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ARTICLE

Effects of uric acid on vascular endothelial function from bedside to bench

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Abstract

This study was designed to investigate the effects of uric acid on vascular endothelial function in measurements carried out either at the bedside or the laboratory bench. First, we performed reactive hyperemia peripheral arterial tonometry using an EndoPAT 2000 device and measured serum uric acid levels in 92 outpatients with hypertension. The reactive hyperemia index (RHI) showed no correlation with serum uric acid level ($R = -0.125$, $P = 0.235$) in either overall patients or in a high-risk group of 51 patients with complications such as cardiovascular and cerebrovascular diseases, chronic kidney disease, and/or diabetes ($R = -0.025$, $P = 0.860$). However, in the remaining 41 patients in the low-risk group, RHI correlated negatively with serum uric acid level ($R = -0.335$, $P = 0.032$). Multiple regression analysis showed that serum uric acid level predicted RHI ($R = -0.321$, $P = 0.043$) in the low-risk group independent of age, body mass index, systolic blood pressure, and low density lipoprotein-cholesterol level. We then performed an in-vitro study using the WST-8 assay in human umbilical vein endothelial cells, which showed that hypoxic conditions reduced cell viability. Treatment with uric acid caused a further reduction in cell viability, while ascorbic acid improved viability. Using Western blot analysis, we observed that uric acid reduced endothelial nitric oxide synthase phosphorylation during hypoxic conditions. Serum uric acid level is associated with peripheral vascular endothelial function in patients with low-risk hypertension and uric acid could directly impair endothelial function under hypoxic conditions. These results are relevant to the interventional studies examining the cardiovascular protective effect of hypouricemic agents.

Introduction

Uric acid is an end product of purine metabolism. Its immediate precursor, xanthine, is converted to uric acid by an enzymatic reaction involving xanthine oxidoreductase. Uric acid is then degraded to allantoin by urate oxidase and excreted freely in the urine in most mammals, including humans [1]. In humans, however, the uricase gene is a nonfunctioning pseudogene, resulting in overproduction or decreased excretion of uric acid resulting in high levels of serum uric acid, i.e., hyperuricemia [2–4]. Uric acid is considered an anti-oxidant with potentially beneficial anti-atherosclerotic effects. However, during uric acid formation, xanthine oxidoreductase produces reactive oxygen species (ROS), possibly contributing to increased oxidative stress and vascular endothelial dysfunction, both of which are associated with atherosclerosis risk. On the other hand, uric acid itself has been shown also to induce vascular endothelial dysfunction via oxidative stress and inflammatory responses [5–7]. Many studies have demonstrated that hyperuricemia has a close relationship with cardiovascular disease and chronic kidney disease and is an independent risk factor for these diseases [8–10]. Notably, several other studies reported no association with cardiovascular disease [11, 12]. Therefore, the role of hyperuricemia in

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cardiovascular disease remains controversial. Furthermore, it remains unclear whether the effects of hyperuricemia on the cardiovascular system are caused by uric acid itself or by oxidative stress derived from xanthine oxidoreductase.

This study was designed to investigate the effects of uric acid on vascular endothelial function. We first assessed the clinical relationship between serum uric acid level and vascular endothelial function parameters in patients with hypertension, and then assessed in-vitro cell viability of cultured human umbilical vein endothelial cells (HUVECs), and the level of endothelial nitric oxide synthase (eNOS) phosphorylation and xanthine oxidoreductase activity during hypoxic conditions, with or without treatment with uric acid.

Methods

Clinical study

We recruited 92 consecutive outpatients with hypertension who had regularly visited the general internal medicine clinic of a community hospital associated the Dokkyo Medical University, Shimotsuga Medical Association Hospital, between February and November 2013. All patients were measured for height, weight, blood pressure, and vascular endothelial function, and a blood sample was collected during a clinic visit. Body mass index was calculated as weight / height^2 (kg/m^2). Blood pressure was measured in duplicate using a mercury sphygmomanometer with an appropriately sized cuff. The measurements were taken after the patients had rested in the seated position for at least 5 min, with at least 5 min between the two measurements. All values were averaged for analysis.

Fasting venous blood was collected from the antecubital vein and the serum uric acid level was determined using the uricase peroxidase method. Serum creatinine level was measured using an enzymatic method, and estimated glomerular filtration rate (eGFR) was calculated using the formula of the Japanese Society of Nephrology CKD Practice Guide: eGFR (ml/min/1.73 m^2) = 194 × (serum creatinine level [mg/dl])$^{-1.094}$ × (age [y])$^{-0.287}$. Total cholesterol and triglyceride levels were determined using enzymatic methods and high density lipoprotein (HDL)-cholesterol by the precipitation method. Fasting blood glucose was assayed using the glucose oxidase method and glycohemoglobin (Hb) A1c was measured using high-performance liquid chromatography and expressed as the value of the National Glycohemoglobin Standardization Program. Low density lipoprotein (LDL)-cholesterol was calculated using the Friedewald formula: (LDL-cholesterol = total cholesterol − HDL-cholesterol – triglyceride / 5).

Peripheral vascular endothelial function was assessed in the morning by reactive hyperemia peripheral arterial tonometry (RH-PAT) using an EndoPAT 2000 device (Itamar Medical, Caesarea, Israel), as described previously [13]. Briefly, vasoactive drugs were withheld on the day of measurement. Patients were required to fast at least 4 h before the measurement and not consume caffeine or smoke during this period. They then rested in the supine position for at least 15 min in a quiet, temperature-controlled room. A blood pressure cuff was placed on one upper arm, while the contralateral arm served as a control. PAT probes were placed on a finger of each hand. After a 5 min equilibration period, the cuff was inflated to 60 mmHg above systolic pressure (if systolic blood pressure was < 140 mmHg) or to 200 mmHg (if systolic blood pressure was >140 mmHg) for 5 min and then deflated to induce reactive hyperemia. The reactive hyperemia index (RHI) was calculated as the ratio between the signals measured at baseline and 5 min after upper-arm occlusion, relative to the response in the contralateral arm. The local institutional review board of the Dokkyo Medical University approved the study protocol, and written informed consent was obtained from each patient.

Cell viability assay

Uric acid was purchased from Sigma-Aldrich (St. Louis, MO, USA), and ascorbic acid and adenosine from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were obtained from the usual commercial sources. HUVECs were obtained from the Japanese Collection of Research Bioresources Cell Bank, National Institute of Biomedical Innovation, Health and Nutrition (Ibaraki, Japan). The WST-8 assay (Dojindo, Kumamoto, Japan) was used to assess cell viability [14], according to the manufacturer's instructions. Briefly, HUVECs were seeded in 100 µl of EGM-2 medium in flat-bottom 96-well plates at a concentration of 1×10^4 cells per well and maintained with 5% CO_2 and 95% air at 37 °C in a CO_2 incubator. After 24 h, the medium was replaced with DMEM (10% FBS) and cells were cultured in either 21% (normoxic condition) or 1% O_2 (hypoxic condition) with either uric acid (0, 0.1, 0.3, or 1.0 mM), ascorbic acid (0, 30, 100, or 300 µM), or adenosine (0, 3, 10, or 100 µM). Concentrations of uric acid, ascorbic acid, and adenosine were set based on previous reports [15–17]. After 24 h of incubation, a Cell Counting Kit-8 was added to each well (1:10 final dilution), and the cells were incubated at 37 °C for up to 4 h. The optical density of each well was measured using an infinite F200 PRO microplate reader (TECAN, Japan) at a wavelength of 450 nm. The data were expressed as the ratio of the experimental sample relative to

the control sample, measured simultaneously in the same plate.

Western blot analysis

Cell lysates were prepared using 60 µl of lysis buffer (50 mM Tris-HCl at pH 7.8, 150 mM NaCl, 5 mM sodium pyrophosphate, 1 mM sodium vanadate, 3 mM β-glycerophosphate, 10 mM NaF, 5% deoxycholic acid, 1% Triton X-100, 5 mM EDTA). Samples (5 µg total protein) were separated by SDS–PAGE with the use of polyacrylamide gradient gel (4–15%) and then transferred to Immobilon-P® polyvinylidene difluoride membranes (Merckmillipore, Bedford, MA, USA). The blots were blocked with Tris-buffered saline / 0.1% Tween 20 (TBS-T) solution containing 5% nonfat dry milk or 4% bovine serum albumin (BSA) for 1 h; subsequently they were incubated overnight at 4 °C with the primary antibodies. The membranes were washed three times with TBS-T, and the antibodies were probed with an HRP-conjugated secondary antibody using standard methods. Immunoreactive proteins were visualized by enhanced chemiluminescence using a CCD imager (Amersham Imager 600) and blots were quantified by Image J software (NIH, Bethesda, MD, USA). Anti-eNOS antibody and anti-phospho-Ser1177-eNOS (p-eNOS) antibody were obtained from Cell Signaling Technology (Danvers, MA, USA), anti-xanthine oxidoreductase antibody from Abcam (Cambridge, MA, USA), anti-β-actin antibody from Medical & Biological Laboratories (Nagoya, Japan), and HRP-conjugated anti-rabbit IgG from GE Healthcare (Little Chalfont, UK). Normoxic and hypoxic conditions were created in the same manner as the cell viability assay and uric acid was added at the concentrations of 0, 0.3, or 1.0 mM. Quantitative analysis was achieved by normalization of the integrated signal density of each protein.

Statistical analysis

Data were expressed as mean ± SD. The normality of the distribution of the variables was assessed using the Kolmogorov–Smirnov test with Lilliefors' correlation. Inter-group comparisons were performed using Student's unpaired t test for continuous variables and the χ-square test for categorical variables. Serial changes in variables were assessed using repeated measures analysis of variance and the correlation between two variables by simple linear regression. Multiple regression analysis was performed to assess independent predictors of RHI including serum uric acid level and other risk markers. All statistical analyses were performed using the statistical package for the Social Science (Dr. SPSS II for Windows, SPSS Inc., Tokyo, Japan). P values < 0.05 were considered statistically significant.

Table 1 Clinical characteristics of all patients ($n = 92$)

Age (year)	70 ± 10
Male gender, *n* (%)	50 (54)
Body mass index (kg m^{-2})	25 ± 4
Complications, *n* (%)	
Cardiovascular diseases	24 (27)
Cerebrovascular diseases	5 (5.4)
Chronic kidney disease	5 (5.4)
Other risk factors, *n* (%)	
Current smoking	18 (20)
Diabetes	29 (32)
Dyslipidemia	62 (67)
Medications, *n* (%)	
ACE inhibitor / ARB	78 (85)
Calcium channel blocker	62 (67)
Statins	47 (51)
Hypouricemic agents	12 (13)
Systolic blood pressure (mmHg)	133 ± 16
Diastolic blood pressure (mmHg)	80 ± 12
Creatinine (mg dl^{-1})	0.80 ± 0.27
eGFR (ml min^{-1} × 1.73 m^{-2})	71.3 ± 20.3
Uric acid (mg dl^{-1})	5.8 ± 1.5
LDL-cholesterol (mg dl^{-1})	113 ± 34
HDL-cholesterol (mg dl^{-1})	53 ± 13
Triglyceride (mg dl^{-1})	120 ± 68
Fasting blood glucose (mg dl^{-1})	107 ± 22
Hemoglobin A1c (%)	6.0 ± 0.6
Reactive hyperemia index	1.77 ± 0.47

ACE angiotensin converting enzyme, *ARB* angiotensin receptor blocker, *eGFR* estimated glomerular filtration rate, *LDL* low density lipoprotein, *HDL* high density lipoprotein

Results

Relationship between serum uric acid level and vascular endothelial function in patients with hypertension

The clinical characteristics of the 92 study patients are shown in Table 1. Of these, 24 (27%) had cardiovascular diseases (coronary artery disease 14, atrial fibrillation 11, congestive heart failure 3, dilated cardiomyopathy 1, aortic dissection 1, and post-surgical aortic valve replacement 1), 5 (5.4%) cerebrovascular diseases (cerebral infarction 3, cerebral hemorrhage 1, subarachnoid hemorrhage 1), and 5 (5.4%) chronic kidney disease. Other risk factors included diabetes mellitus in 29 patients (32%) and dyslipidemia in 62 (67%) patients. RHI did not correlate with serum uric acid level ($R = -0.125$, $P = 0.235$) in the overall patient cohort (Fig. 1a). Patients were then divided into a high-risk group ($n = 51$ patients with complications such as

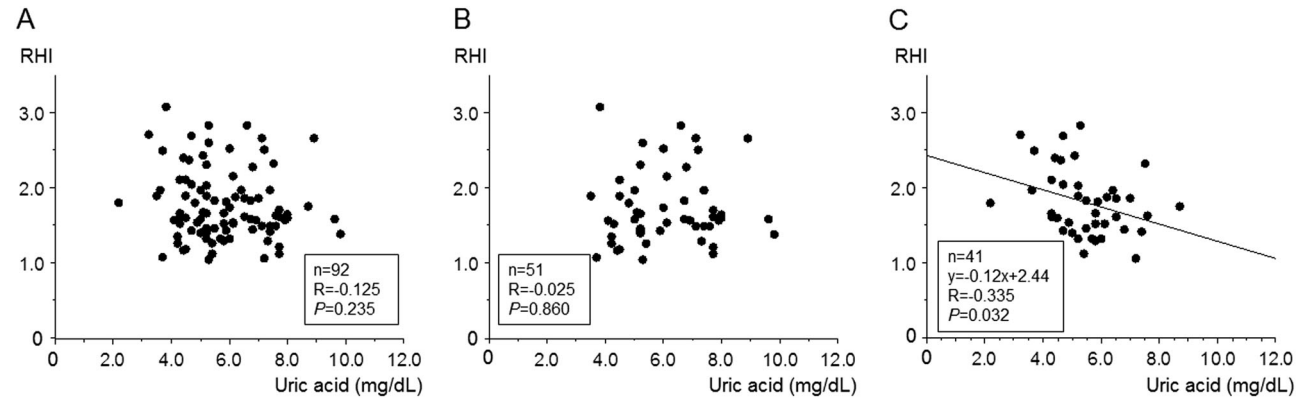

Fig. 1 a–c Relationship between serum uric acid level and reactive hyperemia index (RHI) in patients with hypertension. In the combined patient cohort, RHI did not correlate with serum uric acid level (**a**). RHI did not correlate with serum uric acid level in patients in the high-risk group, who had complications such as cardiovascular diseases, cerebrovascular diseases, chronic kidney disease, and / or diabetes (**b**). RHI negatively correlated with serum uric acid level in patients in the low-risk group (**c**)

Table 2 Comparison of clinical characteristics between the high- and low-risk groups

	High-risk group ($n = 51$)	Low-risk group ($n = 41$)	P value
Age (year)	71 ± 8	68 ± 12	0.197
Male gender, n (%)	32 (63)	18 (44)	0.071
Body mass index (kg m^{-2})	25 ± 4	24 ± 3	0.284
Systolic blood pressure (mmHg)	132 ± 15	136 ± 17	0.215
Diastolic blood pressure (mmHg)	78 ± 10	83 ± 14	0.03
Creatinine (mg dl^{-1})	0.86 ± 0.31	0.72 ± 0.18	0.013
eGFR (ml min^{-1} × 1.73 m^{-2})	68.7 ± 22.9	74.5 ± 16.2	0.175
Uric acid (mg dl^{-1})	6.1 ± 1.6	5.5 ± 1.3	0.033
LDL-cholesterol (mg dl^{-1})	108 ± 28	119 ± 39	0,121
HDL-cholesterol (mg dl^{-1})	51 ± 12	56 ± 14	0.034
Triglyceride (mg dl^{-1})	123 ± 72	115 ± 65	0.601
Fasting blood glucose (mg dl^{-1})	113 ± 26	100 ± 11	0.006
Hemoglobin A1c (%)	6.2 ± 0.6	5.7 ± 0.3	0.0001
Reactive hyperemia index	1.73 ± 0.49	1.81 ± 0.44	0.447
Receiving hypouricemic agents, n (%)	7 (14)	5 (12)	0.848

cardiovascular diseases, cerebrovascular diseases, chronic kidney disease and/or diabetes mellitus) and a low-risk group ($n = 41$ patients with no complications) and clinical characteristics were compared between groups (Table 2). The proportion of males was greater in the high-risk group. Diastolic blood pressure and HDL-cholesterol level were lower and serum uric acid, fasting blood glucose, and hemoglobin A1c levels were higher in the high-risk group than in the low-risk group. The proportion of patients receiving hypouricemic agents was similar between the two groups. The RHI did not correlate with the serum uric acid level in the high-risk group ($R = -0.025$, $P = 0.860$) (Fig. 1b), but had a significant negative correlation in the low-risk group ($R = -0.335$, $P = 0.032$) (Fig. 1c). Multiple regression analysis showed that serum uric acid level predicted RHI in the low-risk group, ($R = -0.321$, $P = 0.043$), independent of age, body mass index, systolic blood pressure, and LDL-cholesterol level, but was not correlated in the high-risk group (Table 3).

Direct effects of uric acid on endothelial cell viability under hypoxic conditions

We assessed in-vitro cell viability of HUVECs after uric acid treatment in five experiments using the WST-8 assay. Uric acid treatment for 24 h under normoxic conditions did not change cell viability (Fig. 2a). Hypoxic conditions alone reduced viability to 82 ± 4% ($P < 0.01$) of normoxic control conditions. Under hypoxic conditions, uric acid treatment further reduced cell viability in a concentration-dependent manner, with this decrease being statistically significant at 1.0 mM (64 ± 4% of control, $P < 0.05$) (Fig. 2b). On the other hand, ascorbic acid treatment caused a concentration-dependent improvement in hypoxia-induced reduction in cell viability, with this change being statistically significant at 300 µM (78 ± 7% to 102 ± 18% of control, $P < 0.01$) (Fig. 2c). Treatment with adenosine, which shares similar molecular characteristics with uric acid as another purine metabolites, did not change cell viability under hypoxic conditions (Fig. 2d).

Effects of uric acid on eNOS phosphorylation and xanthine oxidoreductase expression in endothelial cells

We performed Western blot analysis to assess in-vitro eNOS phosphorylation and xanthine oxidoreductase

Table 3 Multiple regression analysis for predicting reactive hyperemia index using serum uric acid level and other risk markers

	High-risk group ($n = 51$)		Low-risk group ($n = 41$)	
	Standard regression coefficient	P value	Standard regression coefficient	P value
Age (year)	−0.116	0.484	−0,222	0.161
Body mass index (kg m^{-2})	−0.101	0.535	−0.049	0.767
Systolic blood pressure (mmHg)	0.078	0.603	−0.085	0.618
LDL-cholesterol (mg dl^{-1})	0.229	0.134	0.25	0.113
Uric acid (mg dl^{-1})	−0.321	0.801	−0.321	0.043

expression in the HUVECs in three experiments. Hypoxic conditions alone did not change eNOs or p-eNOS levels in the cell lysates, compared to normoxic conditions (control). Under hypoxic conditions, uric acid treatment dose-dependently reduced p-eNOS level, while the eNOS level did not change. The p-eNOS/eNOS tended to be dose-dependently reduced by uric acid, and was significantly reduced by a dose of 1.0 mM under hypoxic conditions, compared with the uric acid-free state (101 ± 31% to 73 ± 32% of control, $P < 0.05$) (Fig. 3a). Hypoxic conditions alone did not change xanthine oxidoreductase level, compared with controls. Under hypoxic conditions, uric acid tended to dose-dependently but not significantly increase the level of xanthine oxidoreductase (96 ± 2% to 121 ± 36% of control) (Fig. 3b).

Discussion

This study demonstrated that serum uric acid level correlated negatively with peripheral vascular endothelial function measured as RHI by the RH-PAT method in patients with low-risk hypertension. Our in-vitro study demonstrated that uric acid directly reduced HUVEC viability and inhibited eNOS phosphorylation under hypoxic conditions.

Although hyperuricemia is strongly associated with an increased risk of atherosclerosis, its significance as an independent risk factor for clinical outcomes remains controversial [18, 19]. Serum uric acid level has been shown to correlate with vascular endothelial function measured by flow mediated vasodilation (FMD) [20]. However, there has been little previous data to support an association between uric acid and RHI measured by RH-PAT. While FMD reflects the endothelial function of large conduit arteries [21], RHI measured by RH-PAT is considered to represent the endothelial function of the microvasculature [22]. The clinical significance of FMD and RHI is therefore different, although both parameters predict cardiovascular events [23, 24]. In our study, serum uric acid level correlated negatively with RHI in patients with low-risk hypertension, i.e., with hypertension alone or in combination with dyslipidemia. In addition, multiple regression analysis indicated that serum uric acid level predicted the RHI independent of age, body mass index, systolic blood pressure, and LDL-cholesterol level. However, the correlation between uric acid level and RHI was absent in patients with high-risk hypertension, who had complications such as cardiovascular and cerebrovascular diseases, chronic kidney disease, and/or diabetes. In this class of patients, serum uric acid level was not an independent predictor of the RHI in the multiple regression analysis. These results suggest that hyperuricemia may be a risk factor for vascular endothelial dysfunction of small resistance vessels, independent of aging, obesity, high blood pressure and increased LDL-cholesterol level, but that its power as an atherogenic risk factor may be masked by stronger risk factors such as cardiovascular, cerebrovascular or, chronic kidney diseases and/or diabetes.

It has long been thought that uric acid provides antioxidant defense in humans, and therefore may protect against oxidative stress in the cardiovascular system, including vascular endothelial cells [25–27]. In contrast, many studies have shown that uric acid induces vascular endothelial dysfunction via oxidative stress and inflammatory responses [5–7]. Recently, Cai et al. [28] demonstrated that uric acid inhibited the expression of eNOS and the production of nitric oxide (NO) in HUVECs. It is well known that hypoxia induces impairment of endothelial function, with oxidative stress playing an important role. Prolonged hypoxia reduces eNOS activity and eNOS mRNA levels via activation of hypoxia inducible factor-2 (HIF-2) [29, 30]. At the post-transcriptional level, hypoxia destabilizes eNOS mRNA in human endothelial cells, in part, via the Rho kinase pathway [29]. Hypoxia also increases arginase II activity in endothelial cells, leading to degradation of L-arginine, an essential substrate for NO production by eNOS [31]. Oxidation of the eNOS cofactor tetrahydrobiopterin (BH4) by ROS such as peroxynitrite appears to be an important mechanism linking oxidative stress to endothelial dysfunction [32]. Therefore, it is possible that uric acid inhibits eNOS activation which promotes hypoxia-induced impairment of vascular endothelial function, and that oxidative stress may play a role in this process. In our *in-vitro* study using the WST-8 assay in

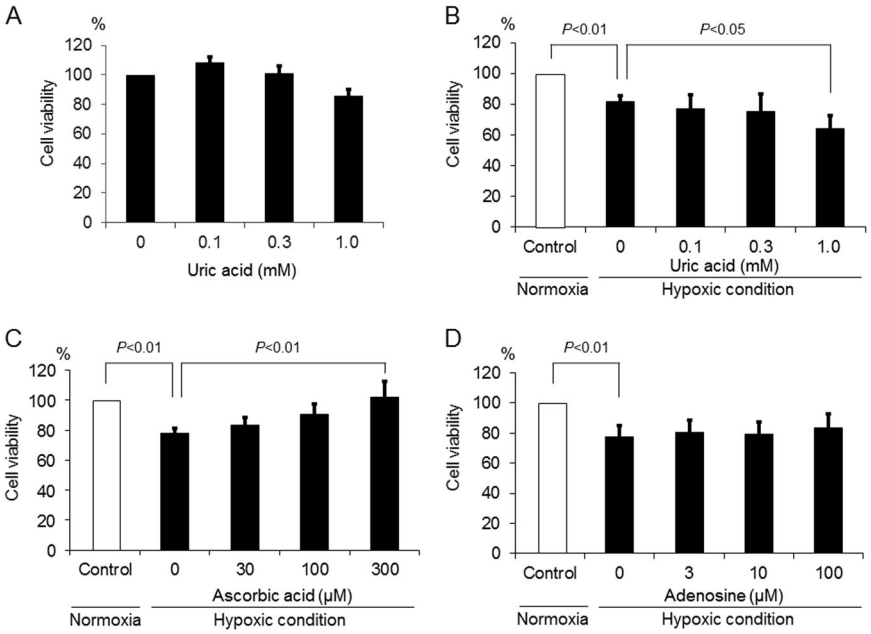

Fig. 2 a–d Effects of uric acid on the viability of HUVECs. Under normoxic conditions, uric acid treatment for 24 h did not change cell viability (**a**). Hypoxic conditions reduced cell viability compared with normoxic conditions. Under hypoxic conditions, uric acid treatment further reduced cell viability in a concentration-dependent manner (**b**). Ascorbic acid treatment improved hypoxia-induced reduction in cell viability in a concentration-dependent manner (**c**). Adenosine treatment did not change cell viability under hypoxic conditions (**d**)

HUVECs, hypoxia reduced cell viability and uric acid treatment further promoted hypoxia-induced cell death, while the anti-oxidant ascorbic acid had an inhibitory effect. This result suggests the possibilities that antioxidants improved vascular endothelial dysfunction for hyperuricemia. Despite a rationale for the important role of ROS in the development of atherosclerosis, however, there is still no clinical evidence that scavenging ROS prevents progression of atherosclerosis [33, 34]. In addition, our Western blot analysis indicated that uric acid reduced eNOS phosphorylation. These results support previously published studies. In our study, another purine metabolite, adenosine, which has a similar molecular structure to uric acid, did not affect cell viability under hypoxic conditions. This result suggests that the direct effect of uric acid on endothelial cell viability may be specific for uric acid and is not a class effect of purine metabolites.

When considering hyperuricemia as a cardiovascular risk factor, it is important to include a role for xanthine oxidoreductase activity in addition to the direct effect of uric acid itself. Xanthine oxidoreductase is constitutively expressed in the dehydrogenase form and its serum level in humans is usually very low. However, the level may be elevated in certain pathological conditions in which xanthine oxidoreductase is released from damaged cells into the circulation, where it is converted to the oxidase form. Circulating xanthine oxidoreductase may bind to endothelial cells, where it causes remote organ injury and endothelial dysfunction [35]. Hyperuricemia may therefore cause oxidative stress by both uric acid itself and by xanthine oxidoreductase activity, potentially leading to endothelial dysfunction. Our Western blot finding that uric acid tended to enhance xanthine oxidoreductase expression in HUVECs under hypoxic conditions may support this hypothesis.

Study limitations and clinical implications

Our study has several limitations. The clinical study was based on cross-sectional data collected at one clinic visit, and no data from follow-up visits was included. Although measurement of RH-PAT was performed in the fasting state, in the morning, and in the absence of vasoactive drugs, caffeine, and smoking, seasoning variations could not be examined. In addition, the sample size was too small to make definitive conclusions regarding the relationship between serum uric acid level and endothelial function. If we assume −0.3 as the regression coefficient between uric acid level and RHI, 85 patients are needed to detect a statistically significant result at 80% power. Therefore, the absence of a relationship between uric acid level and RHI in the 51 high-risk patients might be a type II error. However, since the estimated regression coefficient was −0.25, the finding of a significant relationship is unlikely, even if the number of patients is increased to 85. In addition, multiple regression analysis to assess uric acid as an independent predictor of RHI showed statistically significant results in the low-risk group but not in the high-risk group. In this analysis, age, body mass index, systolic blood pressure, and LDL-cholesterol level are incorporated as the independent variables. As Table 2 shows, however, there are several other variables, such as diastolic blood pressure, creatinine, HDL-cholesterol, fasting blood glucose and hemoglobin A1c, about which significant differences exist between the

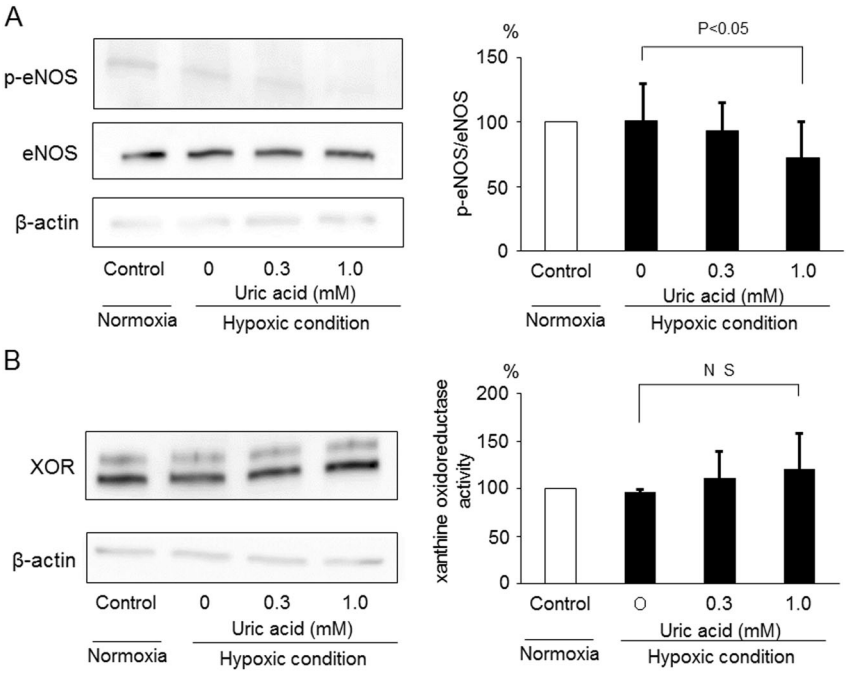

Fig. 3 Western blot analysis to assess in-vitro eNOS phosphorylation and xanthine oxidoreductase activity. Hypoxic conditions did not change eNOs or p-eNOS levels, compared with normoxic conditions (controls). Under hypoxic conditions, uric acid treatment dose-dependently reduced p-eNOS level, while the eNOS level did not change. The p-eNOS/eNOS tended to be dose-dependently reduced by uric acid and was significantly reduced by a dose of 1.0 mM under hypoxic conditions, compared to the uric acid-free state (**a**). Hypoxic conditions did not change xanthine oxidoreductase activity level, compared with controls. Under hypoxic conditions, uric acid tended to dose-dependently increase the xanthine oxidoreductase activity level (**b**)

two groups. It cannot be denied that these variables could have confounded the results of this analysis. Our in-vitro study included the simple observation that uric acid directly promoted a reduction in hypoxia-induced viability of vascular endothelial cells, and reduced eNOS phosphorylation. These experiments alone cannot sufficiently address the role of hyperuricemia on cardiovascular disease and could not conclude whether the effects of hyperuricemia on the cardiovascular system are caused by uric acid itself or by oxidative stress derived from xanthine oxidoreductase. Future experiments are needed to determine more precise mechanisms, including the involvement of intracellular signaling pathways.

Uric acid lowering therapy with a potent xanthine oxidase inhibitor, allopurinol, has been shown to improve vascular endothelial function as measured by FMD in patients with hyperuricemia [36] and in those with associated chronic heart failure [37]. The effect has been attributed to the strong anti-oxidant action of the drug. In a recent clinical trial, however, allopurinol failed to improve heart failure [38]. More recently, novel xanthine oxidase inhibitors such as febuxostat and topiroxostat have been developed [39, 40]. This class of drugs is primarily metabolized by the liver, and has a stronger uric acid lowering effect than allopurinol. However, the cardiovascular effects of these drugs remain to be established. At present, our group is conducting an ongoing trial to evaluate the effects of febuxostat on atherosclerosis in patients with hyperuricemia by measuring changes in carotid intima-media thickness [41]. We believe this trial would provide us an important message for clinical significance of uric acid-lowering treatment by febuxostat on atherosclerosis. In the present study, 14% of high-risk and 12% of low-risk hypertension patients received hypouricemic agents. It cannot be ignored that anti-oxidant effects of hypouricemic agents, which are directly induced by xanthine oxidase independent of uric acid reduction, somewhat affected our clinical results indicating negative correlation between serum uric acid level and RHI in patients with low-risk hypertension. Anyway, the results of the present study provide the basis of future interventional studies on hypouricemic agents targeting cardiovascular protection.

Conclusions

Serum uric acid level is associated with peripheral vascular endothelial function in patients with low-risk hypertension. Uric acid impairs directly endothelial function under hypoxic conditions. Our results provide the basis for future

interventional studies on hypouricemic agents targeting cardiovascular protection.

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Compliance with ethical standards

Conflict of interest Employment: NU (RIKEN, Osaka University, Merck, Takeda, OPKO Health Inc, Ono Pharmaceutical Co., Ltd., Sato Pharmaceutical Co., Ltd., Seren Pharmaceuticals Inc., Bonac Corp.); Stock: NU (Merck); Honoraria: TI (Mochida, Bayer, Boehringer Ingelheim), KN (Daiichi Sankyo, Merck, Phizer, Eli Lilly, Astellas Pharma, Boehringer Ingelheim, Mitsubishi Tanabe Pharma); Research funding: NU (AMED, Fukuoka Prefecture), KN (Bayer, TEIJIN PHARMA, Mitsubishi Tanabe Pharma); Scholarship or donation: KN (Astellas Pharma, Daiichi Sankyo,Sumitomo Dainippon Pharma, Takeda, Mitsubishi Tanabe Pharma, Boehringer Ingelheim). The remaining authors declare that they have no conflict of interest.

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