Hyperuricemia and Atrial Fibrillation
Possible Underlying Mechanisms

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SUMMARY

The importance of atrial fibrillation (AF) as a cause of mortality and morbidity has prompted research on its pathogenesis and treatment. Recognition of AF risk factors is essential to prevent it and reduce the risk of death. Hyperuricemia has been widely accepted to be associated with the incidence of paroxysmal or persistent AF, as well as to the risk of AF in post cardiovascular surgery patients. The possible explanations for this association have been based on their relation with either oxidative stress or inflammation. To investigate the link between hyperuricemia and AF, it is necessary to refer to hyperuricemia-induced atrial remodeling. So far, both ionic channel and structural remodeling caused by hyperuricemia might be plausible explanations for the occurrence of AF. Inhibition of xanthine oxidase and nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, or the use of antioxidants, along with serum uric acid (SUA) level reduction to prevent inflammation, might be useful. Uric acid transporters (UATs) play a key role in the regulation of intracellular uric acid concentration. Intracellular rather than serum uric acid level is considered more important for the pathogenesis of AF. Identification of UATs expressed in cells is thus important, and targeting UATs might become a potential strategy to reduce the risk of hyperuricemia-induced atrial fibrillation. (Int Heart J 2016; 57: 395-399)

Key words: Oxidative stress, Inflammation, Atrial-remodeling, Uric acid transporter (UAT)

As approximately 33.5 million persons globally are suffering from AF, it is considered to be the most frequent cardiac arrhythmia. Among the different types of cardiac arrhythmia, AF is of great concern because it contributes significantly to cardiac mortality and morbidity.19 Patients with AF show an increased risk of stroke, heart failure, and dementia. Aging, male gender, rheumatic heart disease, hypertension, congestive heart failure, hyperthyroidism, chronic kidney disease, and diabetes mellitus have been recognized as risk factors of AF.4,5 Although the pathophysiology underlying AF remains to be elucidated, accumulated evidence suggests the involvement of inflammation and oxidative stress.6-8

Uric acid is the end product of purine metabolism in humans. The serum concentration of uric acid is significantly higher in humans compared to that in other mammals due to loss of uricase activity.9 Uric acid monovalent sodium salt (urate) is soluble in plasma at pH 7.4, accounts for approximately 98% of all uric acid in plasma, and at physiological concentrations acts as an antioxidant. Yet, in a hyperuricemic state it can act as a pro-oxidant. Hyperuricemia refers to the condition when the level of serum uric acid is above 7 mg/dL in adult men, or 6 mg/dL in adult women.10 Hyperuricemia is usually caused by alterations in the balance between uric acid synthesis and urinary excretion, although it is predominantly due to impaired renal excretion of urate.10-13 Various kinds of diseases, such as hypertension, metabolic syndrome, diabetes mellitus, and chronic kidney disease may be related to hyperuricemia.11-13

Hyperuricemia and Atrial Fibrillation

The relationship between hyperuricemia and AF has been well established according to several studies. The association between an increased serum level of uric acid (SUA) and permanent AF was initially found in a cross-sectional study described by Letsas, et al.14 Serum uric acid level was significantly correlated with the new onset of AF in a concentration-dependent manner.15 A meta-analysis of 6 cross-sectional and 3 cohort studies confirmed the association of high SUA with AF.16

Hyperuricemia has also been associated with AF under several clinical conditions such as hypertension,17 heart failure,18 hemodialysis,19 ischemic heart disease,20 sleep apnea syndrome,21 or type-2 diabetes mellitus.22 Hyperuricemia has been associated with left atrial remodeling, leading to an increase of its size, which might be a risk factor of AF.23 A population-based study involving 45,378 patients and equal numbers of matched-control subjects indicated that gout, a known
consequence of hyperuricemia, was independently associated with a higher risk of AF. A mechanism related to gender-specificity might play a role in the association between hyperuricemia and atrial fibrillation. Suzuki, et al reported in 2011 that the effect of SUA level on AF was observed in women but not in men, even after adjustment for well-known cardiovascular risk factors. The Atherosclerosis Risk in Communities (ARIC) Study showed that elevated SUA was associated with a higher risk of AF, particularly in African Americans and women. The underlying mechanisms remain unknown. A study on flow-mediated dilation (FMD) showed that hyperuricemia is independently associated with endothelial dysfunction in post-menopausal, but not pre-menopausal women, suggesting that uric acid can be used as a risk marker of endothelial dysfunction in the female population. This indicates that uric acid could be an independent risk factor for cardiovascular disease including AF, particularly in postmenopausal women but not in premenopausal women.

High serum uric acid might be a predictor for the occurrence of AF, since a cohort study involving 6,308 men and women in Norway showed that baseline SUA was associated with an increased risk of future AF. Besides being a significant middle-term predictor of tachyarrhythmia, SUA also seems to be a predictor of myocardial infarction and left ventricular hypertrophy. The presence of hyperuricemia increases the risk of AF, particularly in patients who have undergone cardiac surgery, such as a coronary artery bypass graft (CABG). Thus, SUA level can increase sensitivity and specificity in predicting atrial fibrillation after CABG surgery.

Hyperuricemia-Induced Atrial Remodeling: Possible Mechanisms

To elucidate the possible mechanisms underlying hyperuricemia-induced AF, we should first understand the pathophysiology of AF. The electrophysiological properties of AF can be composed of trigger factors for ectopic beat and substrate responsible for reentry circuit in the atrium, which is attributed to 4 mechanisms: electrical remodeling, structural remodeling, autonomic nervous system changes, and Ca²⁺ handling abnormalities; referred to as atrial remodeling. Each mechanism is caused by either aging or disease-related remodeling. AF needs a trigger factor to initiate AF and an appropriate substrate for the reentrant circuit to maintain AF, which could be caused by electrical and structural remodeling of the atrium. Electrical remodeling defined as changes in ion channel expression and function can shorten the action potential duration, ie, the effective refractory period slows the conduction velocity in the atrium. Electrical remodeling was shown to increase outward potassium channels and decrease Ca²⁺ channels, shortening the atrial refractory period while reducing the Na⁺ channel to slow the atrial conduction and induce the unidirectional conduction block, establishing thereby a reentrant circuit in the atrium. The trigger factor might be delayed after depolarization (DAD) due to abnormal Ca²⁺ release from the sarcoplasmic reticulum in the atrium. Haïssaguerre, et al found that the automaticity that originated in the pulmonary vein could be an important trigger of atrial fibrillation. Structural remodeling could present as an enlarged LA size, which is a well-known factor that facilitates the initiation and maintenance of AF. Changes in the atrial structure might slow conduction velocity and thereby allow re-entry as well. Furthermore, AF itself might promote atrial remodeling; namely, AF begets AF. While electrical and structural remodeling is the most important process involved in the pathogenesis of AF, oxidative stress and inflammation are the most likely contributing factors for AF.

The roles of inflammation: Although it is well-known that inflammation plays an important role in the development of AF, its role might be very complex. It can modulate atrial electrophysiological and structural substrates to increase the risk of AF.

Various investigators have assessed the state of the inflammatory response in patients with AF. It was found, for instance, that left appendage tissues obtained from AF patients showed more prominent presence of CD45-reactive cells, CD68-positive macrophages, and less CD3-positive T cells, compared to tissues obtained from patients with a sinus rhythm. In the atrium, inflammation induces its enlargement and the development of atrial fibrosis. Atrial fibrosis promotes AF by interrupting the continuity of fiber bundles and causing thereby atrial conduction disturbance. Several reports have indicated that hyperuricemia induced protein expression in the cells, causing inflammation through activation of uric acid transporters. Chao, et al found that hs-CRP expression and insulin resistance were higher and LA diameter was larger in patients with a high SUA level. These findings indicated that increased inflammation and insulin resistance may be involved in the relationship between high SUA level and LA size.

The renin-angiotensin-aldosterone system (RAAS) has also been reported to induce atrial inflammation. Mice treated with angiotensin-II showed an increase of neutrophil infiltration in their atrial tissue, which depends on CD11b and CD18 integrins, since angiotensin II has been reported to stimulate the production of pro-inflammatory cytokines [such as IL-6, IL-8, and tumor necrosis factor (TNF)] through activation of angiotensin I receptors. Although the relationship between uric acid and the RAAS system in cardiac atrial myocytes has never been tested, there is a large body of experimental evidence indicating that uric acid could stimulate the circulating and local RAAS system in the cardiovascular system. Yu, et al reported that uric acid up-regulated the cellular expression of angiotensinogen, angiotensin-converting enzyme, and angiotensin II receptors and increased angiotensin II levels, which were ameliorated by ACE inhibitors, uric acid transporter blockers, and antioxidants. On the other hand Corry, et al found that uric acid stimulated cell proliferation as well as angiotensin II production, which was inhibited by PD98059, a MAP kinase inhibitor. It also increased oxidative stress in vascular smooth muscle cells (VSMCs) through tissue RAS. Taken together, these findings suggest that uric acid could facilitate the activation of the RAAS system at the local level. Therefore, uric acid would cause cardiovascular disorders by stimulating vascular RAS by making use, at least partially, of the MAP kinase pathway. Hyperuricemia has been reported to increase the mRNA expression of renin in rats and to play a role in the development of hypertension, suggesting the involvement of circulating RAAS system activation by uric acid. In clinical settings, the angiotensin receptor blocker losartan, which has a uric acid lowering action, has been shown to significantly reduce the onset of atrial fibrillation.
suggested that losartan combined with uric acid lowering agents might further decrease the risk of AF, through cancelling RAAS system activation by hyperuricemia. The roles of oxidative stress: Xanthine oxidoreductase (XOR) is a key enzyme in uric acid metabolism, which can be a source of reactive oxygen species (ROS). The role of XOR in the pathogenesis of AF has been investigated in several studies.

AF increases $O_2^-$ production in both the left atrium and left atrial appendage. Increased NAD(P)/H oxidase and xanthine oxidase activities contribute to the observed increase in left atrial appendage $O_2^-$ production. This increase in $O_2^-$ and its reactive metabolites through activation of xanthine oxidase may contribute to the pathological consequences of AF such as thrombosis, inflammation, and tissue remodeling. An in vivo study using a canine model of atrial pacing-induced left ventricular dysfunction suggested the prevention of AF by the xanthine oxidase inhibitor allopurinol. One week of preventive therapy by daily allopurinol reduced atrial vulnerability by inhibiting both electrical and structural atrial remodeling in a canine model of ventricular tachypacing.

The relationship between hyperuricemia and changes in cardiac structure (remodeling) might also be explained from findings of a study in mice fed a Western diet. The increase of serum uric acid after 16 weeks was accompanied by an increase in cardiac tissue xanthine oxidase activity, which induced cardiomyocyte hypertrophy, myocardial oxidative stress, interstitial fibrosis, and impaired diastolic relaxation through activation of the S6 kinase-1 growth pathway, profibrotic TGFβ1/Smad2/3 signaling pathway, and macrophage proinflammatory polarization. These conditions improved with allopurinol treatment.

Several reports indicated that xanthine oxidase played a pivotal role in the generation of superoxide free radicals in the human atrium as well. However, so far, there has been no prospective clinical trial to test whether xanthine oxidase inhibitors could prevent the onset of AF.

Intracellular Accumulation of Uric Acid Causes Pathological Changes in Various Organs

There is still need for further research directly addressing the effects of UA on atrial cardiomyocytes and occurrence of AF. Nevertheless, there is growing experimental evidence that intracellular uric acid activates particular pathways to cause pathological changes in several types of cells, and that uric acid transporters are involved in the intracellular accumulation of UA.

While uric acid transporters in renal proximal tubular cells play a pivotal role in the regulation of serum urate levels, UATs are reported to be expressed not only in renal tubular cells, but also in vascular smooth muscle cells, endothelial cells, adipocytes, and pancreatic $\beta$-cells.

Intracellular accumulation of uric acid through activation of UATs could be responsible for C-reactive protein expression implicated in cell proliferation and impaired nitric oxide production in hVSMCs, which could be attenuated by the UAT inhibitor probenecid. Uric acid induced senescence and apoptosis of human umbilical vein endothelial cells, which could be blocked by not only probenecid but also enalaprilat or telmisartan. These effects are mediated by local activation of oxidative stress and the renin-angiotensin system as described elsewhere. Under oxidative stress, UA caused endothelial dysfunction in vascular cells by direct interaction with nitric oxide (NO) in a rapid irreversible reaction. This reaction of UA with NO occurs preferentially, even in the presence of oxidants such as peroxynitrite and hydrogen peroxide. Another report by Park, et al suggested that uric acid decreased eNOS activity and NO production through an impaired interaction between eNOS and CaM in endothelial cells. As for the association between hyperuricemia and type 2 diabetes, Jia, et al and Zhang, et al reported that uric acid activated NF-κB as well as the AMPK and ERK signaling pathways, causing pancreatic $\beta$-cell dysfunction, while pretreatment with UAT inhibitors could block the effects of uric acid. Zhi, et al reported that cardiac cells showed insulin resistance due to increased oxidative stress, when cells were exposed to high uric acid under in vitro and in vivo conditions.

Uric acid transporters play a key role on the regulation of intracellular urate concentration. It is important to identify UATs expressed in cells, since manipulation of UAT activities could lower intracellular UA concentration. Mouse atrial myocytes were found to express at least 3 UATs: URATv1, ABCG2, and MRP4, while human embryonic stem cell-derived cardiomyocytes expressed at least 4 UATs: URATv1, ABCG2, MRP4, and MCT9. In addition to cardiomyocytes, these 4 transporters are commonly expressed in other types of mouse tissues, such as brain, intestines, pancreas, liver, skeletal muscle, and vascular cells, suggesting their ubiquity and important roles. Figure 1 shows the expression of UATs in several mouse organs (Maharani N, Hisatome I, unpublished data).

In a recently published study, we observed the indirect effect of UAT inhibition on hyperuricemia-related changes on cardiac ion channels. While inhibition of uric acid-eflux transporter ABCG2 by KO143 increased intracellular concentrations of uric acid, the accumulated intracellular urate increased urate-induced damage of the cells via an increase of oxidative stress and activation of the ERK1/2 pathway. In mouse atrial myocytes, uric acid enhanced the protein expression of Kv1.5 channel, resulting in an increase of the ultra-rapid delayed-rectifier current (I_Kur) associated with shortening of the atrial ac-
tion potentials. Inhibition of uric acid-influx UATs by benzbro-marone attenuated the UA-induced enhancement of Kv1.5 protein expression through a decrease of intracellular uric acid. This effect was oxidative stress-dependent, since the enhancement of Kv1.5 protein expression by uric acid was reversed by the antioxidant N-acetylcysteine and the NADPH-oxidase inhibitor apocynin. This process involved the ERK1/2 pathway as part of the downstream signaling of urate-derived ROS, which was consistent with the findings of other studies. 

In agreement with the involvement of oxidative stress, treatment with an antioxidant abolished urate-induced enhancement of the Kv1.5 protein channel. The possible mechanisms underlying uric acid-induced enhancement of Kv1.5 protein expression are shown in Figure 2.

Conclusion

It has been well-established that hyperuricemia is independently associated with the occurrence of atrial fibrillation. Accumulation of uric acid inside atrial cardiomyocytes might cause ionic and structural remodeling of the atria, referred to as atrial remodeling. UATs as the regulator of intracellular uric acid concentration mediate hyperuricemia-induced atrial remodeling. This atrial remodeling provides a vulnerable substrate for the onset of AF, and is mostly caused by either oxidative stress, inflammation, or both. Reduction of oxidative stress through inhibition of xanthine oxidase using allopurinol or other similar drug, of NADPH-oxidase using apocynin, or the use of N-acetylcysteine, might be beneficial. On the other hand, prevention of structural and ionic remodeling through inhibition of atrial inflammation might also provide benefits. Targeting UATs might also be another option for a comprehensive hyperuricemia management to reduce the risk of atrial fibrillation. More studies are needed to obtain a more comprehensive understanding of the pathophysiology of AF and measures to prevent AF.

Figure 2. Schematic representation of the mechanisms underlying urate-induced enhancement of the Kv1.5 ion channel protein and function. Increased extracellular urate concentration would activate UATs, and more urate would enter the cells. Intracellular urate would induce ROS production, which is NADPH-oxidase-dependent.