daily in 6,144 subjects with recent ACS and found no significant improvement in cardiovascular outcome [27]. While there was an increased rate of atrial fibrillation with elevated lipid levels and blood pressure, there was no corresponding reduction of IL-6 and CRP secondary to succinobucol.

### SELECT-ACS Trial

In the SELECT-ACS trial (ClinicalTrials.gov identifier: NCT01327183) that studied the P-selectin antagonist inclacumab among 544 NSTEMI subjects following percutaneous coronary intervention [28], inclacumab at the dose of 20 mg/kg single infusion reduced the rate of myocardial damage compared with placebo.

### SELECT-CABG Trial

In the SELECT-CABG trial (ClinicalTrials.gov identifier: NCT01245634) [29], a total of 384 ACS patients undergoing elective or urgent coronary artery bypass graft surgery were randomized to anti-P-selectin monoclonal antibody inclacumab (20 mg/kg) or placebo. Inclacumab did not demonstrate a significant effect on CABG outcome, specifically the rate of saphenous vein graft failure. However, based on a *post hoc* analysis, it is suggested that baseline P-selectin values may play a role in the final outcome of these ACS patients.

### VIA-2291 Trial

In a dose-ranging study evaluating the effect of 5-lipoxygenase inhibitor VIA-2291 (atreleuton) versus placebo on various inflammatory biomarkers in patients with recent acute coronary events (ClinicalTrials.gov identifier: NCT00358826) [30], a total of 191 post-ACS patients were followed for a duration of 3 months. A significant lower rate of new coronary plaques was reported secondary to atreleuton (100 mg) compared with placebo. Non-calcified plaques volume was also improved significantly in patients receiving atreleuton.

### LoDoCo Trial

In the LoDoCo Trial (ANZCTR identifier: ACTRN12614000093684) [31], adding colchicine to anti-platelets and statins in patients with stable coronary disease significantly reduced the risk of acute coronary syndrome, out-of-hospital cardiac arrest, or non-cardioembolic ischemic stroke. The anti-inflammatory properties of colchicine, most notably the inhibition of neutrophil function, are reflected by corresponding reduction in high-sensitivity C-reactive protein (hsCRP) concentrations and are considered to account for its cardiovascular benefits.

### CANTOS Trial

In the landmark CANTOS trial (ClinicalTrials.gov identifier: NCT01327846) [12], a total of 10,061 patients with a history of myocardial infarction and elevated hsCRP levels were randomized to receive placebo or canakinumab, a monoclonal antibody targeting the interleukin 1 $\beta$  (IL-1 $\beta$ ). Canakinumab at the dosage of 150 mg once every three months reduced the rate of the primary endpoint of myocardial

infarction, stroke, or cardiovascular death by 15% independent of lipid profile alterations [12]. Furthermore, long-term IL-1 $\beta$  blockade with canakinumab was associated with augmented cardiovascular benefits among subjects with on-treatment hsCRP levels controlled below 2 mg/L: a 27% reduction in thrombotic events and 31% reduction in all-cause mortality or cardiovascular mortality [32].

### Ongoing Studies

Four major trials currently underway are closing the loop of the inflammatory hypothesis for atherothrombosis: COLCOT (Colchicine Cardiovascular Outcomes Trial), LoDoCo2, CIRT (Cardiovascular Inflammation Reduction Trial), and CLEVER-ACS (Controlled Level EVERolimus in ACS) trials. The COLCOT trial (Colchicine Cardiovascular Outcome Trial) (ClinicalTrials.gov identifier: NCT02551094) will investigate the therapeutic anti-inflammatory effects of colchicine in coronary artery disease (CAD) patients [33]. Similarly, the LoDoCo2 trial (EudraCT identifier: 2015-005568-40) will study the incremental benefit of low-dose colchicine (0.5 mg daily) to the routine regimen for secondary cardiovascular prevention [34]. The CLEVER-ACS trial (Controlled Level EVERolimus in ACS) (ClinicalTrials.gov identifier: NCT01529554) is currently gathering data from post-STEMI patients on the atheroprotective effect of everolimus (7.5 mg for 3 days, followed by 5 mg for 2 days in a 5-day regimen), which inhibits the inflammation through the mTOR pathway [35]. Lastly, the CIRT trial (Cardiovascular Inflammation Reduction) (ClinicalTrials.gov identifier: NCT01594333) from the National Heart, Lung, and Blood Institute will assess the effect of low-dose methotrexate (15 to 20 mg weekly) on cardiovascular atherothrombosis events in 7,000 patients with a history of stable coronary artery disease plus type 2 diabetes or metabolic syndrome [36]. These results will be essential for expanding the evidence base of anti-inflammatory agents that aim to further improve cardiovascular outcomes on top of the standard medical treatment.

### Conclusions

Inflammation is not only a pivotal mediator but also a malleable therapeutic target in atherothrombosis. The role of inflammation in atherosclerosis has been supported by a wealth of evidence demonstrating higher incidence of cardiovascular events in patients with higher levels of inflammatory markers. In addition, it has been demonstrated that anti-inflammatory therapy independent of lipid-lowering effects may offer effective protection against recurrent cardiovascular events. Based on these compelling results, exploration of potential indications for anti-inflammatory therapy targeting the IL-1 $\beta$  pathway can be facilitated by the use of biomarker-driven adaptive design that allocates a larger proportion of participants to the biomarker-positive study arm with a more prominent treatment benefit.

### Conflict of Interest

The work is not funded and the authors declare no conflicts of interest.

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