Uric Acid Puzzle: Dual Role as Anti-oxidant and Pro-oxidant

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Hyperuricemia is known to be associated with the presence of cardiovascular and metabolic syndrome and with the development of incipient kidney disease and an accelerated renal progression. However, an elevated uric acid level was not generally regarded as a true etiology or mediator, but an indicator of these diseases. Uric acid has recently regained the clinical interest and popularity based on emerging data suggesting the causative role of hyperuricemia in cardiovascular and renal disease. Experimental data demonstrates oxidative stress is one of the earliest phenomena observed in vascular, renal, liver cells and adipocytes exposed to uric acid. Since uric acid is one of the major antioxidants of plasma acting as a free radical scavenger and a chelator of transitional metal ion, uric acid-induced oxidative stress seems paradoxical. Data regarding the clinical implication of hyperuricemia is even more confusing, which defines hypouricemia as a useless parameter to be eliminated from routine follow-up or a major risk factor to be therapeutic target. With a review of experimental and epidemiologic data, the presence of molecular switch to regulate the role of uric acid as anti- or pro-oxidant in different compartment of our body is suggested, which may shed light on understanding the paradoxical role of uric acid and solving the “uric acid debate”.

Key Words: Uric acid, Oxidative stress, Anti-oxidant

Introduction

An association of elevated serum uric acid with hypertension, cardiovascular, renal and metabolic disease has been reported throughout the 20th century after the first description regarding high prevalence of hypertension in gout patients in the 1870’s1). A recent meta-analysis reveals that a 1 mg/dL increase in serum uric acid level is associated with a significant increase of incident hypertension (relative risk 1.13, 95% confidence interval 1.06 to 1.20) after adjusting for confounding risk factors2). Indeed, an elevated serum uric acid is currently the most reliable predictor for the development of hypertension. There is also a solid data supporting the role of serum uric acid as a determinant of the change in estimated glomerular filtration rate in diverse spectrum of population from healthy participants with normal kidney function to normoalbuminuric diabetics3-8). Higher uric acid levels were associated with subsequent worsening of kidney function after adjustment for theorized confounders such as body mass index, blood pressure and urine albumin-creatinine ratio6,7,9). Many epidemiologic studies demonstrated uric acid level as a major predictor for the development of incident kidney disease3-8).

Since a simple association based on epidemiologic or observational studies did not support the role of hyperuricemia as a predictor or causative factor in the development of cardiovascular and renal disease, the “uric acid debate” has been going on for decades. This debate is generated by two major concerns, an absence of randomized controlled study using uric acid-lowering therapy in
a large population and a lack of mechanisms by which uric acid imposes a detrimental effect on individual organ system. Randomized controlled studies to examine the etiologic role of hyperuricemia on the progression of cardiovascular, renal and metabolic diseases are now on-going or recruiting the participants, which will provide the answer to clinical implication of hyperuricemia in near future.

In this review, potential mechanism of uric acid-related complications in cardiovascular, renal and metabolic diseases will be discussed with a special emphasis on uric acid-induced oxidative stress. According to a hypothesis by Ames et al, the silencing of uricase gene with an increase in serum uric acid level in humans provided an evolutionary advantage for our ancestors. This hypothesis is based on the results of in-vitro experiment demonstrating uric acid as a powerful scavenger of free radical. On the other hand, many epidemiologic studies clearly show an association of hyperuricemia and clinical conditions described above. The dual role of uric acid as an anti-oxidant and pro-oxidant which can be determined by specific factors and the interrelation among factors, may explain the controversy regarding the role of uric acid, however there is no clear explanation for this uric acid paradox.

What makes serum uric acid elevated?

Uric acid is produced from metabolic conversion of either dietary or endogenous purines, primarily in the liver, muscle, and intestine. The immediate precursor of uric acid is xanthine, which is degraded into uric acid by xanthine oxidoreductase. Xanthine oxidoreductase may attain two inter-convertible forms, xanthine dehydrogenase or xanthine oxidase. Most xanthine oxidoreductase is in the xanthine dehydrogenase form in vivo, which is transformed into xanthine oxidase by irreversible proteolytic cleavage or reversible oxidation in specific environment including hypoxia. Xanthine oxidase uses molecular oxygen as electron acceptor and generates superoxide anion and other reactive oxygen species as by-products in the process of uric acid degradation whereas xanthine dehydrogenase generates the reduced form of nicotinamide-adenine dinucleotide (Fig. 1). Both exogenous (present in fatty meat, organ meats, and seafood) and endogenous purines are major sources of uric acid in humans. Approximately two thirds of total body urate is produced endogenously while the remaining one third is originated from dietary purines.

The primary site of excretion of uric acid is the kidney. The normal urinary urate excretion is in the range of 250 to 750 mg per day, approximately 70% of the daily urate production. Although urate (the form of uric acid at blood pH of 7.4) is freely filtered in the glomerulus, there is evidence that both reabsorption and secretion occur in the proximal tubule, and as a consequence the fractional urate excretion is only 8% to 10% in the normal adult. Some adaptation occurs with renal disease, in which the fractional excretion of urate will increase to the 10% to 20%. The remainder of uric acid excretion occurs through the gut, where uric acid is degraded by uricolytic bacteria. Ideas of the handling of uric acid by the kidney have changed greatly over the past few decades, with the identification, characterization, and isolation of transporters and channels mainly or exclusively restricted to urate transport.

The serum urate concentration reflects the balance between urate production and elimination. Hyperuricemia has been arbitrarily defined as >7.0 mg/dL in men and
>6.5 mg/dL in women. “Normal” serum uric acid levels in the population appear to be rising throughout the last century, likely as a consequence of changes in diet, and mean levels in men in the United States are now in the 6.0 to 6.5 mg/dL range. Hyperuricemia may also result from diets high in purines, from ethanol, and from fructose. The effect of alcohol is in part related to increased urate synthesis, which is due to enhanced turnover of adenosine triphosphate (ATP) during the conversion of acetate to acetyl-CoA as part of the metabolism of ethanol. In addition, acute alcohol consumption causes lactate production, and because lactate is an antiuricosuric agent, it will reduce renal urate excretion and exacerbate hyperuricemia. Fructose (a simple sugar present in sucrose, table sugar, high fructose corn syrup, honey, and fruits) can also induce a rapid rise in serum uric acid, due in part to its rapid phosphorylation in hepatocytes with the stimulation of adenosine monophosphate (AMP) deaminase and ATP consumption. It has been proposed that the marked increase in fructose intake may have a role in the rising levels of serum uric acid and obesity worldwide. Uric acid may also be affected by exercise, with moderate exercise reducing urate levels (probably by increasing renal blood flow) and severe exercise causing a rise in uric acid (probably due to ATP consumption with adenosine and xanthine formation).

**What happens first when cells are exposed to uric acid?**

One of the earliest phenomena observed in uric acid-exposed cells is the generation of oxidative stress. Reactive oxygen species (ROS) is known to be associated with local inflammation, an impairment of nitric oxide (NO) generation, an activation of the renin-angiotensin system, insulin resistance and fat accumulation. Uric acid-induced ROS production seems paradoxical since uric acid has generally been considered to be one of the important anti-oxidants that protect the cardiovascular system from oxidative stress. Uric acid prevents per-oxynitrite-induced protein nitrosylation, lipid and protein peroxidation and an inactivation of tetrahydrobiopterin, which results in scavenging free radical and chelating transitional metal ions. Consistent with an intrinsic anti-oxidant activity, uric acid administration in healthy volunteers and athletes reduced ROS production. Nonetheless, many experimental and human studies demonstrated the role of uric acid as pro-oxidants. Animals with hyperuricemia was reported to develop hypertension associated with an evidence for oxidative stress, and the hypertension was blocked by anti-oxidant treatment.

Experimental studies demonstrated an activation of oxidative stress by uric acid in various cells.

### 1. Vascular endothelial and smooth muscle cells

Uric acid is already known to activate critical proinflammatory pathways and stimulate cell proliferation in vascular smooth muscle cells. In endothelial cells, uric acid decreases NO bioavailability and inhibits cell migration and proliferation, which are mediated in part by the expression of C-reactive protein and oxidative stress. Chronic fructose consumption also stimulates uric acid synthesis. It has been proposed that the marked increase in fructose intake may have a role in the rising levels of serum uric acid and obesity worldwide. Uric acid may also be affected by exercise, with moderate exercise reducing urate levels (probably by increasing renal blood flow) and severe exercise causing a rise in uric acid (probably due to ATP consumption with adenosine and xanthine formation).

### 2. Renal tubular cells

In cultured renal tubular cells, uric acid induced epithelial-to-mesenchymal transition (EMT) which was blocked by probenecid, an inhibitor of organic anion transporter. Anti-oxidants ameliorated uric acid-induced
EMT and apoptosis of renal tubules. N-acetylcysteine inhibited E-cadherin degradation and the expression of Snail24, suggesting EMT by uric acid was mediated by oxidative stress-driven inhibition of E-cadherin synthesis as well as E-cadherin degradation.

3. Adipocytes

Sautin et al. reported soluble uric acid per se stimulated an increase in NADPH oxidase activity and ROS production in mature adipocytes as early as 30 minutes23. An interesting phenotype of these differentiated adipocytes is a substantial uptake rate for uric acid with an expression of urate transporters (URAT1 and OAT4) and high basal level of ROS production. Uric acid stimulated NADPH oxidase-dependent ROS production, an activation of MAP kinases p38 and ERK 1/2, a decrease in NO bioavailability, and an increase in protein nitrosylation and lipid oxidation. A recent study demonstrated uric acid-induced ROS production in adipocytes was mediated by an activation of the local renin-angiotensin system31.

Oxidative stress in the adipose tissue has recently been recognized as a major cause of insulin resistance and cardiovascular disease32, and therefore hyperuricemia-induced alterations in oxidative homeostasis in the adipose tissue suggested the potential role of uric acid as a cause of insulin resistance.

4. Hepatocytes

We demonstrated uric acid-induced oxidative stress as a major mechanism of fat accumulation in cultured hepatocytes, HepG2 cells25. Uric acid (>6 mg/dL) increased H$_2$O$_2$ production and NOX activity in HepG2 cells from 30 minutes, and antioxidants including diphenylene iodonium and rotenone blocked uric acid-induced triglyceride accumulation, suggesting both NADPH oxidase-dependent and mitochondria-mediated oxidative stress generated by uric acid were responsible for hepatic fat accumulation33.

What are the lessons from epidemiologic studies?

Many epidemiologic studies suggest that serum uric acid is at best a very weak predictor of cardiovascular disease after an adjustment of other risk factors in the general population34,35. This is mainly due to a strong correlation between serum uric acid level and other cardiovascular risk factors including blood pressure, body mass index, creatinine, glucose, insulin, and lipid profiles. In contrast, uric acid appears to be a significant independent predictor of vascular and renal disease in hypertensive patients and in subjects with high risk factors, such as diabetes, patients with chronic kidney disease or congestive heart failure36-39. This discrepancy regarding the effect of uric acid in relatively healthy populations and those in high-risk individuals raises two questions about clinical implication of hyperuricemia. First, if a certain factor is strongly related with other risk factors, it can never be interpreted as an independent risk factor by conventional statistics. Second, what is the molecular switch that convertsuric acid from anti-oxidant to pro-oxidant. It is particularly interesting since a mild elevation of uric acid, often observed in patients with uncomplicated obesity or hypertension, seems to impose no major effect on cardiovascular and renal complication34,35. It is necessary to explore the molecular environment in which uric acid induces oxidative stress and an inflammatory reaction in hypertensive and/or diabetic subjects with high cardiovascular risk factors.

Conclusion

Based upon the data from experimental and clinical research, uric acid seems to play a dual role as pro- and anti-oxidants. Interestingly, intracellular uric acid generally imposes the detrimental effects as a pro-oxidant in cultured cells and animal model of hyperuricemia, which is supported by the protective effects of an inhibitor of organic anion transporter via blocking the entry of uric acid into the cells and amelioration of oxida-
tive stress. On the other hand, uric acid acts as anti-oxidant only in the hydrophilic environment. The differential role of hyperuricemia in a healthy population and subjects with other risk factors for cardiovascular and metabolic disease suggests the presence of a molecular switch which controls the role of uric acid as anti-oxidant versus pro-oxidant. Regulation of this switch could be determined by the microenvironment in different compartment of human organism. Further studies will be necessary to understand the Janus face of uric acid or yin and yang of hyperuricemia with a definition of molecular mechanism by which uric acid activates membranous and intracellular oxidative stress which is incompletely understood. Controlled clinical studies to investigate the effect of uric acid-lowering therapy while monitoring the markers of oxidative stress in subjects with different clinical characteristics, will provide valuable information regarding the clinical implication of hyperuricemia.

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