

REVIEW ARTICLE



The Role of Colchicine in the Prevention of Cerebrovascular Ischemia



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Abstract: Introduction: Despite the proven efficacy of anti-thrombotic, lipid-lowering, anti-hypertensive therapies and lifestyle modification changes for secondary ischemic stroke prevention, the risk of recurrent stroke, coronary events and vascular death remains substantial even for patients treated with high rates of established secondary preventive medications.

Methods: In the present review, we summarize available literature data on the association between systemic inflammation and symptomatic atherosclerosis including recurrent cerebral ischemia. We also highlight the potential role of colchicine in the suppression of atherosclerosis-induced inflammation, plaque stabilization and thromboembolism prevention.

Results: Accumulating evidence suggests that inflammation is of key importance in the pathophysiology of atherosclerotic plaque de-stabilization and thromboembolism, with inflammatory cells being involved in all stages of atherosclerosis development. Therefore, anti-inflammatory therapies targeting the atherosclerotic plaque inflammation may be important contributors in plaque stabilization and in the prevention of thromboembolic events. Colchicine is known to have multiple anti-inflammatory properties including inhibition of microtubule polymerization, leading to reduced secretion in monocyte-macrophages. Currently the randomized controlled CONVINC trial is enrolling stroke patients to evaluate the effect of a daily low-dose of colchicine in reducing the rate of recurrent stroke and major vascular events.

Conclusion: Inflammatory pathways seem to be key mediators in the development of atherosclerotic process, atheromatous plaque destabilization and thromboembolism. Colchicine as a novel therapeutic agent could be a safe and effective inhibitor of the inflammation cascade in patients with extra- or intracranial atherosclerosis or arteriolosclerosis, resulting in reduced vascular events.

Keywords: Colchicine, inflammation, stroke, cerebral ischemia, transient ischemic attack, macrophages, prevention.

1. INTRODUCTION

Cerebrovascular disease is the most common cause of disability in older adults with rapidly increasing incidence rates in low-income and middle-income countries [1]. Despite the proven efficacy of anti-thrombotic, lipid-lowering, anti-hypertensive therapies and lifestyle modification changes for secondary ischemic stroke prevention, sub-optimal secondary prevention is common among patients with history of ischemic stroke (IS) or transient ischemic attack (TIA) [2, 3]. Moreover, the risk of recurrent stroke, coronary events and vascular death remains substantial even for those patients treated with high rates of established secondary preventive therapies. Data from recent clinical trials and stroke registries indicate an annual recurrent stroke risk of 2.5%-4% in patients treated with high rates of statin and antiplatelet therapies after IS or TIA [4-6]. A recent meta-analysis reported that one in five patients with IS/TIA have antiplatelet resistance to either aspirin or clopidogrel and these patients are at a significantly higher risk of IS/TIA

recurrence [7]. A systematic review estimated that the cumulative pooled risk of recurrent stroke risk is 11.1% in the first 1 year and 26.4% in the first 5 years after a cerebrovascular event [8]. Additionally, the annual incidence rate of vascular death not attributed to stroke and non-fatal myocardial infarction in stroke survivors has been estimated to 2.1% and 0.9% respectively [9].

2. ARTERY ATHEROTHROMBOSIS AND ARTERIOLO-SCLEROSIS: A TARGET FOR INTERVENTION

Early stroke recurrence risk is higher in patients with large-artery atherosclerosis compared with other stroke etiologies [10-13]. Population-based and hospital registry studies have reported the presence of symptomatic ipsilateral carotid stenosis of more than 50% of arterial lumen in 12%-20% of IS patients [14-19], and intracranial atherosclerosis in 8%-10% of North American/European and 30%-50% of Asian patients [20-22]. Additionally, in the North Dublin Population Stroke Study, 73% of cerebrovascular ischemic events classified as cryptogenic according to TOAST criteria [23] had evidence of aortic or cranio-cervical atherosclerosis [24]. The presence of thick plaques (>4mm) in the aortic arch and descending aorta is also frequent in patients with cryptogenic stroke [25-28]. In one prospective study protocol, patients

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with cryptogenic IS/TIA were found to have 5 times higher odds of aortic arch plaques >4mm compared to patients with stroke of identified cause [29], supporting the hypothesis that aortogenic plaque embolism might have contributed to the cerebral ischemic event.

Formation of micro-atheroma is an important contributor to small vessel cerebrovascular ischemia, accounting for up to 20% of total IS. Although the pathophysiology of cerebral ischemic events in small vessel disease is heterogeneous and not completely understood, existing data suggest that micro-atheroma of larger penetrating arterioles causes distinct symptomatic acute lacunar infarcts, while lipohyalinosis ('arteriolosclerosis') is responsible for the diffuse white matter hyperintensities on neuroimaging and neuropathological evidence of cerebral demyelination [30].

3. SYMPTOMATIC ATHEROMATOUS PLAQUES: A TRIGGER OF SYSTEMIC INFLAMMATION CASCADE

Recent experimental and clinical-pathological studies have shown that microscopic cholesterol crystals form in the early stages of atherosclerotic plaque development, and may be a potent inflammatory stimulus for neutrophils and macrophages [31]. Studies in apolipoprotein E (ApoE)-deficient mice have demonstrated that cholesterol crystals activate monocytes and macrophages via the intracellular nucleotide-binding leucine-rich repeat-containing pyrin receptor (NLRP) inflammasome protein complex [32]. This causes increased interleukin-1 β (IL-1 β) expression, which in turn is considered as a key event in the initiation of local and systemic inflammatory cascades, evidenced by increased systemic levels of interleukin-6 (IL-6) and C-Reactive Protein (CRP) [33].

The Oxford Plaque Study found that 66% of 526 patients with symptomatic carotid stenosis had evidence of marked inflammation in resected plaques from the ipsilateral internal carotid arteries, while 64% had other features of plaque instability (thin fibrous cap, large lipid core, cap rupture, or superficial thrombus) [34]. Infiltration of many inflammatory cells, large lipid core, and thin fibrous cap are the main features of an unstable atheromatous plaque that is prone to rupture and thrombosis [35]. Extensive macrophage infiltration, cap thickness <200 μ m, intraplaque hemorrhage, and large lipid core were independently associated with plaque rupture. In a similar study of 44 consecutive patients with ipsilateral carotid stenosis who underwent endarterectomy within 10 days following the IS/TIA index event, stroke recurrence in the days between index event and surgery was independently associated with extensive macrophage and lymphocyte infiltration [36]. These important observations provide direct evidence linking plaque lipid metabolism and inflammation.

Indirect in-vivo support for the aforementioned findings have been provided by a positron emission tomography (PET) study in patients with symptomatic carotid stenosis, where 18-fluorodeoxyglucose (18-FDG) uptake in symptomatic plaque was associated with total and LDL cholesterol, and inversely associated with HDL cholesterol, after adjustment for confounding variables [37]. FDG-PET is a validated technique for imaging plaque metabolic activity, mainly related to inflammatory monocyte-macrophage activity. Symptomatic carotid plaques had high FDG uptake that correlated strongly with plaque macrophage density [38]. Moreover, cross-sectional studies have reported that plaque FDG uptake is associated with other markers of plaque instability, including microembolic signals on transcranial Doppler ultrasound, unstable plaque morphology, and expression of inflammatory cytokines and proteases [39-41]. Carotid plaque FDG uptake was identified as an independent predictor of early pre-endarterectomy stroke recurrence in patients with IS/TIA and ipsilateral carotid stenosis [42-44].

4. INFLAMMATORY BIOMARKERS: INDICATORS OF PLAQUE DE-STABILIZATION AND PREDICTORS OF THROMBOEMBOLISM IN PATIENTS WITH ATHEROSCLEROSIS

Accumulating evidence supports the view that inflammation is of key importance in the pathophysiology of atherosclerotic plaque de-stabilization and thromboembolism, with inflammatory cells being involved in all stages of atherosclerosis development [45, 46]. Retention of LDL lipids in the extracellular matrix of the arterial wall is accompanied by differentiation of monocytes and lipid uptake to lipid-laden 'foamy' macrophages. Trans-endothelial migration of circulating monocytes is a key event in the initiation of atherosclerotic plaque induced inflammatory process, resulting in local expression of pro-inflammatory cytokines, particularly IL-1 β and tumor necrosis factor-alpha (TNF- α) [45]. Other produced cytokines, such as platelet-derived growth factor (PDGF), promote recruitment and proliferation of smooth muscle cells into the endothelial cell layer, which in turn promote the expression of matrix proteins such as collagen and elastin. Expression of cytokines and collagenolytic enzymes, such as metalloproteinases from macrophages and other cells, contributes to erosion and rupture of the fibrous cap, which results in exposure of circulating platelets and coagulation factors to the pro-thrombotic core, and subsequent thromboembolism [46].

Increased inflammatory cells, including macrophages and activated microglia, expressing matrix metalloproteinases (MMPs) are present around affected arterioles and areas of ischemic demyelination in patients with small artery disease [47, 48]. Numerous studies have associated CRP levels and leucocyte count not only with pathological features of plaque instability (rupture, inflammation), but also with higher risk of recurrent stroke in patients with IS or TIA due to extracranial or intracranial atherosclerosis [49-55]. In 1,244 patients with lacunar stroke in the SPS3 trial, baseline CRP was associated with a 2.32-fold increase in the adjusted risk of recurrent stroke and vascular events [56]. In the Chinese CHANCE trial, which included 3,044 patients with non-severe stroke of non-cardioembolic origin, high CRP levels at baseline were associated with a 1.46-fold increase in the adjusted risk of early recurrent stroke at 90 days [57]. Likewise, in 1,232 patients with recent IS/TIA in the Oxford Vascular Study, CRP measured in the acute phase was an independent predictor of 90-day stroke recurrence [58]. Similar findings have been observed with other inflammatory biomarkers, including fibrinogen and IL-6 [59]. Epidemiological studies have consistently described dose-dependent associations between circulating blood cytokines IL-1, IL-6, TNF- α and vascular disease, including stroke [60].

5. ASSOCIATION OF STATIN-INDUCED SUPPRESSION OF INFLAMMATION WITH ATHEROSCLEROTIC PLAQUE STABILIZATION

Even though the exact mechanisms explaining the higher risk of cerebrovascular ischemia in patients with arterial atherosclerosis yet remain unclear, it has been hypothesized that inflammation-related plaque instability may be an important contributing factor [61], with recent data indicating that anti-inflammatory therapies targeting the atherosclerotic plaque inflammation may be important contributors to plaque stabilization and in the prevention of thromboembolic events [62].

Randomized-controlled clinical trials in patients with non-cardioembolic IS or TIA have demonstrated benefit of targeting plaque-related lipid accumulation with high-dose atorvastatin and platelet activation to prevent recurrent stroke and coronary events

[63-65]. In addition to their lipid-lowering effect, statins are known to influence other biological pathways including the upregulation of endothelium nitric oxide synthase (eNOS) and other anti-inflammatory effects [66]. Our study group has recently shown that statin pretreatment in patients with ischemic stroke due to large artery atherosclerosis is dose-dependently associated with several improved outcomes: neurologic improvement, disability, survival, and stroke recurrence. [67]. We also documented that statin pretreatment was associated with reduced presence of microembolic signals (MES) on transcranial Doppler monitoring and lower MES burden with an apparently dose-dependent association in patients with extra- or intra-cranial atherosclerosis [68]. Likewise, other study protocols have demonstrated reduction in blood biomarkers and plaque FDG uptake in a dose-dependent manner by statins [69, 70].

These real-time in-vivo observations provide additional data on the positive role of statins on atherosclerotic plaque stabilization and cerebrovascular vasculature, and thus further support the beneficial effect of statins on recurrent ischemic events [66]. Interestingly, in the JUPITER trial, treatment with rosuvastatin reduced LDL by 50%, CRP levels by 37% and major vascular events by 46% [71]. Likewise, in the IMPROVE-IT trial addition of ezetimibe to simvastatin was associated with significantly lower values of both LDL and high sensitivity CRP (hsCRP), and with lower incidence rates of primary endpoints (cardiovascular deaths, major coronary events, or strokes) compared to simvastatin alone after multivariable adjustment [72].

6. ASSOCIATION OF CANAKINUMAB-INDUCED INHIBITION OF INFLAMMATION WITH CARDIOVASCULAR EVENT REDUCTION

It has been estimated that in approximately 25% of patients with ischemic heart disease the risk of future vascular events is directly associated with inflammatory atherosclerosis, measured using the hsCRP biomarker [73]. Thus, the immune system may represent a novel and promising target for the prevention and treatment of atherothrombosis [74].

ACZ885 (canakinumab) is a selective, high-affinity, fully human monoclonal antibody inhibiting IL-1 β , a key cytokine in the pathway of inflammatory atherosclerosis [75-78]. The efficacy, safety and tolerability of quarterly subcutaneous injections of canakinumab in combination with standard of care for the prevention of recurrent cardiovascular events in patients with a prior myocardial infarction and hsCRP levels $\geq 2\text{mg/L}$ has been evaluated in the the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) trial [79]. According to a recently published media report CANTOS met the primary composite endpoint of heart attack, stroke and cardiovascular death after recruiting a total of 10,061 participants with a median follow-up of 3.8 years [80]. The positive results of the CANTOS trial not only show that canakinumab reduces cardiovascular risk in people with a prior heart attack and inflammatory atherosclerosis [80], but also provide invaluable support to the inflammatory hypothesis of atherothrombosis and expand even further on the contribution of innate immunity to cardiovascular health [81].

7. COLCHICINE: A POTENTIAL DRUG TARGETING ON ATHEROSCLEROSIS INDUCED INFLAMMATION AND SECONDARY STROKE PREVENTION

Colchicine has been the cornerstone treatment for gout, Behcet's Disease and Familial Mediterranean Fever for decades [82]. Colchicine is readily-absorbed after oral administration in the jejunum and ileum, by a P-glycoprotein (ABCB1-transporter) dependent process. It undergoes significant 1st-pass hepatic metabolism, resulting in oral bioavailability averaging 45% (range 24-88%) in healthy volunteers. It is excreted primarily (80-90%) *via* biliary secretion (*via* the cytochrome P450 CYP3A4 system), and

also partly by the renal route (10-20%), and *via* intestinal epithelium [82].

Its primary cellular action is binding to α - and β -tubulin proteins, which are highly-expressed in neutrophils and monocyte-macrophage inflammatory cells. It has multiple anti-inflammatory properties including inhibition of microtubule polymerization, with inhibition of E-selectin mediated leucocyte rolling and endothelial adhesion, and leucocyte motility, phagocytosis, and cytokine secretion. *In vitro*, colchicine inhibits crystal-induced activation of the NLRP inflammasome, possibly via inhibition of microtubule polymerisation, which is a pre-requisite for inflammasome assembly. This leads to inhibition of proteolytic cleavage of pro-IL1 β by caspase-1, leading to reduced secretion in monocyte-macrophages (Fig. 1) [83]. On the other hand, evidence on the potential use of colchicine in chronic low-grade inflammatory conditions is still limited [84].

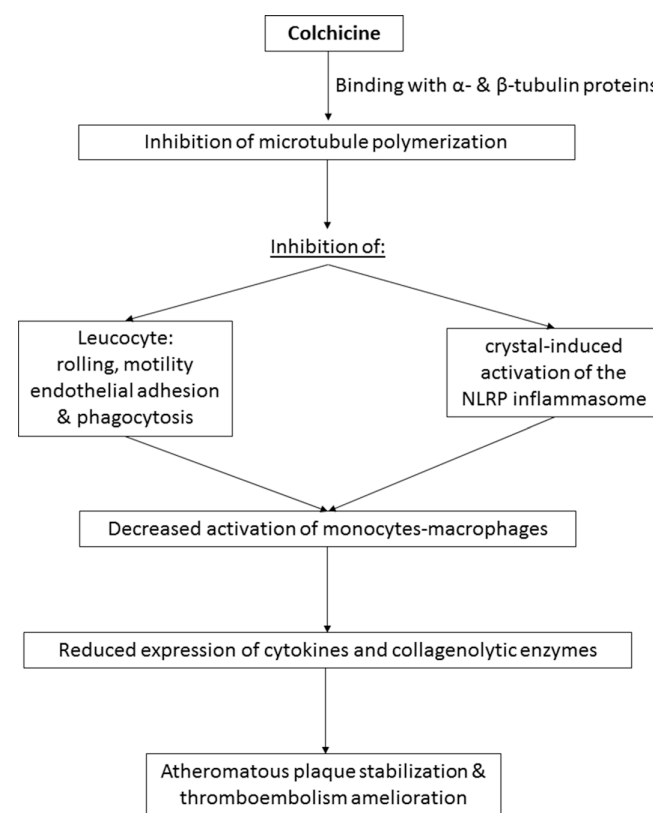


Fig. (1). Potential pathophysiological mechanisms of colchicine on plaque stabilization and thromboembolic event prevention.

Observational data have reported lower rates of coronary artery disease in patients with gout and Familial Mediterranean Fever receiving colchicine therapy [85]. In the LoDoCo RCT of 532 patients with chronic stable coronary artery disease, low dose of colchicine (0.5mg/day) reduced the risk of major cardiovascular events by 67% over a median follow-up period of 36 months. In this trial colchicine had an excellent safety profile, with the more frequently reported adverse events being intestinal upset (2.5%), myalgia (0.9%) and myositis, rash, itch, alopecia, or peripheral neuropathy (<0.5%), and no excess of death observed [86]. Recent trials have also demonstrated benefit of low-dose colchicine (0.5-1mg/day) for prevention of recurrent pericarditis and reduced infarct size when used acutely in patients with ST-elevation myocardial infarction [87, 88]. A recent systematic review of 39 trials and 4,992 total patients focusing on the safety and efficacy of long term colchicine use on cardiovascular disease reported that colchicine was related

Table 1. Outcomes of interest reported in the meta-analysis of available randomized clinical trials by Hemkens *et al.* [76] on the effect of Colchicine on the prevention of cardiovascular events.

Outcome	No of Participants (Studies)	Relative Effect (95%CI)	Heterogeneity
Stroke	874 (3 studies)	OR 0.38 (0.09 to 1.70)	I ² =0%
Myocardial infarction	652 (2 studies)	RR 0.20 (0.07 to 0.57)	N/A
Cardiovascular mortality	1132 (7 studies)	RR 0.34 (0.09 to 1.21)	I ² =9%
All-cause mortality	4174 (30 studies)	RR 0.94 (0.82 to 1.09)	I ² =27%
Total adverse events	1313 (11 studies)	RR 1.52 (0.93 to 2.46)	I ² =45%

RR: risk ratio, OR: odds ratio

with an 80% decrease on the risk of myocardial infarction, with no increased risk of serious adverse events [89]. The main findings of this meta-analysis are summarized in Table 1.

Moreover, in another RCT a short perioperative course of colchicine was effective in attenuating postoperative myocardial injury of patients undergoing coronary artery bypass grafting [90]. Similarly, diabetic patients with contraindication to a drug-eluting stent undergoing percutaneous coronary intervention randomized to receive colchicine 0.5 mg twice daily for 6 months were found to have significantly lower rates of bare-metal stent restenosis compared to those receiving placebo [91]. Within the setting of other published RCTs colchicine was effective and safe treatment for the prevention of early atrial fibrillation recurrence after pulmonary vein isolation in the absence of antiarrhythmic drug treatment, with this effect being strongly associated with a significant decrease of the inflammatory mediators IL-6 and CRP [92], while several other RCTs that will evaluate the role of colchicine in the prevention of atrial fibrillation after cardio-thoracic surgery are still ongoing [93, 94]. Treatment with colchicine was also associated with reduction in inflammation biomarker levels in patients with stable chronic heart failure [95].

Colchicine for prevention of Vascular Inflammation in Non-CardioEmbolic stroke (CONVINCE) trial is an ongoing RCT that will test the hypothesis that daily low-dose colchicine (0.5mg/day) combined with usual care (lifestyle changes, antiplatelet, antihypertensive and lipid-lowering therapy) will have benefit in the prevention of recurrent stroke and major vascular events compared with usual care alone [96, 97]. Similar to other stroke secondary prevention trials of antiplatelet and lipid-lowering therapy, CONVINCE will include patients with non-severe (modified Rankin score 3 or less) stroke or TIA, randomized between 72 hours and 28 days of symptom onset and followed for a median of 36 months. Patients with cardioembolic stroke/TIA, those with other identified non-atherosclerotic stroke mechanisms (e.g. arterial dissection), or contra-indication to colchicine therapy will be excluded. The tolerability and safety of colchicine will be closely monitored, with a pre-specified review after completion of randomization of the initial 10% of the trial sample [96, 97]. The study primary endpoint aims to investigate the efficacy of low dose colchicine (0.5mg/day) in this study population to prevent non-fatal recurrent ischemic stroke, myocardial infarction, cardiac arrest, and vascular death. Other secondary endpoints include the safety of colchicine, the effect of

colchicine on each component of the composite primary outcome measure and the effect of treatment on the primary outcome across different patient categories [94]. Moreover, CONVINCE trial will investigate the effect of colchicine on direct health care costs, incorporating quality-adjusted life years [96, 97].

CONCLUSION

Experimental, epidemiological and clinical data suggest that inflammatory pathways are key mediators in the development of atherosclerotic process, atheromatous plaque destabilization, thromboembolism and arteriolosclerosis leading to cerebrovascular ischemia and other vascular events. Recent RCT data indicate that inhibition of inflammatory pathways in patients with coronary artery disease and elevated CRP levels may result in additional prevention of cardiovascular and cerebrovascular events. Colchicine could be a safe and effective inhibitor of the inflammation cascade induced by extracranial or intracranial atherosclerosis, providing thus an additional therapeutic agent in secondary non-cardioembolic stroke prevention. CONVINCE trial will test the role of colchicine in the secondary prevention of non-cardioembolic stroke and hopefully improve our knowledge on the role of inflammation in stroke pathogenesis.

DISCLOSURE

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CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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