Pilot Study

Colchicine as a Novel Therapy for Suppressing Chemokine Production in Patients With an Acute Coronary Syndrome: A Pilot Study

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ABSTRACT

Purpose: Existing literature reports that colchicine inhibits inflammasome activation and downstream inflammatory cytokine production and stabilizes coronary plaque. However, colchicine's effect on chemokines, which orchestrate multiple atheroinflammatory pathways, is unknown.

Methods: Patients with acute coronary syndrome (ACS) were randomly assigned to colchicine (1.5 mg PO) (n = 12; mean age, 65.2 years) or no treatment (n = 13; mean age, 62.2 years). Blood samples were collected during cardiac catheterization within 24 hours of colchicine administration from the coronary sinus, aortic root, and right atrium. Patients with colchicine-naive stable angina (SAP) (n = 13; mean age, 66.8 years) were additionally sampled. Serum chemokine levels were analyzed with ELISA. In parallel, monocytes from healthy donors were isolated and subjected to colchicine treatment.

Findings: Transcoronary (TC) levels of chemokine ligand 2 (CCL2) and C-X3-C motif chemokine ligand 1 (CX3CL1) were significantly elevated in patients with ACS versus patients with SAP ($P < 0.01$). TC chemokine ligand 5 (CCL5) levels were not significantly ($P = 0.084$) elevated in patients with ACS versus patients with SAP. Colchicine treatment markedly reduced TC levels of CCL2, CCL5, and CX3CL1 in patients with ACS ($P < 0.05$). *In vitro* colchicine suppressed *CCL2* gene expression in stimulated monocytes ($P < 0.05$). Colchicine treatment reduced the intracellular concentration of all 3 chemokines ($P < 0.01$) and impaired monocyte chemotaxis ($P < 0.05$).

Implications: Here, we report for the first time that short-term colchicine therapy significantly reduces the local production of coronary chemokines, in part by attenuating production of these mediators by monocytes. These data provide further evidence of colchicine's beneficial role in patients with ACS. (*Clin Ther*. 2019;41:2172–2181) © 2019 Published by Elsevier Inc.

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INTRODUCTION

Chemokines are a class of chemotactic cytokines characterized by their ability to induce cell migration. Beyond their chemotactic function, chemokines are

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involved in a plethora of proatherogenic processes, including cell survival, leukocyte adhesion, foam cell differentiation, and endothelial to mesenchymal transition.[1] Chemokine ligand 2 (CCL2), chemokine ligand 5 (CCL5), and C-X3-C motif chemokine ligand 1 (CX3CL1) are commonly expressed chemokines implicated in atherosclerotic plaque progression and instability. Combined inhibition of CCL2, CCL5, and CX3CL1 axes abolishes atherosclerosis in hypercholesterolemic mice,[2] whereas blockade of a single pathway stunts plaque progression and produces a more stable disease phenotype.[3–5] Furthermore, antagonism of the CCL2 receptor was reported to reduce serum C-reactive protein in humans at high risk of coronary artery disease (CAD).[6] Despite these promising results, there is yet to be a tolerable, effective, and accessible therapeutic option available to favorably modify chemokine expression in humans.

Colchicine is a potent, yet inexpensive, anti-inflammatory medication used commonly in the management of gout and pericarditis. Although colchicine's precise mechanism of action is ill defined, it is known to inhibit microtubule polymerization.[7] As such, colchicine influences numerous cellular processes, including inflammasome function, cytokine release, and phagocytosis.[7–9] Low-dose colchicine treatment was found to reduce the incidence of secondary cardiovascular events and to promote atherosclerotic plaque stability.[10,11]

Given colchicine's purported broad mechanism of action, this pilot study was designed to test its effects on inflammatory chemokines in patients presenting with an acute coronary syndrome (ACS). We hypothesized that (1) the local cardiac production of CCL2, CCL5, and CX3CL1 would be higher in patients with ACS than in patients with stable CAD; (2) colchicine would suppress transcoronary (TC) production of these chemokines; and (3) this reduction in chemokine levels would be due to a direct effect of colchicine on monocyte processes.

PATIENTS AND METHODS

Patient Recruitment

Adult patients indicated for cardiac catheterization at Royal Prince Alfred Hospital (Sydney, NSW, Australia) were recruited for this study. Patients were divided into 2 cohorts, according to clinical presentation (as per the American Heart Association guidelines)[12] as follows: (1) ACS cohort in which patients presented with recent, acute-onset chest pain associated with ECG changes and/or positive cardiac enzymes (creatine kinase or troponin T) and (2) stable CAD cohort in which patients presented with symptomatic chest pain on exertion for a period >3 months or asymptomatic patients with >50% stenosis and positive function tests.

The ACS cohort was randomly assigned, in a 1:1 fashion, to receive either 1 mg, followed by 0.5 mg (1 hour later), of colchicine or no colchicine, 6 to 24 hours before undergoing cardiac catheterization, as per our previous publication.[8] Staff within the catheterization laboratory, but not the patient, were blinded to the assigned treatment. Individuals with stable CAD served as a control cohort and were not treated. Exclusion criteria included (1) cardiogenic shock or hemodynamic instability, (2) known hypersensitivity to colchicine, (3) use of colchicine or other anti-inflammatory medications besides aspirin (ie, corticosteroid drugs); (4) evidence of concurrent inflammatory or infective conditions that may affect serum concentration of acute-phase proteins (ie, rheumatoid arthritis and peripheral vascular disease), (5) use of potent cytochrome p450 inhibitors, (6) moderate renal dysfunction (creatine clearance < 45 mL/min) or hepatic dysfunction (alanine aminotransferase 1.5× upper limit of normal range), and (7) thrombocytopenia or leukopenia.

Demographic and clinical data were recorded. The local Ethics Review Committee approved the study protocol, and all patients gave written informed consent before participating.

Sample Collection

Blood sampling was performed as per our previously published method.[8,13] Briefly, a standard coronary angiogram was performed in all patients. Subsequently, right femoral vein access was obtained with a 5F sheath and a 5F Simmons catheter was advanced into the right atrium. Blood (20 mL) was drawn from the coronary sinus, lower right atrium–inferior vena cava junction (venous), and ascending aorta (arterial). Blood samples were collected in vacuum containers that contained EDTA. Samples were subsequently centrifuged at 1500 rpm at room temperature for 10 minutes; plasma was then immediately collected and stored at −80°C until required for processing. TC concentrations (defined as

coronary sinus concentration minus the central aortic concentration) for all 3 chemokines were calculated.

Cell Culture

Human-derived mononuclear cells (HDMCs) were isolated via elutriation from the buffy coat of healthy individuals. After isolation, HDMCs were seeded in 6-well plates at a density of 3×10^6 cells/well in RPMI. Cells were incubated at 37°C for 2 hours to facilitate adhesion. Subsequently, the medium was replaced with either colchicine (50 nM) or ethanol as a vehicle control in RPMI that contained 10% fetal bovine serum. Cells were incubated for 4 hours before lipopolysaccharide (LPS; 25 ng/mL) stimulation for 4 hours. After this, cells were lyzed by either TRI Reagent (Sigma–Aldrich, Castle Hill, NSW, Australia) or CelLytic (Sigma–Aldrich) for RNA and cellular protein extraction, respectively. All samples were stored at −80°C until required.

Chemokine Quantification

The concentration of plasma and intracellular CCL2, CCL5, and CX3CL1 was analyzed by ELISA (R&D Systems, Minneapolis, Minnesota). The intracellular concentration of the 3 chemokines was assessed by subjecting the cell lysates to bicinchoninic acid analysis and loading equal protein quantities (10 μg) from each sample. ELISAs were performed as per the manufacturer's guidelines.

RNA Isolation and Quantitative Reverse Transcriptase Polymerase Chain Reaction

RNA was isolated from HDMCs with the use of TRI Reagent (Sigma–Aldrich) as per the manufacturer's instructions. RNA was reverse transcribed with the use of the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, California). Quantitative reverse transcriptase polymerase chain reaction was performed with the use of IQ Sybr mix in a CFX384 thermocycler (Bio-Rad Laboratories). Relative changes in mRNA expression were determined with the $^{\Delta\Delta}$CT method normalized to the housekeeping gene *GAPDH*. The primer sequences used for mRNA amplification are given in Table I.

Migration Assay

Supernatant from HDMCs treated with colchicine (50 nM) or ethanol (vehicle control) and stimulated with LPS (25 ng/mL) for 4 hours was collected and centrifuged at 550 × *g* and 600 μL was added to 12 wells of a 24-well plate. Freshly isolated HDMCs were stained with CellTrace CFSE (Thermo Fisher, Waltham, Massachusetts) as per the manufacturer's instructions. Once stained, 5×10^4 HDMCs in 100 μL of RPMI were added to the inserts (Corning, Tewksbury, Massachusetts; 3-μm pores) of the transwell plate and incubated at 37°C for 1 hour to facilitate adhesion. Thereafter, the inserts were added to the wells that contained the conditioned media and incubated for 2 hours to allow migration. After migration, the transwell inserts were removed, washed 3 times in phosphate-buffered saline, and fixed in 2% formalin for 10 minutes. Once dry, the insert was imaged with the use of the Zeiss Axio Imager Z2, and 3 images of each membrane were obtained with the use of the 10× objective. Migrated cells were counted manually with the use of Image J software (National Institute of Health, Bethesda, Maryland).

Statistical Analysis

Continuous data are reported as mean [SD] or [SEM] when appropriate. Categorial variables are displayed as number and percentage. Proportional differences in categorical data were tested by Pearson χ^2 test. Differences in means of continuous variables were analyzed by 1-way ANOVA or independent *t* test when appropriate. All tests were 2-tailed with the

Table I. Human primer sequences used in gene amplification.

Gene	Forward	Reverse
CCL2	TCATAGCAGCCACCTTCATT	TCGGAGTTTGGGTTTGCTT
CCL5	TCCTCATTGCTACTGCCCT	TTGGCGGTTCTTTCGGGTGA
CX3CL1	TATCTCTGTCGTGGCTGCT	TCCTTGACCCATTGCTCCTT
GAPDH	TTCAACAGCGACACCCACT	TTCCTCTTGTGCTCTTGCT

acceptable type 1 error set at $P < 0.05$. All statistical analyses were performed with SPSS Statistics 25 software (IBM, Armonk, New York), and graphs were produced with GraphPad Prism 7.0 (GraphPad Software, San Diego, California).

RESULTS

Between March 2017 and October 2017, 38 patients were enrolled in the study. This included 25 patients presenting with an ACS and 13 with stable CAD. Of patients within the ACS cohort, 12 were randomly assigned to colchicine, whereas the remaining 13 patients received no treatment. Colchicine treatment was well tolerated, and no adverse reactions were reported.

Baseline Characteristics

Baseline characteristics for the 3 cohorts, untreated ACS, colchicine-treated ACS, and stable CAD, are presented in Table II. Compared with the untreated ACS cohort there were fewer active smokers in the ACS plus colchicine group ($P = 0.035$) and fewer patients currently taking a β-blocker in the stable CAD group ($P = 0.047$). As expected, patients with ACS had significantly higher levels of C-reactive protein and troponin compared with the stable CAD cohort ($P = 0.015$ and $P < 0.001$, respectively).

Patients with ACS Have Increased Tc Chemokine Levels

A significant association was found between TC chemokine levels and clinical presentation. Patients

Table II. Baseline characteristics for study participants.

Characteristic	ACS (n = 13)	ACS + Col (n = 12)	Stable CAD (n = 13)
Age, years	62.2 [12.1]	65.2 [11.1]	66.8 [8.2]
Female	1 (7.7)	3 (25.0)	1 (7.7)
Diabetes	6 (46.2)	5 (41.7)	4 (30.8)
Hypertension	9 (69.2)	9 (75.0)	10 (76.9)
Dyslipidemia	10 (76.9)	8 (66.7)	10 (76.9)
Current smoker	6 (46.2)	1 (8.3)*	4 (30.8)
Previous MI	4 (30.8)	7 (58.3)	2 (15.4)
Previous PCI	4 (30.8)	4 (33.3)	2 (15.4)
Previous CABG	3 (23.1)	1 (8.3)	1 (7.7)
Medications			
Aspirin	13 (100)	12 (100)	12 (92.3)
β-Blockers	10 (76.9)	10 (83.3)	5 (38.5)*
ACE-I	4 (30.8)	8 (66.7)	2 (15.4)
Insulin	0 (0.0)	2 (16.7)	1 (7.7)
Statin	11 (84.6)	9 (75.0)	11 (84.6)
Admission blood value			
Troponin T, μg/L	89 (18–1300)	206.5 (38.5–1083)	7 (4.5–10.5)*
CRP, mg/L	5.2 (2.8–18.9)	2.7 (1.1–19.45)	1.2 (0.7–2.2)*

Data are presented as mean [SD], n (%), or median (IQR).
ACE-I = angiotensin converting enzyme inhibitor; ACS = acute coronary syndrome; CABG = coronary artery bypass graft; CAD = coronary artery disease; Col = colchicine; CRP = C-reactive protein; MI = myocardial infarction; PCI = percutaneous coronary intervention.
*$P < 0.05$ compared with the untreated ACS cohort; χ^2 test used for binary variables; non-normally distributed data (troponin and CRP) were log transformed, and subsequent analysis was performed with an independent *t* test.

with ACS had significantly higher TC levels of CCL2 and CX3CL1 (12.2 versus −10.0 pg/mL and 73.4 versus −25.0 pg/mL; $P = 0.049$ and $P = 0.007$, respectively) compared with patients with stable CAD. Patients with ACS had elevated levels of TC CCL5, although this did not reach statistical significance (12.6 versus −1.0 ng/mL; $P = 0.16$). When aortic, coronary sinus, and venous levels of the 3 chemokines were compared independently, no significant differences were found (data not shown).

Colchicine Treatment Acutely Suppresses TC Chemokine Levels in Patients with ACS

A single dose of colchicine (1.5 mg) had no significant effect on the concentration of chemokines at the individual sampling sites (data not shown). However, calculation of the TC values revealed that colchicine acutely suppressed TC levels of all 3 chemokines: CCL2 (12.2 versus −12 pg/mL; $P = 0.049$), CCL5 (12.6 versus −16.2 ng/mL; $P = 0.010$), and CX3CL1 (73.4 versus −7.8 pg/mL; $P = 0.016$) as illustrated in Figure 1. Because there were significantly more active smokers in the untreated ACS group, a sensitivity analysis with adjustment for numbers of smokers was performed. This resulted in similar significant differences in chemokine levels between the groups.

Colchicine Suppresses *CCL2* Gene Expression in Activated HDMCs

Colchicine treatment of HDMCs *in vitro*, before LPS stimulation, inhibited *CCL2* gene expression by 59% (4.6 versus 11.1; $P = 0.028$) (Figure 2A). Moreover, colchicine exposure reduced the relative increase in CCL2 intracellular protein concentration associated with LPS stimulation by ~3-fold (0.4 versus 1.3; $P = 0.002$) (Figure 2B).

Unlike CCL2, colchicine treatment had no significant effect on CCL5 or CX3CL1 mRNA expression versus the ethanol-treated control group (data not shown). Despite this, colchicine treatment significantly reduced the relative increase in CCL5 and CX3CL1 intracellular protein concentration associated with LPS stimulation (2.5 versus 9.7; $P = 0.005$; 0.9 versus 1.3; $P = 0.009$, respectively) (data not shown).

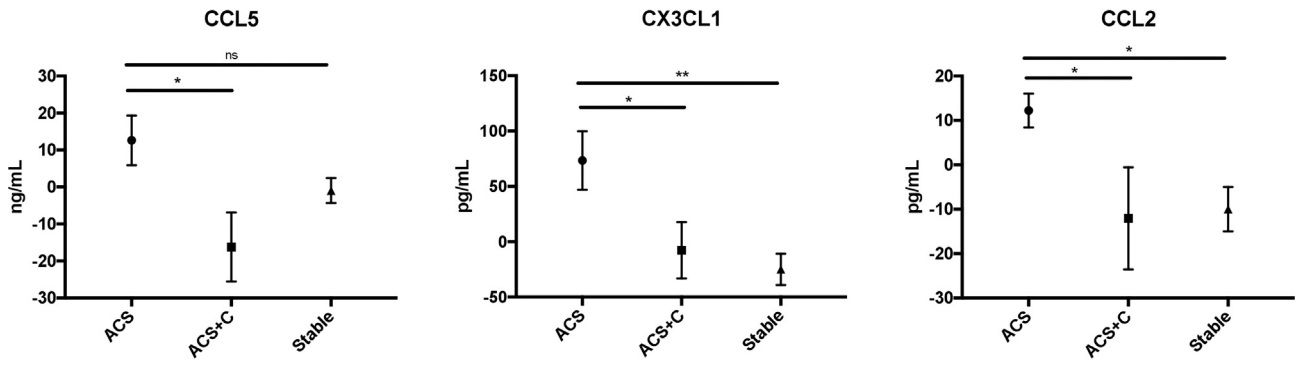

Figure 1. Transcoronary (TC) chemokine levels. During cardiac catherization, 6 to 24 hours after colchicine administration, blood was collected from the coronary sinus and aortic root. The plasma was isolated, chemokine concentrations were analyzed by ELISA, and the TC gradient was calculated (coronary sinus − aortic). *P* values were calculated with a 1-way ANOVA with multiple comparisons; multiplicity was adjusted for by using the Holm−Sidak method. ACS = acute coronary syndrome; ASC+C = acute coronary syndrome plus colchicine; CCL2 = chemokine ligand 2; CCL5 = chemokine ligand 5; CX3CL1 = C-X3-C motif chemokine ligand 1.

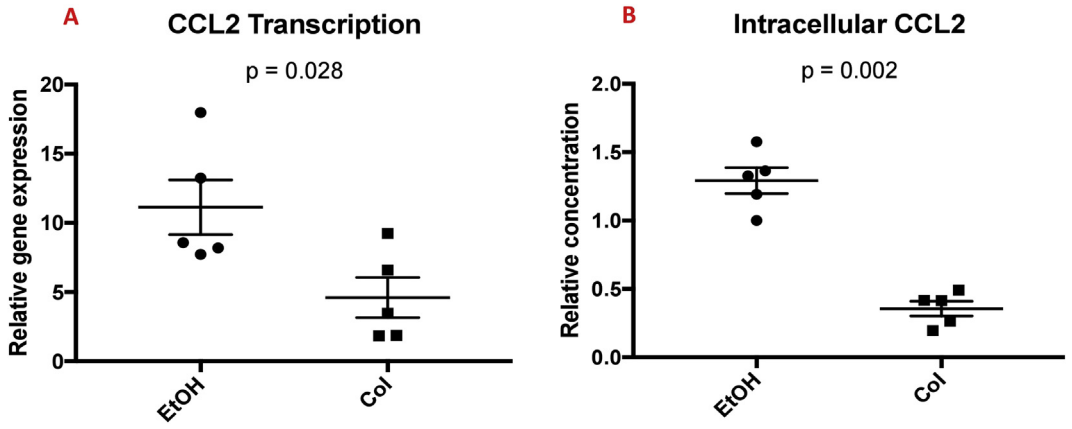

Figure 2. Effect of colchicine (Col) on chemokine ligand 2 (CLL2) expression. Col treatment significantly reduced CCL2 (A) mRNA and (B) intracellular protein levels compared with vehicle (ethanol; EtoH) control. Human-derived mononuclear cells (HDMCs) were isolated from buffy coat and treated with either 50 nM Col (n = 5) or EtOH (n = 5) as a vehicle control for 4 hours. Cells were stimulated with lipopolysaccharide (25 ng/mL) for a further 4 hours before being harvested. The mean difference between treatment groups was analyzed by paired *t* test.

Colchicine Inhibits The Generation of a Functional Chemotactic Gradient

We next used a migration assay to assess the functional consequences of reduced chemokine production. Monocyte migration toward conditioned media generated from colchicine-treated HDMCs was reduced by 46% ($P = 0.035$) compared with the ethanol-treated controls (Figure 3).

DISCUSSION

This pilot study is the first, to our knowledge, that measures TC chemokine levels in patients with ACS and the effect of colchicine on these factors. There are several novel findings. First, patients with ACS have increased TC levels of CCL2 and CX3CL1 compared with patients with stable CAD. Second, a one-off colchicine dose acutely suppressed the TC levels of CCL2, CCL5, and CX3CL1. Finally, colchicine reduced CCL2 expression and chemotaxis in activated HDMCs, suggesting a direct inhibitory effect of colchicine on these cells.

Local Cardiac Production of Chemokines is Elevated in Patients with ACS

Expectedly, these results found that the local cardiac production of CCL2, CCL5, and CX3CL1 is higher in patients with ACS than in patients with stable CAD; however, results for CCL5 were statistically indeterminant. From this trend, a higher powered study might be able to detect a significant difference in the TC levels of CCL5 between these 2 cohorts. As previously reported by our group,[8] venous levels do not track with increased TC production, indicating the importance of measuring the TC gradient to identify local cardiac changes. Although augmented chemokine expression is known to disrupt plaque stability, results of this study are unable to confirm whether the increase in TC chemokine levels were the cause of plaque rupture or consequent to it.

CCL2, CCL5, and CX3CL1 are instrumental in the progression of atherosclerosis and have been suggested to promote instability of atherosclerotic lesions.[14] CCL2 is primarily responsible for inducing monocyte migration into plaque, although it is also involved in regulating matrix metalloproteinase expression; therefore, it directly influences plaque stability.[15,16] In

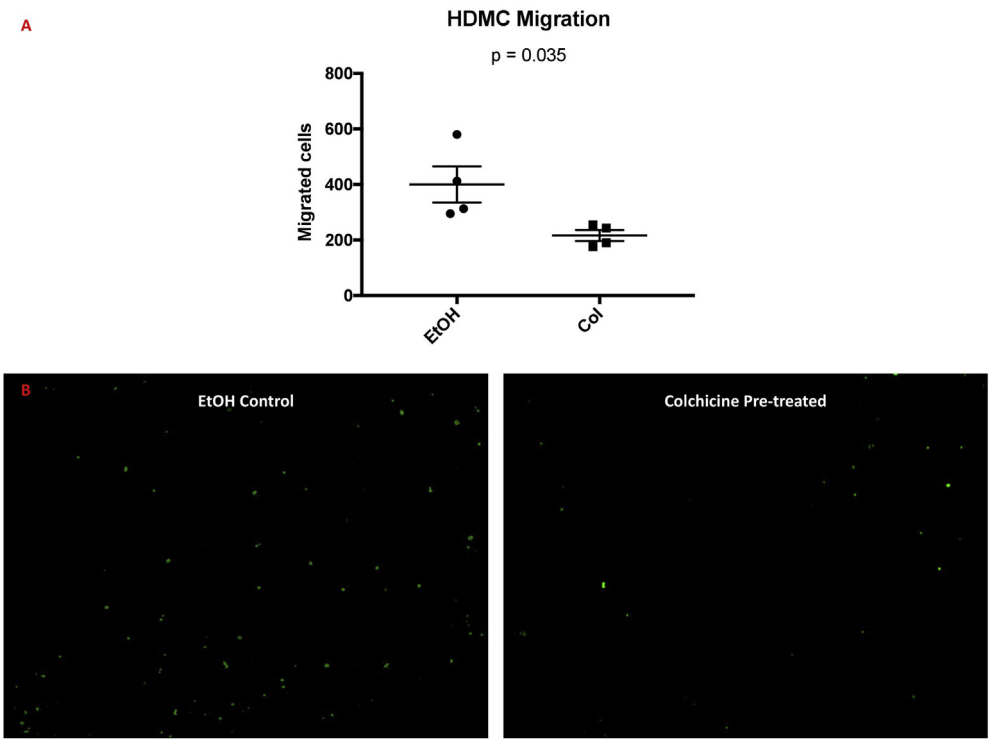

Figure 3. Colchicine (Col) reduced human-derived mononuclear cell (HDMC) migration. (A) Graphical representation of HDMC migration in response to conditioned medium with and without Col. (B) Representative images of carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled HDMCs (green) that have migrated to the underside of the transwell membrane. HDMCs treated with either 50 nM Col or ethanol (EtOH) for 4 hours were stimulated with lipopolysaccharide (25 ng/mL). The supernatant was subsequently collected, centrifuged, and added to a 24-well plate. Freshly isolated CFSE-stained HDMCs were then added to transwell inserts and allowed to migrate toward the conditioned media (n = 4).

mice, CCL2 overexpression accelerates plaque progression[17]; whereas CCL2 blockade stunts progression and limits destabilization of preexisting atherosclerotic lesions.[18] In humans, elevated circulating CCL2 levels after ACS are associated with an increased risk of recurrence and a higher mortality.[19,20] CCL5 induces migration of monocytes, T lymphocytes, and neutrophils toward atherosclerotic lesions. Inhibition of CCL5 signaling attenuates plaque progression and promotes plaque stability in mice.[4] In humans, CCL5 is increased during cardiac ischemia, and elevated CCL5 levels after ACS are associated with progressive atherosclerotic lesions and future cardiovascular adverse events.[21,22] The dual-function chemokine CX3CL1 acts as both a soluble ligand and a membrane-bound adhesion molecule. In atherosclerosis, CX3CL1 is known to facilitate monocyte recruitment to the inflamed endothelium, activate platelets, and promote leukocyte survival.[1,23] Not surprising, increased serum levels of CX3CL1 are associated with an unstable plaque morphology and a higher risk of ACS.[24–26]

Colchicine Reduces TC Chemokine Levels

We found that colchicine administration acutely suppressed the local cardiac production of CCL2, CCL5, and CX3CL1. This reduction in intracardiac chemokine expression has significant therapeutic implications. ACS is most commonly preceded by

plaque rupture and subsequent thrombosis, a process that is driven by the continual recruitment of leukocytes to atherosclerotic lesions. By reducing this chemotactic stimulus, colchicine may increase stability of preformed atherosclerotic lesions and may reduce plaque disruption rates. Encouragingly, the use of colchicine in patients with ACS and patients with CAD has been supported by findings from the LoDoCo trial[10] and the COLPLAST-ACS study[11] which found that colchicine stabilizes coronary plaque and reduces ACS recurrence rates. As such, these current findings combined with previous data from our group indicating suppression of inflammasome-specific cytokines found that colchicine has a broad antiatherogenic mechanism(s) of action.

Colchicine Modulates Chemokine Expression in HDMCs

With the use of an *in vitro* model, we aimed to elucidate the mechanism by which colchicine reduces TC chemokine levels and whether this effect has a functional consequence. Our data found that colchicine regulates HDMC migration, in part by reducing CCL2 expression, a major process that mediates atherogenesis. In line with a previous report from Li et al,[27] this data found that colchicine acts directly on HDMCs to reduce CCL2 mRNA expression. One possible mechanism may be modulation of transcription factor nuclear translocation by disruption of microtubule networks,[28,29] although confirmation of this is beyond the scope of this article. We also found that colchicine reduces CCL5 and CXC3L1 protein levels without affecting transcription; this may be due to interference with post-transcriptional microtubule-dependent packaging and transport of these chemokines, thereby reducing their production. Again, dedicated mechanistic studies are required to confirm these findings. To determine whether the observed reduction in intracellular chemokine expression translated to a reduction in secretion and thus chemotaxis, we used a transwell migration assay. We found that by reducing chemokine production, colchicine inhibits HDMC chemotaxis. This evidence provides a mechanism by which colchicine may improve plaque stability by reducing intraplaque immune cell accumulation. Overall, these data suggest that colchicine suppresses monocyte activation and therefore chemotaxis, as found by the blunted response to LPS stimulation.

This pilot study was designed to identify potential anti-inflammatory mechanisms of colchicine in patients presenting with an ACS, rather than to assess colchicine's efficacy in cardiovascular disease or its effect on plaque stability. As such, this study has 3 important limitations. First, the sample size of this study was relatively small. Consequently, the distribution of baseline characteristics was not entirely equal, and results may be influenced by confounders not identified in this pilot study. Second, we did not measure the effect of colchicine on chemokines in patients with stable CAD. Finally, we only investigated the impact of colchicine on HDMCs, whereas *in vivo* serum chemokine levels are regulated by many cell types. To completely understand colchicine's mechanism of action *in vivo* it would be essential to investigate all relevant cell types, including endothelial cells and macrophages, which also secrete large amounts of chemokines.

CONCLUSION

In summary, these data provide a novel mechanism by which colchicine exerts its anti-inflammatory effects in patients with ACS. These results found that colchicine inhibits chemokine expression and thus monocyte migration. In combination with the existing literature, this evidence highlights the need for further large-scale, outcome-based clinical trials to assess the efficacy of colchicine in cardiovascular disease.

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Bradley Tucker was responsible for drafting and revising the manuscript. Rahul Kurup and Jennifer Barraclough were responsible for patient recruitment and sample collection. Bradley Tucker, Rodney Henriquez, and Sian Cartland performed the laboratory-based experimental procedures. Clare Arnott, Ashish Misra, and Mary Kavurma provided further technical support and aided with data analysis and presentation. Gonzalo Martínez and

Sanjay Patel were responsible for study design and development. Sanjay Patel was the senior author and approved the final manuscript. All authors contributed to editing of the final manuscript.

DISCLOSURES

We received no support from industry or organizations that may have influenced this work, and there were no study sponsors involved. The authors have indicated that they have no conflicts of interest regarding the content of this article.

REFERENCES

1. Zernecke A, Weber C. Chemokines in atherosclerosis: proceedings resumed. *Arterioscler Thromb Vasc Biol*. 2014;34:742–750.
2. Combadiere C, Potteaux S, Rodero M, et al. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation*. 2008;117:1649–1657.
3. Bot I, Ortiz Zacarias NV, de Witte WE, et al. A novel CCR2 antagonist inhibits atherogenesis in apoE deficient mice by achieving high receptor occupancy. *Sci Rep*. 2017;7:52.
4. Braunersreuther V, Steffens S, Arnaud C, et al. A novel RANTES antagonist prevents progression of established atherosclerotic lesions in mice. *Arterioscler Thromb Vasc Biol*. 2008;28:1090–1096.
5. Lesnik P, Haskell CA, Charo IF. Decreased atherosclerosis in CX3CR1-/- mice reveals a role for fractalkine in atherogenesis. *J Clin Invest*. 2003;111:333–340.
6. Gilbert J, Lekstrom-Himes J, Donaldson D, et al. Effect of CC chemokine receptor 2 CCR2 blockade on serum C-reactive protein in individuals at atherosclerotic risk and with a single nucleotide polymorphism of the monocyte chemoattractant protein-1 promoter region. *Am J Cardiol*. 2011;107:906–911.
7. Vaidya K, Martinez G, Patel S. The role of colchicine in acute coronary syndromes. *Clin Ther*. 2019;41:11–20.
8. Martínez GJ, Robertson S, Barraclough J, et al. Colchicine acutely suppresses local cardiac production of inflammatory cytokines in patients with an acute coronary syndrome. *J Am Heart Assoc*. 2015;4, e002128.
9. Robertson S, Martinez GJ, Payet CA, et al. Colchicine therapy in acute coronary syndrome patients acts on caspase-1 to suppress NLRP3 inflammasome monocyte activation. *Clin Sci (lond)*. 2016;130:1237–1246.
10. Nidorf SM, Eikelboom JW, Budgeon CA, Thompson PL. Low-dose colchicine for secondary prevention of cardiovascular disease. *J Am Coll Cardiol*. 2013;61:404–410.
11. Vaidya K, Arnott C, Martinez GJ, et al. Colchicine therapy and plaque stabilization in patients with acute coronary syndrome: a CT coronary angiography study. *JACC Cardiovasc Imag*. 2018;11(2 Pt 2):305–316.
12. King 3rd SB, Smith Jr SC, Hirshfeld Jr JW, et al. 2007 Focused update of the ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice guidelines. *J Am Coll Cardiol*. 2008;51:172–209.
13. Martínez GJ, Bailey BP, Celermajer DS, Patel S. A safe and easy technique to sample the coronary sinus–facilitating a closer look at cardiac disease. *Int J Cardiol*. 2014;176:1321–1322.
14. Ravindran D, Ridiandries A, Vanags LZ, et al. Chemokine binding protein 'M3' limits atherosclerosis in apolipoprotein E-/- mice. *PLoS One*. 2017;12, e0173224.
15. Werle M, Schmal U, Hanna K, Kreuzer J. MCP-1 induces activation of MAP-kinases ERK, JNK and p38 MAPK in human endothelial cells. *Cardiovasc Res*. 2002;56:284–292.
16. Yang CQ, Li W, Li SQ, et al. MCP-1 stimulates MMP-9 expression via ERK 1/2 and p38 MAPK signaling pathways in human aortic smooth muscle cells. *Cell Physiol Biochem*. 2014;34:266–276.
17. Aiello RJ, Bourassa PA, Lindsey S, et al. Monocyte chemoattractant protein-1 accelerates atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 1999;19:1518–1525.
18. Inoue S, Egashira K, Ni W, et al. Anti-monocyte chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice. *Circulation*. 2002;106:2700–2706.
19. de Lemos JA, Morrow DA, Blazing MA, et al. Serial measurement of monocyte chemoattractant protein-1 after acute coronary syndromes: results from the A to Z trial. *J Am Coll Cardiol*. 2007;50:2117–2124.
20. de Lemos JA, Morrow DA, Sabatine MS, et al. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. *Circulation*. 2003;107:690–695.
21. Blanchet X, Cesarek K, Brandt J, et al. Inflammatory role and prognostic value of platelet chemokines in acute coronary syndrome. *Thromb Haemost*. 2014;112:1277–1287.
22. Kraaijeveld AO, de Jager SC, de Jager WJ, et al. CC chemokine ligand-5 (CCL5/RANTES) and CC chemokine ligand-18 (CCL18/PARC) are specific markers of refractory unstable angina pectoris and are transiently raised during severe ischemic symptoms. *Circulation*. 2007;116:1931–1941.

23. Schafer A, Schulz C, Eigenthaler M, et al. Novel role of the membrane-bound chemokine fractalkine in platelet activation and adhesion. *Blood*. 2004;103:407–412.
24. Damas JK, Boullier A, Waehre T, et al. Expression of fractalkine (CX3CL1) and its receptor, CX3CR1, is elevated in coronary artery disease and is reduced during statin therapy. *Arterioscler Thromb Vasc Biol*. 2005;25:2567–2572.
25. Niessner A, Marculescu R, Haschemi A, et al. Opposite effects of CX3CR1 receptor polymorphisms V249I and T280M on the development of acute coronary syndrome. A possible implication of fractalkine in inflammatory activation. *Thromb Haemost*. 2005;93:949–954.
26. Zhong ZX, Li B, Li CR, et al. Role of chemokines in promoting instability of coronary atherosclerotic plaques and the underlying molecular mechanism. *Braz J Med Biol Res*. 2014;48:161–166.
27. Li JJ, Lee SH, Kim DK, et al. Colchicine attenuates inflammatory cell infiltration and extracellular matrix accumulation in diabetic nephropathy. *Am J Physiol Ren Physiol*. 2009;297:F200–F209.
28. Li Q, Martinez JD. P53 is transported into the nucleus via an Hsf1-dependent nuclear localization mechanism. *Mol Carcinog*. 2011;50:143–152.
29. Mackenzie GG, Keen CL, Oteiza PI. Microtubules are required for NF-kappaB nuclear translocation in neuroblastoma IMR-32 cells: modulation by zinc. *J Neurochem*. 2006;99:402–415.

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