

# Mechanism of Action of Colchicine in the Treatment of Gout

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## ABSTRACT

**Purpose:** The aims of this article were to systematically review the literature about the mechanism of action of colchicine in the multimodal pathology of acute inflammation associated with gout and to consider the clinical utility of colchicine in other chronic inflammatory diseases.

**Methods:** The English-language literature on PubMed was searched for articles published between 1990 and October 2013, with a cross-reference to citations across all years. Relevant articles pertaining to the mechanism of action of colchicine and the clinical applications of colchicine in gout and other inflammatory conditions were identified and reviewed.

**Findings:** The molecular pathology of acute inflammation associated with gouty arthritis involves several concurrent pathways triggered by a variety of interactions between monosodium urate crystals and the surface of cells. Colchicine modulates multiple pro- and anti-inflammatory pathways associated with gouty arthritis. Colchicine prevents microtubule assembly and thereby disrupts inflammasome activation, microtubule-based inflammatory cell chemotaxis, generation of leukotrienes and cytokines, and phagocytosis. Many of these cellular processes can be found in other diseases involving chronic inflammation. The multimodal mechanism of action of colchicine suggests potential efficacy of colchicine in other comorbid conditions associated with gout, such as osteoarthritis and cardiovascular disease.

**Implications:** Colchicine has multiple mechanisms of action that affect inflammatory processes and result in its utility for treating and preventing acute gout flare. Other chronic inflammatory diseases that invoke these molecular pathways may represent new therapeutic applications for colchicine. (*Clin Ther.* 2014;36:1465–1479) © 2014 The Authors. Published by Elsevier HS Journals, Inc.

**Key words:** colchicine, gout, inflammatory arthritis, mechanism of action.

## INTRODUCTION

Gout is an inflammatory arthritis that is the result of the precipitation of serum urate into crystallized deposits of monosodium urate (MSU) in and around the joint. These crystals cause recurrent episodes of severe inflammatory arthritis that present as swelling, redness, heat, pain, and stiffness in the joints, most often seen in the first metatarsophalangeal joint. Colchicine is a natural product originally extracted from plants of the genus *Colchicum* (*autumn crocus*) and has been used to treat gouty arthritis for centuries.<sup>1</sup> Clinical trial results have demonstrated that low-dose colchicine is effective for the management of acute gout flares as well as for long-term prophylactic maintenance.<sup>2–4</sup>

Current treatment guidelines recognize the efficacy of colchicine in the treatment of acute gouty arthritis and for the prevention of gout flares. The American College of Rheumatology Guidelines for Management of Gout recommend the pharmacologic treatment of acute gout flares within 24 hours of onset and recommend colchicine, NSAIDs, selective cyclooxygenase-2 inhibitors, and corticosteroids as first-line therapies for treating the pain of acute flares.<sup>5</sup> A recent update of the European League Against Rheumatism (EULAR) guidelines for the management of gout, carried out by a multidisciplinary panel of experts from the United States, also recommends that the initial treatment of acute gout flares begin with low-dose colchicine, NSAIDs, and glucocorticoids.<sup>6</sup> The EULAR guidelines indicate that prophylaxis for acute gout attacks during the first 6 to 12 months of therapy with urate-lowering agents can be achieved with colchicine.<sup>6</sup>

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These changes in treatment guidance have been the result of an increased understanding of the molecular pathology underlying the acute inflammation associated with gout and the potential benefits of early and aggressive treatment. In light of this new information, there is growing evidence that the therapeutic response of colchicine is multifaceted and intervenes at several different pathways involved in inflammation. The objectives of this review were to determine the current views regarding the mechanism of action of colchicine and to consider the potential clinical utility of colchicine in other chronic inflammatory diseases.

## MATERIALS AND METHODS

The PubMed database was searched for relevant studies published between 1990 and October 2013 and restricted to the English language. All medical-subject heading searches were explored using Boolean-based key word search criteria and included the terms *gout*, *inflammation*, *colchicine*, *osteoarthritis*, and *cardiovascular disease*. The focus was on the following questions: (1) What is the process of inflammation in gout?; (2) What is the mechanism of action of colchicine?; and (3) What are the clinical applications of colchicine in gout and other medical conditions? Additionally, references noted in relevant articles were also accessed and reviewed. Studies that included original research and explored recent advances in the understanding of the molecular pathology of inflammation associated with gout, the multimodal mechanism of action of colchicine in response to inflammation, and the potential use of colchicine in other chronic inflammatory diseases were critically discussed.

## RESULTS

A total of 756 scientific and clinical articles published in English were identified through a cross-comparative search. After medical review, 693 were evaluated as outside the scope of the focus of this review. The remaining 63 publications were carefully reviewed to identify potentially relevant articles for retrieval.

### Inflammation and Gout

Awareness of the multiple actions of colchicine in gout requires an understanding of the inflammatory cascade underlying the symptoms of this debilitating disease. Gout is a disease process triggered by interactions between MSU microcrystals and the local tissue environment. The affected synovium of patients

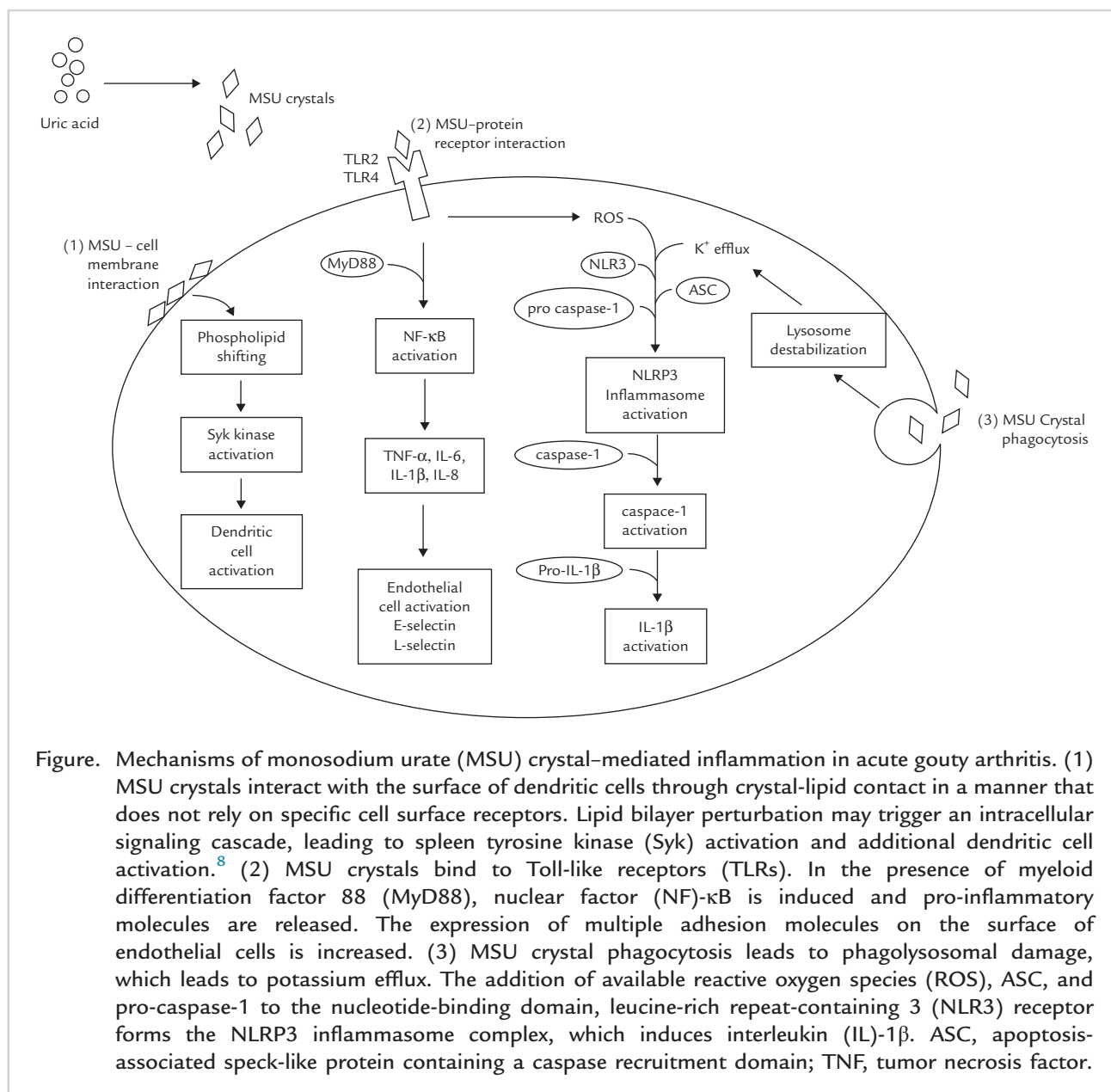
with acute gouty arthritis is infiltrated with neutrophils, mononuclear phagocytes, and lymphocytes, resulting in marked swelling of the tissues and vascular injury.<sup>7</sup> The biochemical mechanisms that link MSU crystal precipitation with joint inflammation have not been definitively elucidated and likely involve a variety of leukocytes, cytokines, and chemokines that participate in the innate immune system response (Figure).

### MSU Crystal Formation

Precipitation of urate into MSU crystals is central in acute gouty arthritis. However, the mechanism by which MSU crystals form directly at the site of joint inflammation is not well understood. Monosodium urate crystallizes when the plasma concentration of urate exceeds its solubility (~7 mg/dL).<sup>9</sup> Factors in addition to plasma concentration that have been shown to affect urate solubility in vitro include pH, temperature, ionic strength, and the binding of urate to plasma macromolecules.<sup>9-13</sup> However, environmental conditions and/or mechanisms favoring/limiting crystal formation in vivo are likely different from in vitro models. The de novo formation of MSU crystals within the joint may be triggered by excessive alcohol or red meat intake and large-scale cell death from trauma, surgery, or anticancer therapies. Plasma macromolecules such as albumin have been suggested as possible MSU crystal-nucleating agents.<sup>11,14</sup> Circulating antibodies, including immunoglobulin (Ig) G and IgM, recognize MSU crystal surfaces, stabilize them, and promote further crystallization.<sup>15-17</sup>

### MSU Crystal Stimulation of Pro-Inflammatory Cells

Endogenous MSU crystals act as danger-associated molecular patterns (DAMPs) that are recognized by the innate immune system, notably neutrophils and macrophages/monocytes, as well as mast cells and dendritic cells.<sup>8,18-21</sup> Uric acid DAMP signaling activates dendritic cells and macrophages to secrete pro-inflammatory cytokines, including interleukin (IL)-1 $\beta$ .<sup>22,23</sup> The mechanism by which pro-inflammatory cells interact with MSU crystals is a major area of research focus and likely involves different pathways operating simultaneously to initiate the inflammatory cascade as described subsequently.



**Figure.** Mechanisms of monosodium urate (MSU) crystal-mediated inflammation in acute gouty arthritis. (1) MSU crystals interact with the surface of dendritic cells through crystal-lipid contact in a manner that does not rely on specific cell surface receptors. Lipid bilayer perturbation may trigger an intracellular signaling cascade, leading to spleen tyrosine kinase (Syk) activation and additional dendritic cell activation.<sup>8</sup> (2) MSU crystals bind to Toll-like receptors (TLRs). In the presence of myeloid differentiation factor 88 (MyD88), nuclear factor (NF)-κB is induced and pro-inflammatory molecules are released. The expression of multiple adhesion molecules on the surface of endothelial cells is increased. (3) MSU crystal phagocytosis leads to phagolysosomal damage, which leads to potassium efflux. The addition of available reactive oxygen species (ROS), ASC, and pro-caspase-1 to the nucleotide-binding domain, leucine-rich repeat-containing 3 (NLR3) receptor forms the NLRP3 inflammasome complex, which induces interleukin (IL)-1β. ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain; TNF, tumor necrosis factor.

### NLRP3 Inflammasome

The nucleotide-binding domain, leucine-rich repeat-containing (NLR) family of receptors plays an important role in the recognition of danger-associated signals. Together with the adaptor apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and pro-caspase-1, NLRs form a multiprotein complex—the inflammasome—which induces pro-inflammatory cytokines, most notably IL-1β.<sup>24</sup> Expressed in myeloid cells, the

inflammasome is a multiprotein oligomer that consists of caspase-1, caspase-5, NLRP, and PYCARD. The inflammasome is a component of the innate immune system and has been shown to be involved in the activation of many inflammatory processes.<sup>25</sup> In 2006, Martinon et al<sup>26</sup> reported that the NLRP3 (formerly NALP3) inflammasome is specifically activated by MSU crystals.

The steps that link cellular contact of MSU crystals with activation of the NLRP3 inflammasome involve

phagocytosis of crystals, potassium efflux, and the generation of reactive oxygen species.<sup>27–31</sup> On activation, the NLRP3 inflammasome recruits and activates caspase-1, which then goes on to produce mature IL-1 $\beta$  from the pro-IL-1 $\beta$  precursor.<sup>32,33</sup> Recent work has implicated the action of microtubules as being central in the assembly and activation of the inflammasome.<sup>34</sup>

#### *Macrophages and Monocytes*

Both resident macrophages and MSU-recruited monocytes that differentiate into macrophages contribute to gout-associated inflammation.<sup>35</sup> Toll-like receptors (TLRs)-2 and -4 and the cytosolic TLR adapter protein myeloid differentiation factor 88 (MyD88) contribute to the activation of macrophages by MSU crystals.<sup>36,37</sup> Once stimulated, TLRs and the IL-1 $\beta$  receptor associate with a number of intracellular adaptor molecules, including MyD88, to trigger a signaling cascade that activates pro-inflammatory transcription factors, such as nuclear factor (NF)- $\kappa$ B, and increases release of pro-inflammatory molecules such as tumor necrosis factor (TNF)- $\alpha$ , IL-6, and IL-8.<sup>18,22,38–40</sup> Results from ex vivo experiments showed that stimulation of resident peritoneal macrophages with MSU crystals increased expression of multiple inflammatory cytokines, including IL-1 $\beta$ , IL-6, and TNF- $\alpha$ . Additional in vivo studies showed that depletion of resident macrophages resulted in decreased cytokine production.<sup>20</sup> Additionally, monocytes recruited to sites of MSU crystal deposition differentiate into pro-inflammatory M1-like macrophages.<sup>35</sup> It has been suggested that stimulation of these macrophages by fresh MSU crystals results in a secondary wave of inflammation in acute gout flares.<sup>35</sup>

#### *Neutrophils*

Secretion of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8 by monocytes that have been stimulated with MSU crystals increases expression of multiple adhesion molecules on the surface of endothelial cells, including E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1. This, in turn, leads to recruitment of neutrophils to sites of crystal deposition.<sup>19</sup> Neutrophils have been shown to contribute to IL-1 $\beta$  production in some inflammatory states, which may also be the case in MSU crystal-induced inflammation.<sup>41</sup> MSU crystals rapidly stimulate tyrosine phosphorylation in neutrophils, leading to the production of superoxide anions necessary for

NLRP3 assembly and neutrophil activation.<sup>42,43</sup> Activation of neutrophils in gout is associated with the formation of pro-inflammatory neutrophil extracellular traps, which are associated with both autophagy and IL-1 $\beta$  production.<sup>44</sup> Prolonged exposure to neutrophil extracellular traps increases the risk for chronic inflammation; the synovial fluid and joint tissue of patients with gout also reveal neutrophil extracellular trap formation, particularly during flares.<sup>45</sup>

#### *Dendritic Cells*

Dendritic cells are antigen-presenting cells that detect DAMPs and propagate signaling cascades, leading to nuclear translocation of transcription factors and escalation of inflammation. MSU crystals interact with the surface of dendritic cells through crystal-lipid contact in a manner that does not rely on specific cell-surface receptors.<sup>8</sup> MSU crystals engage the lipid surface of dendritic cells, thereby perturbing the lipid bilayer and causing lipid and cholesterol shifting. Ng et al<sup>8</sup> proposed that this lipid sorting initiates an intracellular signaling cascade that triggers spleen tyrosine kinase and leads to subsequent dendritic cell activation.

#### *Mast Cells*

Mast cells are involved in the early phase response to MSU crystal-induced inflammation based on results from the rat peritonitis model.<sup>8</sup> On introduction of MSU crystals to the peritoneal cavity, mast cell infiltrates were identified in the subintimal layer of the peritoneal membrane before monocyte/macrophage and neutrophil influx to the membrane.<sup>21</sup> On activation, mast cells release factors such as TNF- $\alpha$ , IL-1 $\beta$ , platelet-activating factor, and histamine to regulate endothelial cell adhesion molecules and promote inflammatory amplification.<sup>46,47</sup>

#### *Complement*

MSU crystal-induced activation of both the classic and the alternative complement pathways contributes to acute gouty inflammation.<sup>48</sup> Complement components including C1q, C1r, and C1s, as well as IgG and IgM, bind to MSU crystals, and the activation process is amplified by the presence of these proteins.<sup>49</sup> MSU-mediated activation of the classic pathway will also occur in the absence of Ig, indicating that MSU crystals can directly initiate the classic cascade.<sup>50</sup>

Activation of the alternative pathway leads to the production of C5a and C5b fragments by a C5 convertase localized on the surface of MSU crystals. These fragments act as potent leukocyte chemo-attractants.<sup>51</sup> Additionally, in response to MSU crystals, the C6-mediated formation of the membrane attack complex has a substantial role in IL-8 production and subsequent neutrophil influx in acute gouty inflammation.<sup>52</sup>

### **Hypernociception**

Severe joint pain is the central experience of individuals with acute gouty arthritis. Neutrophils and associated pro-inflammatory cytokines, including TNF- $\alpha$ , IL-1 $\beta$ , and IL-8, play a crucial role in inflammatory hypernociception.<sup>53,54</sup> Amaral et al<sup>41</sup> injected MSU crystals into the joints of mice to stimulate an inflammatory response in the synovial fluid and surrounding tissue and evaluated concomitant articular hypernociception. Results demonstrated that hypernociceptive responses were dependent on the activation of the NLRP3 inflammasome, IL-1 $\beta$  production, MyD88 activation, and neutrophil accumulation. Caspase-1 has also been shown to induce hypernociception by promoting IL-1 $\beta$  secretion.<sup>54</sup>

### **Resolution of Gout Attacks**

Many gout attacks resolve spontaneously over  $\sim$ 1 week, even without therapeutic intervention. A variety of processes may contribute to the self-limiting nature of gouty attacks, and multiple factors may be utilized. Coating of MSU crystals by interstitial fluid proteins (apolipoproteins B and E) may decrease their ability to initiate inflammation.<sup>55,56</sup> Differentiated macrophages may also contribute to gout attack resolution by phagocytosis of crystals without stimulating inflammatory events.<sup>57</sup> These interactions may help to explain the presence of MSU crystals in synovial fluid after an acute gout attack is resolved and in the synovial fluid of asymptomatic gout patients.<sup>58–60</sup>

Stimulation of antiinflammatory pathways may also play a role in gout-attack resolution. Peroxisome proliferator-activated receptor- $\gamma$  is activated in gout attacks, and ligands for this receptor inhibit transcription of the genes encoding TNF- $\alpha$ , IL-1, IL-6, cyclooxygenase-2, inducible nitric oxide synthase, and matrix metalloproteinases.<sup>61,62</sup> Peroxisome proliferator-activated receptor- $\gamma$  ligands promote monocyte expression of the scavenger receptor CD36, which is involved in the phagocytosis of apoptotic cells. This may increase rapid

phagocytosis of apoptotic neutrophils by macrophages and reduce damage associated with exposure to toxins released from dying cells.<sup>63</sup>

Transforming growth factor (TGF)- $\beta$ 1 is an important cytokine mediator in the resolution of MSU-induced inflammation.<sup>64</sup> Phagocytosis of apoptotic neutrophils by macrophages and live neutrophils triggers TGF- $\beta$ 1 production and release. Increased TGF- $\beta$ 1 production suppresses neutrophil inflammatory response and moderates IL-1 production.<sup>65–67</sup> IL-8 is the principle cytokine involved in neutrophil migration. Scanu et al<sup>68</sup> demonstrated that the level of IL-8 in synovial fluid remains elevated 4 to 7 days after the initiation of gout flare. Maintenance of IL-8 levels allow for continued recruitment of neutrophils to inflamed joints, enabling phagocytosis of apoptotic neutrophils and increased TGF- $\beta$ 1 production. Additionally, suppressors of cytokine signaling, including cytokine-inducible SH2-containing protein and suppressor of cytokine signaling 3, are upregulated. These 2 factors are involved in suppressing IL-1 $\beta$  and TNF- $\alpha$  production and promoting TGF- $\beta$ 1 production, which contribute to the resolution of acute gout attacks.<sup>69</sup>

### **Mechanism of Action of Colchicine in Gouty Arthritis**

Colchicine affects the molecular pathology underlying acute inflammation associated with gouty arthritis in a multimodal manner (Table). In vitro, at micromolar concentrations, colchicine inhibits MSU crystal activation of the NLRP3 inflammasome, blocks the release of IL-1 $\beta$ , and suppresses the expression of genes involved in cell regulation.<sup>26,34,70,71</sup> At nanomolar concentrations, colchicine modulates adhesion protein expression on endothelial cells, inhibits IL-1-induced L-selectin expression, modulates cytokine maturation and release, and diminishes neutrophil chemotaxis to cytokines.<sup>72,76,77</sup> Whereas plasma concentration after single dosing of 0.6-mg colchicine is approximately 3 nmol/L, it has been shown to accumulate in a saturable manner in neutrophils to 40 to 200 nmol/L, well above its  $K_i$  of 24 nmol/L for microtubule polymerization.<sup>78,79</sup> The correlation of the inhibition of microtubule polymerization with the effects on these aforementioned pathways supports the inhibition of microtubule polymerization by colchicine and its effects on downstream pathways as a primary target in the mechanism of action of this molecule in the treatment of gout.<sup>34</sup>

Table. Multimodal effects of the inhibition of microtubule polymerization by colchicine.

Biological Effect of Colchicine	Study
Inhibits activation of the NLRP3 inflammasome in response to inflammatory microcrystals	Martinon et al (2006) <sup>26</sup>
Suppresses the expression of NF-κB	Jackman et al (2009) <sup>70</sup>
Decreases the number of TNF-α receptors on the surface of macrophages and endothelial cells	Ding et al (1990) <sup>71</sup>
Decreases L-selectin expression on neutrophils	Cronstein et al (1995) <sup>72</sup>
Alters the distribution of E-selectin on the surface of endothelial cells	Cronstein et al (1995) <sup>72</sup>
Inhibits superoxide anion production in response to MSU crystals	Roberge et al (1996) <sup>43</sup> ; Chia et al (2008) <sup>73</sup>
Interrupts mast cell degranulation process	Oka et al (2005) <sup>74</sup>
Increases the level of TGF-β1	Yagnik et al (2004) <sup>67</sup> ; Sayarlioglu et al (2006) <sup>75</sup>

MSU = monosodium urate; NF = nuclear factor; NLR = nucleotide-binding domain = leucine-rich repeat-containing; TGF = transforming growth factor; TNF = tumor necrosis factor.

### Tubulin Disruption

Colchicine binds to both  $\alpha$ - and  $\beta$ -tubulin to create a tubulin–colchicine complex that prevents the formation of microtubules.<sup>80,81</sup> The state of microtubule polymerization can control numerous cellular functions, including intracellular organelle and vesicle transport; secretion of cytokines and chemokines; and migration, division, and regulation of gene expression.<sup>82</sup> These actions influence the cell activity known to be involved in inflammatory pathways central to the pathogenesis of gout. Processes that require microtubule-mediated recruitment or cytosolic components, such as mitochondria and proteins such as MyD88, ASC, spleen tyrosine kinase and other kinases, to modulate the generation of cytokines and chemoattractants, are all susceptible to modulation by colchicine treatment. Microtubule disruption by colchicine has been studied extensively and is a primary mechanism by which colchicine intervenes in the molecular processes underlying gout inflammation; however, it is probably not the only site of colchicine action.

### NLRP3 Inflammasome

Colchicine interrupts the process by which MSU crystals activate the NLRP3 inflammasome, thereby preventing the processing of pro-IL-1 $\beta$  and the release

of IL-1 $\beta$ .<sup>26</sup> Colchicine suppression of the active NLRP3 inflammasome stems from disruption of the microtubule-mediated transport of mitochondria (where endogenous ASC is localized) to the endoplasmic reticulum (ER; where NLRP3 is localized). The co-localization of NLRP3 and ASC is required for assembly and activation of the inflammasome to produce mature IL-1 $\beta$ .<sup>34</sup>

### Inhibition of Superoxide Anion Production

Colchicine has also been demonstrated to have an effect in suppressing MSU crystal-induced tyrosine phosphorylation and superoxide anion production in human neutrophils in vitro and in murine peritoneal macrophages.<sup>42,83</sup> The colchicine-mediated inhibition of reactive oxygen species production necessary for inflammasome activation has been shown to be caused by the microtubule-disrupting effect of colchicine.<sup>42,43,73,83</sup> The inhibition of MSU-induced superoxide production by neutrophils can be accomplished in vivo at doses 100-fold lower than those required to inhibit neutrophil infiltration.<sup>73</sup>

### Neutrophil–Endothelial Cell Interactions

Colchicine interferes with neutrophil adhesion and recruitment to inflamed tissues by decreasing neutrophil L-selectin expression and changing the distribution

of E-selectin on endothelial cells.<sup>72</sup> By preventing the formation of intact microtubule structures, colchicine blocks trafficking of E-selectin at the cell surface, leading to changes in endothelial adhesiveness.<sup>72,76</sup> At nanomolar concentrations, colchicine is able to disrupt the topography of E-selectin distribution on the surface of endothelial cells, potentially providing a mechanism for the prophylactic effect of colchicine on gout-related inflammation.<sup>72</sup> At micromolar concentrations, colchicine decreases the expression of L-selectin on the surface of neutrophils, thereby impairing the adhesion between neutrophils and the endothelium. This observation provides a potential mechanism for the therapeutic effect of colchicine on gout flare.

Leukotriene B<sub>4</sub> (LTB<sub>4</sub>) is a chemo-attractant and mediator of inflammation that is required for MSU-induced activation of the NLRP3 inflammasome and subsequent release of IL-1 $\beta$ .<sup>41</sup> LTB<sub>4</sub> is also involved in promoting the adhesion and mobility of neutrophils.<sup>84</sup> Colchicine significantly decreased LTB<sub>4</sub>-induced leukocyte adherence and decreased LTB<sub>4</sub>-induced leukocyte emigration from postcapillary venules.<sup>84</sup> A convergent downstream effect of LTB<sub>4</sub> on inflammatory cells is the microtubule-driven assembly and activation of NLRP3, further supporting the microtubule as a primary target for colchicine.<sup>34</sup>

#### *TNF- $\alpha$*

Colchicine blunts TNF- $\alpha$ -induced activation of macrophages and diminishes the number of TNF- $\alpha$  receptors on the surface of macrophages and endothelial cells but not on the surface of neutrophils. These findings were attributed to colchicine-mediated destabilization of the microtubule network.<sup>71</sup> TNF- $\alpha$ -induced activation of NF- $\kappa$ B is also inhibited by colchicine.<sup>70</sup> Evidence from Jackman et al<sup>70</sup> suggested that microtubules are integral to the regulation of the signaling cascade involved in NF- $\kappa$ B activation. Colchicine-induced disruption of microtubules inhibits signal transduction of the TNF- $\alpha$ -NF- $\kappa$ B pathway.

#### *Mast Cells*

Colchicine has been implicated in the interruption of mast cell degranulation processes, thus preventing the release of inflammatory mediators.<sup>74</sup> This is believed to be the result of colchicine-induced disruption of microtubule-mediated granular transport. In addition, with regard to allergen-mediated degranulation, microtubules are involved in regulating calcium ion signaling

between the ER and the plasma membrane, which is necessary to initiate mast cell degranulation. In concert with its effect on mitochondrial motility during the assembly of NLRP3, colchicine was also shown to disable proper ER arrangement within the cell to permit calcium ion influx, leading to attenuation of the allergic response in vivo.<sup>34,74</sup>

#### *Hypernociception*

One pathway to hypernociception depends on activation of the NLRP3 inflammasome, generation of cytokines and leukotrienes, MyD88 recruitment, and neutrophil chemotaxis.<sup>41</sup> Interruption of these as well as other processes may explain the ability of colchicine to attenuate hypernociception. Additional research indicates that colchicine-mediated microtubule disruption affects sensory neurons attenuating hyperalgesia independent of inflammation.<sup>85-87</sup>

#### ***Effects of Colchicine on Antiinflammatory Mediators***

In addition to interfering with the actions of pro-inflammatory pathways, colchicine also increases levels of antiinflammatory molecules that may contribute to clinical benefit in patients with gout. TGF- $\beta$ 1 has been shown to be elevated in the synovial fluid of patients with gout, and its levels are highest during the resolution of gout attacks.<sup>68,88</sup> Blood levels of this potent antiinflammatory molecule are increased by colchicine, and the decrease in IL-1 $\beta$  levels induced by colchicine treatment may be expected to enhance TGF- $\beta$ 1 signaling as described by Lim et al.<sup>75,89</sup> Colchicine treatment has also been shown to provide protection against oxidative stress and to increase the activity of the antioxidant redox system in patients with remission of familial Mediterranean fever (FMF).<sup>90</sup>

More recent studies have investigated the mechanism of action of colchicine in novel inflammatory diseases, with a specific focus on the cytoskeleton. A study by Taskiran et al<sup>91</sup> investigated the effects of colchicine on pyrin and its interacting proteins as part of the potential pharmacologic effect in patients with FMF. The investigators reported that colchicine directly prevents the formation of reticulated fibrils that are generated by the cytosolic adaptor protein, proline-serine-threonine phosphatase-interacting protein 1, thereby preventing the transport of proline-, glutamic acid-, serine-, and threonine-rich phosphatases to their substrates. Colchicine also reduced ASC speck rates in

transfected cells, as well as downregulated *MEFV* gene expression and reorganization of actin cytoskeleton of THP-1 cells. The investigators concluded that reorganization of the actin cytoskeleton may affect expression of the *MEFV* gene and potentially explain the pharmacologic action of colchicine in FMF. Paschke et al<sup>77</sup> investigated the effects of colchicine on the regulation of cell motility, evaluating the reorganization of subcellular compartments by which colchicine modulates the elasticity, stiffness, and viscosity of neutrophils. Colchicine, at therapeutic doses, was found to significantly affect the deformability and motility of human neutrophils in confined spaces, emphasizing the role of the cytoskeleton as a pharmacologic target during any inflammatory process in which activated neutrophils are involved.

### **Colchicine Metabolism and Adverse Reactions**

In the past, colchicine was administered both by the oral and the intravenous routes. However, the latter dosage formulation is no longer practiced due to serious adverse events.<sup>92</sup> The most common adverse reactions reported with oral colchicine therapy in clinical trials in gout were diarrhea (23%) and pharyngolaryngeal pain (3%).<sup>2-4,93</sup> These events were considered generally mild and resolved with dose reduction. More severe adverse events were observed with overdoses of colchicine, including bone marrow suppression with agranulocytosis. Colchicine is metabolized by the cytochrome P450 (CYP) 3A4 enzyme and it is also a substrate for the P-glycoprotein 1 (P-gp) efflux transporter.<sup>94-96</sup> Concurrent use of interacting drugs or the administration of colchicine to patients with impaired renal function has been associated with myopathy or rhabdomyolysis.<sup>97-99</sup> For example, when P-gp or strong CYP3A4 inhibitors (eg, cyclosporin, tacrolimus, ketoconazole, protease inhibitors, imidazole, and clarithromycin) are prescribed in combination with colchicine, increased intracellular accumulation of colchicine is likely and may lead to adverse pharmacologic or toxic effects, such as muscle weakness or pain, severe diarrhea or vomiting, abdominal pain, increased infections, or unusual bleeding or bruising.<sup>95,100,101</sup> Significant adverse drug interactions have occurred in patients treated with colchicine and lipid-lowering drugs, such as simvastatin, that utilize the CYP3A4 pathway of drug metabolism, causing muscle aches, rhabdomyolysis, and/or myopathy.<sup>102-104</sup> In addition, rhabdomyolysis was reported in a patient who received colchicine with digoxin, a P-gp substrate.<sup>105</sup>

Current prescribing information, based on drug–drug interaction studies only recently conducted, recommend dose reductions when colchicine is used in conjunction with P-gp inhibitors or moderate/strong CYP3A4 inhibitors. Colchicine administration is contraindicated in patients with renal or hepatic impairment receiving both P-gp and strong CYP3A4 inhibitors concurrently.<sup>93</sup>

Both accidental and therapeutic poisoning deaths have occurred with colchicine overdose.<sup>106</sup> High fatality rates have been reported after doses exceeding 0.5 mg/kg. Therapeutic overdose may occur at lower doses, particularly in patients with renal impairment or on P-gp or strong CYP3A4 inhibitors or in patients given colchicine acutely with the high-dose “to-gastrointestinal-toxicity” approach. After colchicine overdose, gastrointestinal symptoms occur within a day after acute ingestion, followed by multiple organ failure 1 to 7 days after ingestion. Myocardial toxicity may lead to acute cardiovascular collapse and ventricular dysrhythmias.<sup>107,108</sup> Treatment options are very limited after acute colchicine overdose. Early gastrointestinal decontamination using gastric lavage and multidose activated charcoal is recommended. Hemodialysis or hemofiltration is ineffective due to the large volume of distribution of colchicine, and after decontamination, treatment is mainly supportive.

### **Areas of Ongoing Clinical Interest**

Colchicine affects many molecular targets and disrupts multiple pathways involved in inflammation associated with acute gout flare. This role in the regulation of inflammatory mediators suggests potential efficacy of colchicine in other conditions involving chronic inflammation, including other forms of arthritis and cardiovascular disease.<sup>109-112</sup>

### **Colchicine in the Treatment of Other Forms of Arthritis**

Gout and osteoarthritis (OA) occur together in many patients.<sup>113</sup> The pathophysiology of OA is characterized by upregulation of a large number of cytokines, including IL-1 $\beta$  and TNF- $\alpha$  in the chondrocyte and resident macrophages of the affected joints, which serve to initiate and accelerate disease progression.<sup>114,115</sup> These pro-inflammatory mediators lead to the activation of destructive pathways involving extracellular matrix–degrading enzymes and bone remodeling driven by the activation of NF- $\kappa$ B and TGF- $\beta$ .<sup>116,117</sup> Several recent studies have indicated



that local elevations of IL-1 $\beta$  and TNF- $\alpha$  can modulate the TGF- $\beta$  pathway from a homeostatic to a pathogenic role leading to OA, suggesting a potential role for colchicine in the treatment of OA.<sup>118</sup>

A small-scale evaluation of colchicine in OA included 61 postmenopausal patients with primary knee OA who were treated with colchicine 0.5 mg BID or placebo. Results from this trial suggested that use of rescue medication (acetaminophen) was significantly lower in the colchicine group compared with the placebo group ( $P < 0.0001$ ). Rates of improvement, as measured using patients' global assessment and physicians' global assessment, at the end of 3 months of follow-up were significantly greater with colchicine compared with placebo (both,  $P < 0.0001$ ).<sup>109</sup> In another study, in 36 patients with knee OA, colchicine 0.5 mg BID or placebo was added to nimesulide (an NSAID), and patients were followed up for 5 months. A 30% improvement rate, as measured by total Western Ontario and McMaster Universities Osteoarthritis Scale scores, at 20 weeks was noted in the group that received colchicine (57.9%) versus the group treated with placebo (23.5%) ( $P < 0.05$ ). The percentage of patients achieving a 30% reduction in index knee pain, as measured by a visual analog scale, was significantly greater in the colchicine group compared with the placebo group (52.6% vs 17.6%;  $P < 0.05$ ).<sup>112</sup> With the known effect on the reduction of IL-1 $\beta$  in the inflammatory state, future evaluation of colchicine in patients with OA in the clinical setting seems appropriate.

Calcium pyrophosphate (CPP) crystal deposition disease (CPPD) has clinical similarities to gout and is sometimes called *pseudogout*. Although the exact mechanism of CPP crystallization is not clear, crystal deposition depends on several factors including the presence of extracellular proteins and the concentration of calcium ions, inorganic phosphate, and inorganic pyrophosphate.<sup>119–121</sup> Once CPP crystals form, the release of IL-1 $\beta$  is triggered by CCP-induced crystal activation of the NLRP3 inflammasome, and the inflammation cascade is initiated by macrophages and mast cells.<sup>19,26,122</sup>

EULAR recently developed recommendations for the management of CPPD and treating acute attacks.<sup>123</sup> Unlike in gout, there is no CPP-lowering therapy available. For CPPD prophylaxis, the EULAR recommendations include the use of colchicine (0.5–1.0 mg/d) in combination with oral NSAIDs. As of

yet, there have been no registered clinical trials of colchicine for the management of CPPD; however, in many of the laboratory studies of colchicine and neutrophil function, colchicine has been shown to prevent neutrophil activation in response to microcrystal activation.<sup>83,123–125</sup>

Despite the central role of pro-inflammatory cytokines such as TNF- $\alpha$  in other forms of inflammatory arthritis, such as psoriatic arthritis, ankylosing spondylitis, and rheumatoid arthritis, these forms of inflammatory arthritis have proven resistant to colchicine treatment.<sup>126,127</sup> These observations suggest that the pro-inflammatory pathways involved in the pathogenesis and maintenance of inflammation in these chronic rheumatic diseases are not molecular or cellular targets for colchicine action.

### **Cardiovascular Risk Reduction With Colchicine**

Patients with gout typically have multiple comorbidities, notably hypertension, that increase the risk for cardiovascular events.<sup>128</sup> Gout itself may be an independent risk factor for cardiac events and mortality.<sup>129,130</sup> Although colchicine has been shown to act on cells and mediators of inflammation, there are limited clinical data regarding its benefit in cardiovascular risk reduction. Results from a study in patients with stable coronary artery disease and elevated high-sensitivity C-reactive protein (CRP) ( $\geq 2$  mg/L), a biomarker of inflammation, indicated that the administration of colchicine 0.5 mg BID for 4 weeks resulted in a 60% relative decrease in high-sensitivity CRP levels that was independent of aspirin or statin use.<sup>131</sup> In contrast, in a study in 80 patients with acute coronary syndromes or acute ischemic stroke, treatment with colchicine 1 mg/d for 1 month had no significant effect on mean CRP concentration or the percentage of patients achieving CRP levels  $< 2$  mg/L. Treatment also had no significant effect on platelet function.<sup>132</sup>

NADPH oxidase mediates the production of superoxide anions by neutrophils. Superoxide anions cause oxidative damage in chronic vascular diseases.<sup>133</sup> Colchicine inhibits MSU-induced superoxide production most likely through a mechanism that involves disruption of microtubule polymerization and subsequent interference with the assembly of the NADPH oxidase complex.<sup>73</sup> Superoxide production by neutrophils can be inhibited using low doses of

colchicine and could be considered as a potential therapy to reduce vascular dysfunction.

Results from a large-scale medical-records review suggest that colchicine treatment may significantly decrease cardiovascular risk.<sup>134</sup> In that study, 1288 patients with gouty arthritis were identified by *International Classification of Diseases, 9th Revision* diagnostic code from a larger sample of 40,107 patients enrolled in the New York Harbor Healthcare System Veterans Affairs network. Of the patients with gout, 576 had a history of colchicine use and 712 did not. The prevalence of myocardial infarction was significantly lower among colchicine users ( $P < 0.03$ ), and there were numerically but not significantly lower mortality and CRP levels among those with a history of colchicine use.<sup>134</sup> The 2 groups had similar demographic characteristics, comorbidities, cardiac risk factors, and concurrent medication use (aside from colchicine); thus, these factors were not held accountable for the difference in outcomes between the 2 groups. Although the results from that cross-sectional study suggested significant cardiovascular risk reduction with colchicine, pathways underlying this effect have not been elucidated. The investigators of the records review suggested that colchicine may support plaque stability and/or reduce the effects of plaque rupture and that these effects may have been due to blockade macrophage activation, endothelial activation, and/or neutrophil influx and activation by colchicine.<sup>134</sup>

A study by Nidorf et al<sup>135</sup> evaluated the benefit of low-dose colchicine in the prevention of cardiovascular events in patients with clinically stable coronary disease. In that prospective, randomized, observer-blinded study in 532 patients with clinically stable coronary disease, patients were randomized to receive colchicine 0.5 mg/d ( $n = 282$ ) or no colchicine ( $n = 250$ ) in addition to standard therapies including aspirin and/or statins. Patients were followed up for a median of 3 years. The primary end point was the composite prevalence of acute coronary syndromes, out-of-hospital cardiac arrest, and noncardioembolic ischemic stroke. Of patients treated with colchicine, 5.3% reported a primary end point incident compared with 16.0% of patients allocated to receive no colchicine ( $P < 0.001$ ). The effect was believed to have been the result of the inhibition of neutrophil activation in atherosclerotic plaques, thereby preventing the initiation of inflammation, improving plaque stability and reducing the risk for plaque enlargement and rupture.

Additional well-controlled clinical trials are required before colchicine can be recommended for the primary or secondary prevention of cardiovascular disease.

## CONCLUSIONS

Colchicine is a widely used and recommended first-line therapy for the treatment of acute gouty arthritis flares and flare prophylaxis. Although colchicine has many actions that predict efficacy in protecting against and treating gout flares, the exact mechanisms of action underlying its efficacy have not been completely elucidated and remain under active investigation. Results obtained to date suggest that colchicine down-regulates multiple pro-inflammatory pathways and increases levels of antiinflammatory mediators. These pleiotropic effects of colchicine may ultimately expand the use of this agent to other therapeutic areas.

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