

Uric Acid as a Mediator of Endothelial Dysfunction, Inflammation, and Vascular Disease

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Recent experimental findings have led to renewed interest in the possible role of uric acid in the pathogenesis of both hypertension and vascular disease. Often considered an antioxidant, biochemical and *in vitro* data indicate that noncrystalline, soluble uric acid also can react to form radicals, increase lipid oxidation, and induce various pro-oxidant effects in vascular cells. *In vitro* and *in vivo* findings suggest that uric acid may contribute to endothelial dysfunction by inducing antiproliferative effects on endothelium and impairing nitric oxide production. Proinflammatory and proliferative effects of soluble uric acid have been described on vascular smooth muscle cells (VSMCs), and in animal models of mild hyperuricemia, hypertension develops in association with intrarenal vascular disease. Possible adverse effects of uric acid on the vasculature have been linked to increased chemokine and cytokine expression, induction of the renin-angiotensin system, and to increased vascular C-reactive protein (CRP) expression. Experimental evidence suggests a complex but potentially direct causal role for uric acid in the pathogenesis of hypertension and atherosclerosis.

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The significance of serum uric acid levels as a risk factor for vascular disease and hypertension has been a matter of intense debate for several decades. To a large extent this has arisen because of the immense difficulty in separating the complex epidemiologic association of uric acid with other risk factors, disease processes, and treatments, from what may be an independent effect of uric acid on the vasculature. As discussed elsewhere of this issue, there is a clear epidemiologic association between hyperuricemia and vascular disease. Uric acid levels have been shown to predict the development of hypertension,^{1,2} the incidence of myocardial infarction,^{3,4} and the outcome in heart failure⁵ and stroke.⁶ What is less clear, and also of great controversy, is the exact role of uric acid in these disorders. Are increased uric acid levels simply an epiphenomenon or does this

ubiquitous by-product actually have a direct role to play in their pathogenesis?

This article reviews the evidence that uric acid may be a participant in atherogenesis and in the development of hypertension. At the outset, however, it is fair to say that despite several pieces of evidence suggesting a causal role for uric acid in vascular disease, there still remains much controversy. This is strikingly exemplified by the response and counterresponse⁷ to the aforementioned stroke study in which Weir et al⁶ suggest the need for intervention trials examining both the administration of urate and the decreasing of urate levels in acute stroke patients.

Whether Uric Acid is an Anti- or Pro-Oxidant may Depend on the Cellular Environment

Uric acid is a product of purine metabolism, formed from the breakdown of adenosine and guanine. It is produced from hypoxanthine and xanthine via the action of the enzyme xanthine oxidase; oxidants also are generated during this process. Local and circulating urate levels are influenced significantly by tissue oxygenation and hemodynamic factors among other things (eg, dietary, genetic, drug-related, and renal factors). When tissue becomes ischemic, adenosine triphosphate breakdown, xanthine production and metabolism, urate formation, and oxidant formation all are increased

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via this pathway. This is one explanation for the view that urate is simply a marker of increased xanthine oxidase activity and increased oxidant generation, and therefore associated with vascular disease risk and prognosis.

With respect to antioxidant actions, soluble uric acid has been shown to scavenge superoxide, singlet oxygen, hydroxyl radical, and peroxyxynitrite, as well as chelate transitional metals.^{8,9} Urate also can prevent extracellular superoxide dismutase degradation,¹⁰ thus increasing superoxide dismutation to hydrogen peroxide, and decreasing the availability of superoxide and its harmful interaction with nitric oxide.

Although these biochemical reactions are consistent with beneficial vascular effects, experimental data also show that uric acid may have pro-oxidant effects, and therefore the potential for adverse effects on blood vessels. Biochemical studies have found that urate can generate aminocarbonyl radicals^{11,12} that are capable of amplifying the oxidation of liposomes and low-density lipoprotein cholesterol. These pro-oxidant effects of urate and urate metabolites appear to be more pronounced in the setting of relative deficiency of other water-soluble antioxidants, such as ascorbic acid.¹³⁻¹⁵ Consistent with this is the finding by Cherubini et al¹⁶ that a worse outcome in acute stroke is associated independently with lower ascorbate and higher uric acid levels. However, this also may signify that other adverse prognostic factors not easily identified somehow are associated with these same serum changes.

Hyperuricemia and Endothelial Dysfunction

Cardiovascular disease commonly is associated with endothelial dysfunction, oxidant generation, and a pro-inflammatory state.¹⁷ Oxidants generated via xanthine oxidase potentially can cause endothelial dysfunction through effects on nitric oxide synthesis and availability. Xanthine oxidase also produces uric acid, which may explain why hyperuricemia, oxidant generation, and endothelial dysfunction all are associated.

What is the exact role of uric acid in this setting? Is it protective, inert, or harmful? The matter does not appear to have been resolved adequately. Although the oxidants produced in the generation of uric acid by xanthine oxidase could be harmful to the vasculature (the usual explanation), there is also suggestive evidence that uric acid itself can have direct adverse effects unrelated to the generation of oxidants.¹⁸⁻²⁰ One also could argue that the level of xanthine oxidase activity is important.

The effect of allopurinol does not allow this issue to be resolved because it blocks xanthine oxidase-mediated generation of both uric acid and oxidants. Thus, the beneficial effects of allopurinol usually have been attributed to antioxidant properties rather than to any effect on uric acid per se. These beneficial effects include a decrease in cardiovascular complications after coronary artery bypass and in patients with dilated cardiomyopathy.²¹⁻²³ Allopurinol also has been

found to correct impaired nitric oxide production in patients with hypertension, diabetes, and heart failure.^{18,24,25}

Of course, allopurinol also decreases uric acid levels and in the studies by Doehner et al,¹⁸ the degree of decrease correlated very strongly with the improvement in endothelial function. Interestingly, allantoin levels—a measure of oxygen free radical generation—did not correlate with these improvements or with the degree of decrease of serum uric acid levels, suggesting a direct role for uric acid itself. Similarly, in the animal studies by Mazzali et al,^{19,20} (to be discussed in sections IV and VII) uric acid itself seems to have been the important factor leading to decreased nitric oxide production, vascular changes in the kidney, and hypertension. Decreased nitric oxide production also was shown in a shorter-term rat model of hyperuricemia (1 week before the onset of hypertension), also consistent with a direct role for uric acid in mediating endothelial dysfunction.²⁶

Very recent studies by Waring et al,²⁷ however, do not support a direct role for uric acid in causing endothelial dysfunction. Uric acid infused into the forearms of resting healthy human subjects had no effect and was not associated with impaired acetylcholine-induced vasodilation or altered endothelial nitric oxide release. Patients with preexisting increased oxidative stress, eg, heart failure or vascular disease, were not examined; this may be of relevance because uric acid may have different effects in this setting. Certainly there is evidence that uric acid has different biochemical effects in different cellular environments. In addition to the increased pro-oxidant activity of uric acid when ascorbate level is low,¹³ there also is evidence that bicarbonate concentrations can influence greatly the effect of uric acid on peroxyxynitrite-mediated nitration reactions.²⁸

Proliferative and Proinflammatory Effects of Uric Acid on Vascular Smooth Muscle

Soluble uric acid has been shown to induce vascular smooth muscle cell (VSMC) proliferation in vitro via a pathway involving increased platelet-derived growth factor-A expression.²⁹ Our studies have confirmed this observation and also shown that uric acid-induced VSMC proliferation is mediated by the activation or induction of extracellular signal-regulated kinase mitogen-activated protein kinases (MAPK) and cyclooxygenase-2.^{30,31}

Adding further to this pathway, we recently showed that physiologic concentrations of crystal and endotoxin-free uric acid increase VSMC expression of the chemokine monocyte chemoattractant protein-1 (MCP-1) in vitro.³² MCP-1 has been implicated in several studies as a major contributor to atherogenesis.³³⁻³⁷ Uric acid-induced MCP-1 expression in VSMCs was dependent on both new MCP-1 messenger RNA production and posttranscriptional modification of existing MCP-1 messenger RNA. In addition, uric acid activated the transcription factors activator protein-1 and nuclear factor kappa B, as well as p38 MAPK.³² Inhibition of p38 MAPK, extracellular signal-regulated kinase 1/2 MAPK, or cyclooxygenase-2

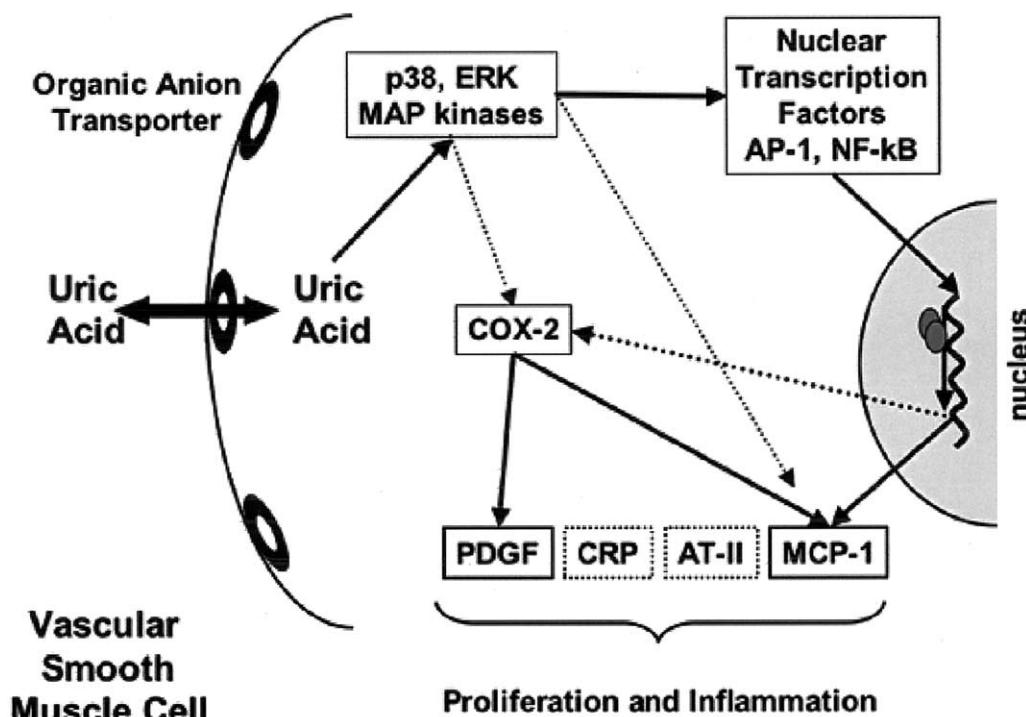


Figure 1 Pathway for uric acid–mediated proinflammatory and proliferative effects on VSMCs. Uric acid appears to enter VSMCs via an anion exchanger/transporter where it alters intracellular redox, activates mitogen-activated kinases (extracellular signal–regulated kinase 1/2 and p38 MAPK), increases cyclooxygenase-2 expression, and activates nuclear transcription factors (nuclear factor κ B and activator protein-1). Downstream of these pathways there is a resulting increase in the production of platelet-derived growth factor and the chemokine MCP-1. Several studies have shown a role for the renin-angiotensin system and angiotensin II (AT-II), whereas recent findings also implicate CRP in the uric acid–induced effects on VSMCs.

ycogenase-2 each significantly suppressed uric acid-induced MCP-1 expression, implicating these pathways in the response to uric acid. Furthermore, both N-acetyl-cysteine and diphenylethylamine (antioxidants) inhibited uric acid-induced MCP-1 production, suggesting involvement of intracellular redox pathways.

We have identified a uric acid-mediated VSMC pro-inflammatory pathway that appears to require uric acid entry via an organic anion transporter (OAT) (Fig 1). Our studies show evidence for such a transporter on rat and human VSMCs.^{38,39} The human VSMC appear to lack OAT1-4 but express messenger RNA and protein for another OAT known as urate transporter 1. Thus, urate appears to enter VSMCs through an OAT and have various effects intracellularly including significant proliferative and proinflammatory actions as outlined in Fig 1.

Other Pro-Atherogenic Pathways: A Possible Link with C-Reactive Protein

We also recently found that uric acid up-regulates C-reactive protein (CRP) expression in cultured human vascular cells, namely VSMCs and endothelial cells.⁴⁰ Given the many recent insights regarding the role of CRP as an active partaker rather than just as a simple marker of vascular inflamma-

tion,⁴¹ uric acid-induced CRP expression by vascular cells may provide further direct evidence for both proinflammatory and proatherogenic effects of uric acid.

Lessons from Animal Models of Hyperuricemia

Several more pieces of evidence suggesting that uric acid is a mediator of endothelial dysfunction, vascular disease, and inflammation have arisen from various animal experiments.^{19,20,30,31,42} Briefly, experimental animal models generated by feeding rats the uricase inhibitor oxonic acid, have mildly increased serum uric acid levels associated with intrarenal vascular disease and salt-sensitive hypertension.^{19,20,30,31,42} Activation of the renin-angiotensin system is evident as is mild inflammation, and there is an associated decrease in nitric oxide synthase expression. Decreasing the uric acid levels with allopurinol or benzbromarone (uricosuric agent) reverses these findings, as does treatment with an angiotensin-converting enzyme inhibitor. Treatment with a thiazide diuretic decreases blood pressure but does not improve uric acid levels or reverse the intrarenal vascular changes.^{19,42} As is discussed elsewhere in this issue, these findings all add credence to the view that uric acid is more than simply a marker, potentially having direct effects on the vasculature.

Conclusion

Many of the studies summarized here have reawakened an old debate regarding the precise significance of increased serum uric acid levels. Although biochemical, cellular, animal model, and epidemiologic evidence exists that appears strongly suggestive of a causal role for uric acid in hypertension and vascular disease, much controversy still remains. Deciphering the complex role uric acid potentially has in the pathogenesis of both vascular disease and hypertension will require further well-designed laboratory-based as well as clinical interventional studies. Perhaps uric acid, a very old molecule, ultimately will be viewed in a similar way to other more novel and seemingly more exciting cardiovascular biomarkers, being both an indicator of and a participant in a complex disease process.

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