Targeting Cholesterol Crystal-Induced Inflammation for the Secondary Prevention of Cardiovascular Disease

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Abstract
Cholesterol crystals are present in nascent and advanced atherosclerotic plaque. Under some conditions, they may enlarge and cause direct plaque trauma or trigger an inflammatory cascade that promotes the growth and instability of atherosclerotic plaque. Therapies that reduce the risk of cholesterol crystal formation or prevent the associated inflammatory response have the potential to improve the clinical outcome of patients with cardiovascular disease. Statins have pleiotropic effects that can reduce the size of the free cholesterol pool contained within atherosclerotic plaques and prevent the formation of cholesterol crystals. Colchicine prevents crystal-induced inflammation by virtue of its ability to inhibit macrophage and neutrophil function. Both statins and colchicine have been demonstrated to reduce the risk of cardiovascular events in patients with stable coronary disease. The efficacy of statins and colchicine for cardiovascular prevention supports the hypothesis that crystal-induced inflammation plays an integral role in the progression and instability of coronary disease. Inhibition of cholesterol crystal-induced inflammation offers a promising new target for the secondary prevention of cardiovascular disease.

Keywords
atherosclerosis, ischemic, heart disease

Introduction
Cholesterol crystals have long been identified within atherosclerotic plaque, and their potential to play a causative role in atherosclerosis was first recognized more than 50 years ago when experiments in animals demonstrated that when injected into the arterial wall they led to the development of typical atherosclerotic lesions.¹

Cholesterol within atherosclerotic plaque is mostly esterified; however, as plaques develop, the amount of free cholesterol within them increases²⁻⁵ and predisposes to the formation of cholesterol crystals.⁶⁻⁸ Recent advances in imaging and staining techniques have demonstrated the presence of cholesterol crystals in nascent plaques⁹,¹⁰ and much higher concentrations of cholesterol crystals in vulnerable and unstable atherosclerotic plaque.¹¹⁻¹²

Although crystals in vivo are usually inert, physiochemical changes in either their structure or their environment can trigger resident macrophages to activate the MEditerranean FeVer (MEFV) and related genes to produce the NLRP3 inflammasome.¹³ This inflammasome is responsible for the production of interleukin 1β (IL-1β) that acts to recruit inflammatory mediators including neutrophils.¹⁴,¹⁵,¹⁶ The magnitude of the inflammatory response appears to be crystal specific; cholesterol crystals induce less IL-1β than monosodium urate (MSU) crystals.¹⁷ Evidence that the NLRP3 inflammasome is activated in macrophages within atherosclerotic plaque¹⁸,¹⁹ has led to the suggestion that cholesterol crystals may trigger the inflammatory cascade that leads to the local production and release of IL-1β and the influx of leucocytes including neutrophils into the atherosclerotic bed.²⁰,²¹ In addition, it is now recognized that large cholesterol crystals can cause direct plaque trauma.²²,²³

Hence, there is increasing evidence to support the hypothesis that cholesterol crystals play an integral role in the early development, growth, and destabilization of atherosclerotic plaque by virtue of their ability to cause direct plaque trauma and to trigger an inflammatory cascade that is distinct from...
Observations that statins prevent the accumulation of cholesterol in foamy macrophages, decrease inflammation and metalloproteinas. By preventing macrophage apoptosis in human plaque in a dose-dependent manner, support this hypothesis. In addition, evidence that statins stunt the growth of cholesterol crystals in vitro provides another mechanism by which they may exert their clinical benefit in vivo.

### Drugs Promoting Reverse Cholesterol Transport

From Atherosclerotic Plaque

In contrast to statins, efforts to improve cardiovascular outcomes in humans by promoting the egress of cholesterol from plaque by inhibiting acyl-coenzyme A, cholesterol acyltransferase (ACAT) and cholesteryl ester transfer protein (CETP), have proved disappointing.

In some animal models, ACAT inhibitors did prevent the transformation of macrophages into foam cells and slowed the progression of atherosclerosis, however, these effects were not reproduced in other animal models of atherosclerosis.

When tested in humans, ACAT inhibitors increased the amount of free cholesterol within plaques, promoted the formation of cholesterol crystals, and resulted in apoptosis of resident macrophages and increased the size of the necrotic plaque core.

Because CETP inhibitors significantly increase serum high-density lipoprotein (HDL) concentrations and lower LDL levels, it was expected that they would lead to a net reverse transfer of cholesterol from plaques, and this was demonstrated in animal models. When trialed in combination with statins in human trials, however, they had no effect on plaque size and were associated with a nonsignificant increase in mortality. As a result, CETP inhibitors are no longer being developed for the secondary prevention of cardiovascular disease.

In summary, the ability of statins to improve clinical outcomes may in part relate to their ability to reduce the accumulation of free cholesterol in atherosclerotic plaques, thereby reducing the risk of cholesterol crystal formation. In contrast, attempts to promote egress of cholesterol from plaque in humans have failed to improve clinical outcomes, possibly because they lead to an increase in the size of the free lipid pool and an increased risk of crystal formation.

### Preventing cholesterol crystal-induced inflammation

It is noteworthy that although steroids, conventional non-steroidal anti-inflammatory drugs and COX-2-selective inhibitors, can suppress active (crystal induced) inflammation, they are unable to prevent the activation of the crystal-induced inflammatory cascade, and when used in patients with cardiovascular disease, their long-term use has been associated with an increased risk of cardiovascular events.

In contrast, colchicine stands in a class of its own as the only oral agent that can prevent crystal-induced inflammation, and there is now increasing evidence that it may also improve the clinical outcome of patients with stable coronary disease.

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**Figure 1. Potential targets for inhibiting cholesterol crystal inflammation:** (1) reducing the ingress of free cholesterol into plaque (statins), (2) reducing the risk of cholesterol crystal formation within plaque (statins and colchicine), (3) inhibiting activation of MEditerranean FeVer (MEFV) gene within macrophages (colchicine), (4) inhibiting assembly of the NLRP3 inflammasome (colchicine), (5) inhibiting the production of IL-1β by macrophages (colchicine), (6) inhibiting action of IL-1β (canakinumab), and (7) inhibiting recruitment and activation of leukocytes (colchicine). IL-1β indicates interleukin 1β.

Reducing the Risk of Cholesterol Crystal Formation Within the Plaque Core

Since the size and composition of the cholesterol pool within atheromatous plaque strongly influence the risk of cholesterol crystallization, it is reasonable to consider that drugs that either reduce the ingress or promote the egress of cholesterol within atheromatous plaque may have the potential to reduce the size of the free cholesterol pool and therefore reduce the risk of cholesterol crystal formation.

**Statins**

Statins have a profound effect on cardiovascular outcomes in patients with coronary disease; however, the mechanism by which they prevent disease progression and promote stability of atheromatous plaque remains unknown.

The striking relationship between their clinical effect and their ability to lower serum low-density lipoprotein (LDL) cholesterol suggests that it is possible that they act to reduce the accumulation of free cholesterol within plaque and so reduce the likelihood of crystal formation and the risk of activating the crystal-induced inflammatory cascade.
More recently, the development of parentally administered agents that specifically block IL-1β, the final step in the crystal-induced inflammatory pathway, has led to a trial of canakinumab in patients with stable cardiovascular disease.

**Colchicine**

For over half a century, colchicine has remained the first-line therapy for the secondary prevention of acute neutrophil-mediated inflammation in patients with familial Mediterranean fever (FMF), and it continues to be recognized as an effective agent for the secondary prevention of crystal-induced inflammation in patients with gout. Recently, its value in the treatment and prevention of pericarditis has also been confirmed.

The clinical effectiveness of colchicine in the secondary prevention of acute inflammatory flares in FMF, and gout is believed to relate to its effects on tubulin and its ability to suppress the expression of the MEFV gene within macrophages, which together impair the production and assembly of the NLRP3 inflammasome and the production of IL-1β thereby reducing the ingress of neutrophils into the inflammatory bed. By targeting tubulin in neutrophils, colchicine also impairs their mobility, adhesion, and activation (Figure 1). These effects are also likely to explain the ability of colchicine to prevent the neutrophil-mediated inflammatory response to cholesterol crystals in vivo.

Colchicine has been demonstrated to prevent the development of atherosclerosis in animals and to directly inhibit the formation of cholesterol crystal in vitro [Personal Communication, Prof G Abels: April 10, 2013]. Although the observed effect of colchicine on atherosclerosis in animal models may relate to its ability to prevent crystal formation and crystal-induced inflammation, its ability to suppress the activity of mast cells, T cells, monocytes, smooth muscle cells derived from vascular plaques, and osteocytes may also play a part in its effects on the disease.

Long-term use of colchicine has also been demonstrated to reverse renal amyloid in patients with FMF, prevent gouty arthropathy, and reduce blood levels of biomarkers normally associated with vascular injury, and these time-dependent, disease-modifying effects of therapy may also be important in slowing the progression of atherosclerosis.

Two retrospective studies have linked continuous use of long-term colchicine with beneficial effects in cardiovascular disease. The first was a case–control study in patients with FMF, which revealed that patients on lifelong therapy had a reduced prevalence of coronary disease. The second was a study in patients with acute gout, which demonstrated that patients who continued to take colchicine for secondary prevention had a reduced risk of myocardial infarction compared with patients who did not continue colchicine therapy.

Although the mechanism of these effects remains speculative, it is noteworthy that low dose of colchicine has been demonstrated to have independent anti-inflammatory effects on patients with stable coronary disease as evidenced by its ability to lower high-sensitivity C-reactive protein (hs-CRP) when added to statins and antiplatelet therapy.

Direct evidence supporting a benefit of colchicine in patients with proven cardiovascular disease was demonstrated in the Low Dose Colchicine (LoDoCo) trial. This was a prospective randomized, observer-blinded (assessment of) endpoint study involving 532 patients with stable coronary artery disease treated with aspirin and statins and followed over a median of 3 years. During this period, patients receiving 0.5 mg/d of colchicine had a significant reduction in the risk of (nonstent related) cardiovascular events (Figure 2) including unstable angina associated with angiographic proof of disease progression and myocardial infarction unrelated to stent disease. The LoDoCo trial therefore strongly supports the hypothesis that a drug that is known for its ability to inhibit crystal-induced inflammation may be effective for the secondary prevention of cardiovascular disease.

**Safety of long-term low-dose colchicine**

Despite concerns about early gastrointestinal (GI) intolerance and late side effects of colchicine, there are advantages in continuing to explore its potential role for the secondary prevention of cardiovascular disease, as issues of dose, safety, tolerance, and resistance to therapy have largely been addressed from its widespread clinical use over more than half a century.

In patients with gout, 0.5 mg/d of colchicine is commonly used for secondary prevention, and in patients with FMF doses up to 2 mg/d are routinely prescribed continuously to prevent acute inflammatory flares. In the LoDoCo trial, 0.5 mg/d of colchicine was used, as it was the lowest dose preparation readily available.

At doses of 0.5 mg/d, colchicine may induce GI intolerance in up to 10% of the people during the first month of treatment; however at this dose, long-term therapy is known to be otherwise safe and well tolerated in children, the elderly individuals, and during pregnancy. At doses of up to 2 mg/d, there have been rare case reports of reversible peripheral neuritis and
myopathy, alopeia, inhibition of spermatogenesis but not fertility, B12 deficiency, and bone marrow suppression. Rhabdomyolysis has rarely been reported with the concomitant use of high-dose colchicine and statin therapy in patients with renal impairment.

The Food and Drug Administration (FDA) has audited all reported fatalities possibly related to colchicine use between 1969 and 2009, which includes 20 years during which statins were widely used in the United States. During that 40-year period, 57 patients were identified whose death was attributed to colchicine unrelated to intravenous use, deliberate over dose, or concomitant use of clarithromycin. No reliable information was available regarding the dose of colchicine or the presence or absence of renal or hepatic dysfunction in these patients. Given that over 3 million scripts of colchicine are filled each year in the United States, these data are reassuring, as they indicate that serious toxicity related to colchicine is rare and when carefully supervised should be safe when administered in patients requiring statins.

Even so, it is recognized that colchicine should be used with caution in patients with advanced renal and liver disease, and with the concomitant use of a number of drugs including cyclosporine, clarithromycin, erythromycin, and ketoconazole, and that overdoses of 40 to 60 mg (80-120 tablets) of colchicine may be lethal.

Further large placebo-controlled trials are required to confirm the results of the LoDoCo study and to determine the optimal dose of colchicine for secondary prevention of cardiovascular disease. Although 0.5 mg/d of colchicine proved effective, trials of higher doses of therapy are likely to be associated with increased GI intolerance. It is possible however that doses <0.5 mg/d may prove effective, as it is known that colchicine is avidly taken up by leukocytes and that cholesterol crystals induce a less intense immune response than MSU crystals. Lower dose therapy may also reduce the likelihood of GI intolerance and the risk of interactions with other therapies including statins.

**Canakinumab**

Over the last decade, advances in the formation of monoclonal antibodies directed against inflammatory mediators have led to the development of canakinumab, a fully humanized antibody directed against IL-1β. Canakinumab has been approved for the treatment of 2 rare immune disorders including periodic syndrome and familial cold autoinflammatory syndrome. Canakinumab has been trialed in rheumatoid arthritis and systemic-onset juvenile idiopathic arthritis with some success and has been extensively studied for the treatment of acute gout and for secondary prevention of gout in patients who appear resistant to low-dose colchicine therapy.

The clinical effect of canakinumab results from its ability to inhibit the action of IL-1β. Its efficacy in the treatment of acute inflammatory flares in patients with FMF and gout who are either resistant or intolerant to colchicine indicates that it is a more potent inhibitor of neutrophil-mediated inflammation than colchicine, and that it should be effective in diseases responsive to colchicine.

In clinical trials in patients with gout, canakinumab has been associated with a small but significant risk of sepsis, neutropenia, renal and liver dysfunction, hypertension, and muscular skeletal effects. These effects appear dose related. Its safety over the very long term and during pregnancy is unknown. Because of the risk of infections, it is recommended that elderly and immune compromised patients be tested for latent and active tuberculosis before starting treatment, and caution is also urged in patients with a history of recurring infections. Therapy should not be started or administered during active infection. Although it is recommended that patients with immune disorders complete all appropriate vaccinations before starting treatment, it is not known whether this recommendation should extend to other patients.

Canakinumab may affect the cytochrome P450 enzyme, and dose adjustment for drugs metabolized by this pathway is required. When used for secondary prevention of gout, canakinumab has been administered monthly by subcutaneous injection.

The effectiveness and safety of canakinumab in patients with coronary disease are currently being evaluated in the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). The CANTOS is a placebo-controlled multi-dosing trial designed to determine whether inhibition of IL-1β with canakinumab can reduce the risk of myocardial infarction, stroke, and cardiovascular death among stable patients following an index myocardial infarction. The study will only recruit patients with a past myocardial infarction who are considered to be at high risk of recurrent events, based upon a hs-CRP >2 mg/L. Canakinumab will be trialed at doses of 50, 150, or 300 mg administered subcutaneously every 3 months, and all patients will be followed over a period of up to 4 years.

In essence, CANTOS will determine whether inhibiting IL-1β, the final effector of crystal-induced inflammation (Figure 1), can improve clinical outcomes in patients with stable coronary disease. In addition, it will explore the safety and practicality of longer term subcutaneous canakinumab. Upon completion, CANTOS will provide the longest experience with the use of the drug for any purpose. If shown to be effective, the results of the dosing comparisons will be of particular interest. It is hoped that it will be more effective at lower doses than required in patients with gout, as in those patients doses of 150 mg were most effective but associated with more adverse effects.

To date, the FDA has not recommended the use of canakinumab for patients with gout in part because of concerns related to its short- and long-term safety for a nonlife-threatening condition. Additional potential barriers to more widespread use of the drug for any other purpose may include its cost and the need for parental administration.

**Other Potential Inhibitors of Crystal-Induced Inflammation in Development**

As understanding of the crystal-induced inflammatory cascade increased, so has the number of agents developed to block this
pathway. The list of agents directed against crystal-induced inflammatory cascade is long and includes anakinra and rilon-cept that inhibit IL-1β, gliburide that blocks the maturation of caspase-1 and pro-IL-1β, and antibodies directed against IL-8, which have inhibitory effects on neutrophil function. It remains to be determined whether any of these agents are effective for the secondary prevention of cardiovascular disease.

Conclusions
There is strong evidence that cholesterol crystals play a causative role in the development, progression, and instability of atherosclerotic plaque by virtue of their ability to cause direct plaque trauma and to trigger the IL-1β-dependent inflammatory cascade.

The observation that drugs that reduce the risk of cholesterol crystal formation and prevent crystal-induced inflammation are associated with improved clinical outcomes in patients with cardiovascular disease offers hope that they will prove safe, effective, additive, and readily available to the majority of patients for secondary prevention of cardiovascular disease.

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References


98. Alten R, Gomez-Reino J, Durez P, et al. Efficacy and safety of the human anti-IL-1β monoclonal antibody canakinumab in


