Review

The relationships among hyperuricemia, endothelial dysfunction, and cardiovascular diseases: Molecular mechanisms and clinical implications

Paolo Puddu (MD), Giovanni M. Puddu (MD), Eleonora Cravero (MD), Luca Vizioli (MD), Antonio Muscari (MD)*

Department of Internal Medicine, Aging and Nephrological Diseases, University of Bologna and S. Orsola-Malpighi Hospital, Bologna, Italy

Received 19 December 2011; received in revised form 17 January 2012; accepted 20 January 2012
Available online 6 March 2012

KEYWORDS
Allopurinol; Atherosclerosis; Oxidative stress; Risk factors; Uric acid; Xanthine oxidoreductase

Summary Uric acid is the end product of purine metabolism. Its immediate precursor, xanthine, is converted to uric acid by an enzymatic reaction involving xanthine oxidoreductase. Uric acid has been formerly considered a major antioxidant in human plasma with possible beneficial anti-atherosclerotic effects. In contrast, studies in the past two decades have reported associations between elevated serum uric acid levels and cardiovascular events, suggesting a potential role for uric acid as a risk factor for atherosclerosis and related diseases. In this paper, the molecular pattern of uric acid formation, its possible deleterious effects, as well as the involvement of xanthine oxidoreductase in reactive oxygen species generation are critically discussed. Reactive oxygen species contribute to vascular oxidative stress and endothelial dysfunction, which are associated with the risk of atherosclerosis. Recent studies have renewed attention to the xanthine oxidoreductase system, since xanthine oxidoreductase inhibitors, such as allopurinol and oxypurinol, would be capable of preventing atherosclerosis progression by reducing endothelial dysfunction. Also, beneficial effects could be obtained in patients with congestive heart failure. The simultaneous reduction in uric acid levels might contribute to these effects, or be a mere epiphenomenon of the drug action. The molecular mechanisms involved are discussed.

© 2012 Japanese College of Cardiology. Published by Elsevier Ltd. All rights reserved.

* Corresponding author at: Department of Internal Medicine, Aging and Nephrological Diseases, University of Bologna — Stroke Unit of the S. Orsola-Malpighi Hospital — Via Albertoni, 15, 40138 Bologna, Italy. Tel.: +39 051 6362280; fax: +39 051 6362210.
E-mail address: antonio.muscari@unibo.it (A. Muscari).

0914-5087/$ — see front matter © 2012 Japanese College of Cardiology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.jjcc.2012.01.013
Introduction

It has been hypothesized that uric acid (UA) provides an antioxidant defense in humans, so that it might be protective against oxidative stress in cardiac, vascular, and neural cells [1–3]. In contrast, several studies in the past two decades have suggested that elevated serum UA (SUA) levels are associated with cardiovascular diseases [4,5]. For example, Neogi et al. [6] found that SUA is not associated with coronary artery calcification, a known marker for early preclinical coronary artery disease (CAD) but, on the contrary, Krishnan et al. [7] recently showed that, after adjustment for many confounding factors, elevated SUA levels correlated with the presence and severity of coronary artery calcification in a cohort of young healthy adults. Thus, the question arises whether the known antioxidant UA exerts protective vascular effects, or is a risk factor for cardiovascular diseases. The aim of this paper is to critically review the paradoxical association between the antioxidant UA and increased cardiovascular morbidity and mortality. Moreover, the molecular aspects of UA formation and its potential role in vascular oxidative stress are described.

The role of xanthine oxidoreductase in endothelial dysfunction

Xanthine oxidoreductase (XOR) is a molybdoflavoprotein enzyme that exists in two interconvertible functionally distinct forms, namely xanthine dehydrogenase (XD) and xanthine oxidase (XO) (Fig. 1). XO is generated from XD by proteolysis and catalyzes the two terminal steps of purine degradation (hypoxanthine → xanthine → UA) in humans. By oxidation of hypoxanthine to xanthine, and xanthine to urate [8–13], XO readily donates electrons to molecular oxygen, thereby producing superoxide radical (O$_2^-$) and hydrogen peroxide. Both XD and XO contain an internal electron transport system that is capable of producing reactive oxygen species (ROS) [14–16]. The physiological substrates, xanthine and hypoxanthine, bind the oxidized enzyme and donate two electrons to the molybdenum cofactor, reducing it from Mo$^{VI}$ to Mo$^{V}$. Substrates are hydroxylated by H$_2$O at the molybdenum site as the electrons travel via two iron-sulfide residues to flavine-adenine dinucleotide (FAD). Reduced FAD can be dually reoxidized by oxygen to produce hydrogen peroxide, or univalently reoxidized in two steps to generate two equivalents of superoxide radical [12,14,17–19]. Superoxide has been identified as the most probable ROS contributing to cardiac dysfunction in the failing myocardium [12,20–23]. The superoxide radical produced by either XO or XD can bind nitric oxide (NO) to form peroxynitrite (OONO$^-$), a potent non-radical oxidant species [24]. NO is usually generated by NO synthase (NOS), although XOR may also be a contributory source of NO under hypoxic conditions [25,26]. Since endothelium-derived NO controls vascular tone, prevents adhesion of leukocytes, inhibits aggregation and adhesion of platelets, and reduces intima proliferation, the reduction of NO bioavailability induces endothelial dysfunction and oxidative stress, a key mechanism of vascular risk and dysfunction. Endothelial dysfunction can be induced by all common cardiovascular risk factors, which are able to activate many enzymatic systems (such as XO, NADPH oxidase, uncoupled eNOS and mitochondrial respiratory chain) thus favoring ROS generation. In particular, XO has been implicated as a source of ROS in the vascular system.

Uric acid as a potentially useful antioxidant

UA, together with ascorbic acid, is considered a major water soluble intracellular free radical scavenger, as well as an antioxidant in human plasma [3,27], since it can react with superoxide anion, hydrogen peroxide, hydroxyl radical, and particularly peroxynitrite. UA reacts preferentially with peroxynitrite anion [28,29], which is a short-lived oxidant species formed by the reaction between NO and superoxide radical.
Uric acid involvement in cardiovascular diseases

Peroxynitrite is capable of inducing cell death or abnormal functioning [30], thus contributing to various forms of cardiovascular diseases [31–37]. In cell culture systems peroxynitrite induced tetrahydrobiopterin oxidation and a decrease in NO generation [28,37]. The addition of UA could prevent oxidative damage in part by restoring NO generation, and in part contributing to maintain extracellular levels of superoxide dismutase (EC-SOD), the only antioxidant enzyme capable of scavenging superoxide anion [38,39].

In peroxynitrite-treated human plasma, Skinner et al. [40] have detected a nitrated UA derivative. This product may be involved in human pathophysiology by releasing NO, which could decrease vascular tone and increase tissue blood flow, thereby suggesting a novel beneficial role for UA. The relationship between UA and NO has been further clarified by the characterization of another reactive product of UA with NO under aerobic conditions [41]. By mass spectrometric data this product was identified as nitrosated UA, which results from the reaction with NO donors, but not with peroxynitrite. Thus UA may act as a vehicle of NO, suggesting that its elevated levels may represent a favorable response to oxidative stress in cardiovascular diseases [3,42].

**Deleterious effects of uric acid and mechanisms involved**

In overt contrast with the above data, since 2005 experimental and clinical studies have suggested that elevated UA levels are associated with a reduction in NO levels with ensuing endothelial dysfunction. UA reduces NO levels in endothelial cell cultures, blocks acetylcholine-induced vasodilatation of aortic rings, and reduces circulating nitrates in experimental animals [43]. Further, rat studies have shown that hyperuricemia-induced hypertension and vascular disease are partially reversed by the supplementation of the NOS substrate, L-arginine [44]. Gersh et al. [45] have demonstrated that UA reacts directly with NO in a rapid irreversible reaction resulting in the formation of 6-aminoacril, with consequent depletion of NO (Fig. 1). This reaction occurs preferentially with NO, even in the presence of peroxynitrite and hydrogen peroxide. The reaction is at least partially blocked by glutathione, showing a potential mechanism by which UA could deplete NO under conditions of oxidative stress, when intracellular glutathione is depleted.

The complexity of the reactions of UA with peroxynitrite has recently been described by Gersh et al. [46] and Imaram et al. [47]. UA can decompose and generate free radicals in various reactions [47], including the reaction with peroxynitrite [48–50]. One such radical has been identified as the aminocarbonyl radical [50], derived from the intermediate triuretcarbonyl radical, which in turn could be an intermediate candidate for the generation of triuret [51,52]. In addition, in this reaction UA can generate alkylated products and allantoin. Thus, the studies of the Gersh group [45,46] suggest a further potential pathway by which the UA-peroxynitrite reaction might lead to deleterious pro-oxidative effects mediated by the formation of reactive intermediates capable of alkylating biomolecules containing OH, labile hydrogens, or reactive functional groups. These effects can be partially prevented by ascorbate [45].

In community-dwelling older persons, significant associations have been shown between SUA levels and inflammatory markers, such as white blood cells, C-reactive protein, interleukin-1 (IL-1), IL-6, IL-6 receptor, IL-10, IL-18, and tumor necrosis factor-alpha [53], suggesting that UA might contribute to the pro-inflammatory state of several chronic conditions typically affecting the old [54]. It has also been proposed that changes in the expression of endothelin-1 are another mechanism by which hyperuricemia might favor cardiovascular diseases [55,56]. Moreover, UA can act as an endogenous danger signal capable of stimulating the innate immune response [57], and it is well known that the immune response plays a role in the development of hypertension, renal, and cardiovascular diseases [58]. Finally, *in vitro* studies with vascular smooth muscle cells have shown that incubation with UA stimulates cell proliferation, angiotensin II production and, oxidative stress. These changes were reversed by the addition of captopril or losartan, which suggests an effect mediated, at least in part, by the renin-angiotensin system [59]. Moreover, smooth muscle cells of human aorta express URAT1, a urate transporter, on their cell membrane [60], indicating a specific functional role for UA in these cells.

The above experimental findings led to renewed interest in the possible role of UA in the pathogenesis of vascular diseases [61].

**Uric acid as a risk factor for vascular disease**

Although many experimental and epidemiological data have suggested a possible role for hyperuricemia in inducing endothelial dysfunction, and particularly impaired NO bioavailability, not all studies have shown a causal role for UA in the development of coronary heart disease or death from cardiovascular causes. Mankovsky et al. [62] have extensively reviewed numerous publications of the past two decades, many epidemiological, concerning the possible relationship between SUA levels and cardiovascular disorders, such as CAD, stroke, hypertension, and metabolic syndrome. The relation between SUA and cardiovascular disease was observed not only with frank hyperuricemia (defined as more than 6 mg/dl in women and 7 mg/dl in men), but also with SUA levels in the normal to high range (5.2–6 mg/dl) [63–68]. Raised levels of SUA, together with high-sensitivity troponin T and N-terminal pro-B-type natriuretic peptide, have been found in most patients with essential hypertension [69]. In patients with chronic heart failure, hyperuricemia is associated with high cardiac event rates [70] and, in combination with six other conventional biomarkers, it may contribute to risk stratification [71]. Recently Neogi et al. [72] have assessed the association of SUA with carotid plaques in a large multicenter study, the NHLBI Family Heart Study. SUA was found to be associated with carotid plaques in men, even in the absence of other cardiovascular risk factors. However, analyzing the same data set [6], the authors were unable to demonstrate any association of SUA with coronary artery calcifications, a known marker for early preclinical CAD (in contrast with the
findings of Krishnan et al. [7]). In 1901 consecutive patients undergoing coronary angiography, De Luca et al. [73] showed that UA was not associated with the extent of CAD and the intima-media thickness of carotid arteries. Similarly, several other studies did not find any association between raised SUA levels and cardiovascular events [74–78].

Thus, the possible significance of hyperuricemia is still widely debated. It remains unsolved whether UA is a causal, compensatory, or coincidental factor [79]. The difficulty in ascertaining the role of UA as an independent risk factor for atherosclerosis is due to the complex associations between hyperuricemia and conventional risk factors. A systematic review with meta-analysis of 26 prospective cohort studies [80] has shown a modest but significant association between hyperuricemia and CAD events, independent of traditional risk factors. The overall risk of CAD death increased by 12% for each increase of 1 mg/dl SUA, although hyperuricemia appeared to significantly increase the risk of death only in women. This gender difference should be confirmed by future research.

It is well known that patients with gout and hyperuricemia frequently experience several comorbidities, such as hypertension, chronic kidney disease, insulin resistance, and obesity, which complicate management and affect long-term prognosis [81]. The extent to which hyperuricemia and gout may be either a cause or a consequence of these conditions is still debated. Further research should also clarify this important question.

**Potential benefits of xanthine oxidase inhibition in the management of cardiovascular risk**

Pacher et al. [82] have reviewed both the molecular biochemistry of XOR and the effects of XO inhibitors in various pathophysiological conditions such as gout, stroke, circulatory shock, global heart failure, and ischemia-reperfusion injury of the myocardium, liver, kidney, and lungs. The putative use of XO inhibitors in the treatment of hypertension, hypercholesterolemia, atherosclerosis, and diabetes has also been discussed [82]. In particular, recent relevant clinical research has focused on the use of the XO inhibitors allopurinol and oxypurinol in the prevention of cardiovascular disease. Allopurinol is a structural analogue of hypoxanthine and is quickly metabolized to oxypurinol which, similarly to allopurinol, can bind XO and inhibit its activity. Febuxostat, a non-purine XO-specific inhibitor, is reported to be significantly more potent than allopurinol [83], which shows that a xanthine-like structural framework is not a prerequisite for high XO inhibitory activity.

Allopurinol has been found to improve endothelial function in type 2 diabetes [84], in hyperuricemic patients with chronic heart failure [85], and in heavy smokers [86]. In 2006, George et al. [87] showed that a steep dose–response relationship exists between allopurinol and its effects on endothelial dysfunction in patients with chronic heart failure (New York Heart Association class II–III): high-dose allopurinol (600 mg/dl) totally abolished the oxidative stress, independently of urate reduction. These data were partially contradicted by the study of Galbusera et al. [14], which showed that allopurinol in vitro generated superoxide radicals during its conversion to oxypurinol, whereas oxypurinol did not produce superoxide. However, it would be difficult to speculate on how much superoxide would be produced in vivo, and whether the superoxide generated from allopurinol contributes to oxidative stress and tissue pathology. Anyway, oxypurinol seems to be more effective than allopurinol as an inhibitor of superoxide production [88]. Hare et al. [89] have studied the effects of oxypurinol in a cohort of patients with symptomatic heart failure (class III–IV New York Heart Association) and left ventricular dysfunction receiving optimal medical therapy. Oxypurinol did not produce clinical improvements in unselected patients with moderate-to-severe heart failure. However, patients with high SUA levels might benefit from XO inhibition. In fact, the degree of SUA reduction correlated with clinical outcome, and the patients that worsened had a relatively smaller reduction in SUA levels. The authors concluded that SUA may serve as a valuable biomarker to target XO inhibition in patients with congestive heart failure. Similarly, according to Wu et al. [90] elevated SUA levels could be an independent predictor of mortality in patients with severe heart failure. On the other hand, in patients with nonelevated SUA and ischemic cardiomyopathy with severely suppressed systolic function, Baldus et al. [91] found that a single intravenous dose of oxypurinol (400 mg) improved myocardial contractility, while benzbromarone [92], a uricosuric agent that does not inhibit XO, did not affect brain natriuretic peptide and left ventricular ejection fraction in patients with chronic heart failure, although fasting insulin and insulin resistance improved.

Overall, these data underscore the role of ROS as key mediators of ischemic heart failure, the significance of XO as a source of ROS and the beneficial effects of XO inhibitors, but they would also show that elevated SUA levels may not be directly associated with myocardial impairment. In fact there are studies showing that SUA has no causal relationship with cardiovascular diseases, so that a reduction in its levels would play no role in preventing them but, on the other hand, other studies suggest that elevated SUA levels may be a marker of the risk for the development of such conditions [67,93–95]. These conflicting results have been widely discussed [82,95,96] and, based on the preponderance of recent epidemiologic studies, it appears that elevated SUA may be at least an independent predictor of the risk of kidney disease, hypertension, and cardiovascular disease, including acute myocardial infarction [66,68,97].

Thus, the beneficial effects of XO inhibitors might be related to decreased ROS production, SUA reduction, or both. Certainly more studies in this interesting area of research are needed to fully elucidate the complex mechanisms connecting XO inhibitors with the prevention and therapy for cardiovascular diseases.

**Conclusions**

Some studies have shown an association between SUA levels and subsequent development of hypertension [98–100]. Moreover, an association between SUA levels and surrogate markers of atherosclerosis is starting to emerge [101–103], and associations with hard atherosclerosis clinical endpoints have also been described [104–109]. However, the role of
UA as an independent risk factor for cardiovascular events is still debated [110,111]. Manzato [112] suggested that, in the presence of elevated SUA levels, other factors, such as insulin resistance, glucose intolerance, dyslipidemia, hypertension, use of diuretics, and obesity, may play the true causal role in increasing vascular risk. The apparent beneficial role of XO inhibitors could be due to their ability to consistently improve endothelial dysfunction by reducing oxidative stress, rather than to urate reduction. Finally, the precise mechanism for UA-induced tissue injury remains unclear, even if UA might play a role in oxidative stress, in the formation of ROS and, consequently, in the impairment of NO production. To date no large blinded, randomized, placebo-controlled studies have clearly demonstrated that lowering SUA can prevent or lessen the risk of cardiovascular disease, renal disease, diabetes, or hypertension, even though they may correlate with hyperuricemia. Future large clinical studies will have to ascertain whether XO inhibition and/or UA reduction are able to deliver long-term benefits, as smaller studies suggest they might.

References


Uric acid involvement in cardiovascular diseases


