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Serum uric acid correlates with extracellular superoxide dismutase activity in patients with chronic heart failure

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Abstract

Increased serum uric acid has been identified as an independent risk factor for cardiovascular disease. However, because of its antioxidant capacity, uric acid may play a beneficial role in endothelial function. This paradoxical relationship between uric acid and endothelial function in chronic heart failure patients remains poorly understood. Thirty-eight chronic heart failure patients (New York Heart Association functional class II–III, mean age 58±10 years and mean left ventricular ejection fraction 25±8%) and twelve age-and-sex-matched healthy controls were studied. Chronic heart failure patients showed higher uric acid levels (7.3±2.3 mg/dL vs. 6.1±0.2 mg/dL, $p<0.05$) and lower extracellular superoxide dismutase activity (136±36 U ml^{-1} min^{-1} vs. 203±61 U ml^{-1} min^{-1}, $p<0.01$) and endothelium-dependent vasodilatation (4.0±1.6% v. 9.1±3.0%, $p<0.01$) when compared with control subjects. In chronic heart failure patients, correlations between both uric acid levels and extracellular superoxide dismutase activity ($r=0.45$; $p<0.01$), and uric acid and endothelium-dependent vasodilatation ($r=0.35$; $p=0.03$) were detected. These correlations were not observed in healthy individuals, suggesting a positive effect of uric acid on endothelial function partially mediated by modulation of extracellular superoxide dismutase activity in chronic heart failure.

Keywords: Extracellular superoxide dismutase; Uric acid; Endothelial function; Chronic heart failure; Oxidative stress

1. Introduction

Uric acid is the final product of purine metabolism in humans [1]. Epidemiological studies have identified a strong correlation between elevated uric acid levels and elevated cardiovascular risk in the general population [2] and high-risk groups, including chronic heart failure (CHF) [3,4]. However, the physiological role of uric acid in cardiovascular disease is poorly understood. In fact, systemic administration of uric acid increases plasma antioxidant capacity at rest [5], reduces exercise-associated oxidative stress in healthy subjects [6] and restores endothelium-dependent, nitric oxide-mediated vasodilation (FDD) in patients with type 1 diabetes and regular smokers [7]. This observation suggest that uric acid could play a role in preserving endothelial function, both in physiological and pathological states [8]. This apparently paradoxical effect could be understood if

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increased uric acid levels such as those present in CHF are interpreted as a coincidental finding rather than a causal risk factor, as has been proposed by some authors [9].

Superoxide dismutase (SOD) represents the major antioxidant defence system against superoxide anions (O_2^-). Extracellular SOD (ecSOD) is secreted and bound to heparan sulfate on the cellular surface [10], accounting for more than 70% of total SOD activity in human vessels [11]. Several studies have shown that the inhibition of ecSOD activity results in a rapid impairment of FDD [12,13]. In patients with CHF, ecSOD activity is dramatically reduced when compared with healthy controls [14,15], contributing to decreased vascular nitric oxide availability and impaired FDD observed in these patients [15,16]. Recent data have shown that uric acid could improve ecSOD activity, avoiding inactivation of ecSOD by hydrogen peroxide at concentrations close to physiological levels in humans [17]. Considering this separate experimental evidence, we hypothesized that increased serum uric acid is an adaptive response to the increased oxidative stress present in CHF patients; leading to a raise in ecSOD activity and thus preserving endothelial function. The aim of this observational study was to evaluate a previously unaddressed question supporting this hypothesis: whether there is a measurable relationship between uric acid levels, ecSOD activity and FDD in CHF patients.

2. Methods

2.1. Study group

We included thirty-eight CHF patients, NYHA functional class II–III attending our university clinical centre between November 2004 and March 2007. Inclusion criteria were: a) left ventricular ejection fraction (LVEF) <40%, measured by radionuclide ventriculography or echocardiogram, b) conventional pharmacological treatment including diuretics, β-blockers, digoxin and angiotensin-converting enzyme inhibitors, c) stable clinical condition during the last four weeks, d) presence of endothelial dysfunction expressed as FDD<8% [18].

Exclusion criteria were: a) acute coronary syndrome in the last six months, b) coronary artery bypass surgery or coronary angioplasty in the last six months, c) uncontrolled arterial hypertension (systolic blood pressure >160 mm Hg or diastolic blood pressure >90 mm Hg), d) hypertrophic cardiomyopathy or congenital cardiopathy, e) use of antioxidants, allopurinol or statins in the previous two months, f) presence of other conditions that affect determination of oxidative stress status, such as renal failure (plasma creatinine >2.0 mg/dL), autoimmune diseases, neoplasia, advanced liver or pulmonary disease, and acute or chronic inflammation.

A group of twelve healthy age-and-sex-matched volunteers were also included as controls. Subjects were ineligible as controls if they were taking any medications, vitamin supplements, antioxidants or if they drank alcohol on a regular basis.

All patients gave written informed consent and the study was approved by our institutional Review Board and Ethics Committee.

2.2. Clinical laboratory measurements

Serum levels of uric acid and creatinine and lipid profile were determined using the standard routine methods at our institution.

For ecSOD measurement at baseline, a venous blood sample was drawn from the antecubital vein of the non-dominant arm. Then, a heparin bolus (5,000 IU) was injected into the brachial artery of the same arm, and blood samples were drawn at fixed intervals from the antecubital vein (1, 3, 5, 7 and 10 min after heparin injection). Plasma SOD activity was measured by epinephrine autoxidation inhibition, as described elsewhere [19]. ecSOD activity was calculated as the area under the curve of plasma SOD activity, as described by Landmesser et al. [15].

2.3. Endothelial function assessment

High-resolution ultrasound was used to measure changes in brachial artery diameter, according to a previously described technique [18]. Ultrasound studies were performed in the morning, after the patients had rested in a supine position for 30 min in a quiet room. A 7.5-MHz linear array ultrasound probe was employed to perform longitudinal section scans of the brachial artery 5 to 3 cm above the elbow pleat. FDD was evaluated by placing a blood pressure cuff around the forearm and inflating it up to 300 mm Hg. After 5 min of arterial occlusion, the cuff was deflated. An increase in vessel diameter of less than 8% was considered evidence of endothelial dysfunction [18]. Endothelial-independent vasodilation was evaluated 10 min after cuff deflation, by measuring brachial artery diameter at baseline and 3 min after administration of isosorbide dinitrate 1.25 mg sublingual spray (Schwarz Pharma AG, Swiss).

2.4. Statistical analysis

Results are presented as mean±SD for continuous variables and percentages for categorical data. Continuous variables were tested for normality using the Kolmogorov–Smirnov test. Baseline data were compared with an age-matched cohort of healthy volunteers using unpaired Student *t* test for continuous data and Fisher's exact test for categorical data. Significant correlations between continuous variables were evaluated using Pearson's method. A $p<0.05$ was considered significant.

3. Results

3.1. Baseline characteristics

Thirty-eight compensated CHF patients and twelve age-and-sex-matched controls were included. Baseline characteristics

Table 1
Baseline characteristics

Parameter	CHF	Control	P
N	38	12	
Mean age, years	58±10	60±6	0.59
Male/female, *n* (%)	32/6 (82/18)	10/2 (83/17)	0.82
LVEF, %	25±8	–	–
NYHA class, *n* (%)			
II	15 (39)	–	–
III	23 (61)	–	–
Aetiology, *n* (%)			
Ischaemic	12 (32)	–	–
Non-ischaemic	26 (68)	–	–
Risk factors, *n* (%)			
Diabetes mellitus *n* (%)	6 (16)	–	–
Hypertension *n* (%)	23 (61)	–	–
Cholesterol, mg/dL (mmol/L)	188±35 (4.9±0.9)		0.85
LDL cholesterol, mg/dL (mmol/L)	107±32 (2.8±0.8)	107±2 (2.8±0.1)	0.99
HDL cholesterol, mg/dL (mmol/L)	44±8 (1.1±0.2)	41±1 (1.0±0.1)	0.12
Triglycerides, mg/dL (mmol/L)	182±36 (2.1±0.4)	181±4 (2.0±0.1)	0.06
Creatinine, mg/dL (μmol/L)	1.1±0.3 (97±27)	0.9±0.2 (80±18)	0.31
Uric acid, mg/dL (mmol/L)	7.3±2.3 (0.4±0.1)	6.1±0.2 (0.3±0.1)	0.04
ecSOD activity, U mL^{-1} min^{-1}	136±36	203±61	0.003
FDD, % change from baseline	4.0±1.6	9.1 ± 3.0	0.0008
Conventional treatment, *n* (%)			
ACE inhibitors	34 (89)	–	–
ß-blockers	30 (79)	–	–
Furosemide	29 (76)	–	–
Digoxin	21 (55)	–	–
Spironolactone	29 (76)	–	–
Hydralazine	6 (16)	–	–

Abbreviations: ACE, angiotensin-converting enzyme; ecSOD, extracellular superoxide dismutase; FDD, endothelial-dependent vasodilation; LVEF, left ventricular ejection fraction; *N*, number.

are detailed in Table 1. In the CHF group, mean age was 58±10 years and thirty-two patients (82%) were male. CHF aetiology was ischaemic in twelve cases (32%) and non-ischaemic dilated cardiomyopathy in twenty-six cases. Twenty-three patients (61%) had a history of hypertension, and six (16%) of diabetes mellitus. Mean LVEF was 25±8%.

Uric acid levels were higher in CHF patients when compared with healthy subjects (7.3±2.3 mg/dL vs. 6.1±0.2 mg/dL; $p=0.04$ vs. control). CHF patients showed a marked impairment in ecSOD activity compared to the control group (136±36 U mL^{-1} min^{-1} vs. 203±61 U mL^{-1} min^{-1}; $p<0.01$). As expected, FDD was impaired in the CHF cohort when compared with the control group (4.0±1.6% vs. 9.1±3.0%, $p<0.01$).

3.2. Correlation between uric acid and endothelial dysfunction

As shown in Fig. 1, a significant correlation was observed between serum uric acid levels and ecSOD activity ($r=0.45$; $p<0.01$). This correlation remained significant after testing for interaction with age, sex, systolic and diastolic blood pressure, creatinine, lipid profile and medical treatment. Higher ecSOD levels were associated with better FDD ($r=0.52$; $p<0.01$, Fig. 2A). Interestingly, higher uric acid

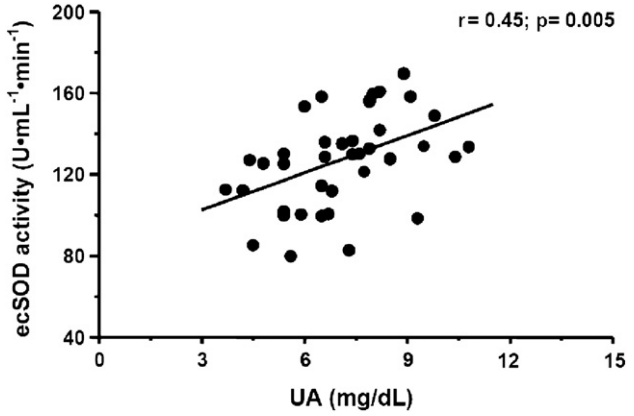

Fig. 1. Correlation between serum uric acid (UA) levels and extracellular superoxide dismutase (ecSOD) activity in chronic heart failure patients. Correlation was evaluated using Pearson's method, $n=38$.

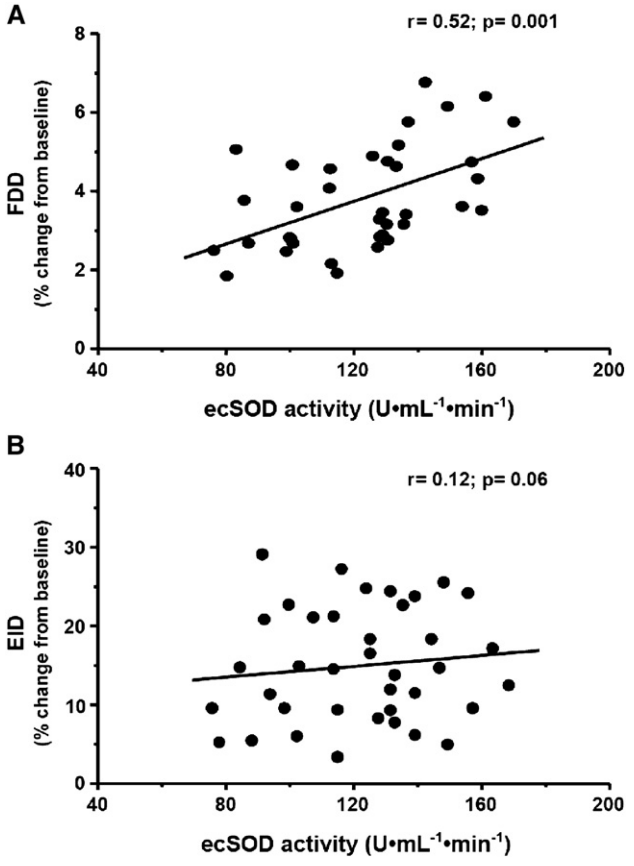

Fig. 2. Correlation between extracellular superoxide dismutase (ecSOD) activity and endothelial function in chronic heart failure patients. Panel A: endothelial-dependent vasodilatation (FDD). Panel B: endothelial-independent vasodilatation (EID). Correlations were evaluated using Pearson's method, $n=38$.

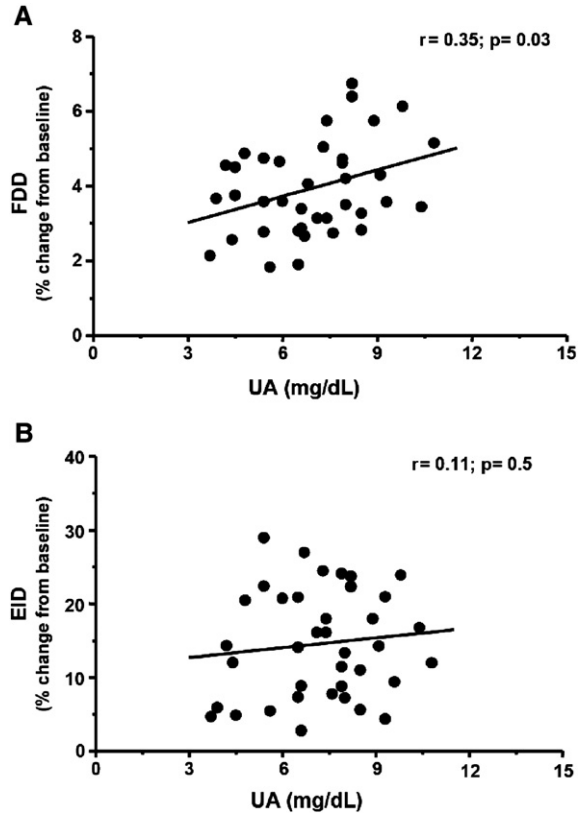

Fig. 3. Correlation between serum uric acid (UA) level and endothelial function in chronic heart failure patients. Panel A: endothelial-dependent vasodilatation (FDD). Panel B: endothelial-independent vasodilatation (EID). Correlations were evaluated using Pearson's method, $n=38$.

levels were associated with improved FDD ($r=0.35$; $p=0.03$, Fig. 3A), even after adjustment for the effect of uric acid on ecSOD levels ($r=0.45$, $p<0.01$). The correlations found between serum uric acid levels, ecSOD activity and FDD were maintained independently of NYHA class and LVEF.

4. Discussion

Endothelial dysfunction has been documented in peripheral and coronary arteries in patients with CHF [20]. It has recently been proposed that local oxidative stress in the endothelial milieu actively participates in the progression of endothelial dysfunction [12–15]. In our group of CHF patients we observed a marked decrease of ecSOD activity, a major antioxidant defence system of the endothelial wall, when compared with healthy controls. This finding closely correlates with the impaired FDD observed in these patients, as has been reported by other authors [15].

Epidemiological studies have identified a strong association between high serum uric acid levels and increased cardiovascular risk [21]. There is considerable debate on the significance of this relationship, and whether increased uric acid is a causal, compensatory, or coincidental risk factor in patients with CHF remains unclear [7,21,22]. A commonly held perception is that high uric acid plays a causal role in the development of cardiovascular disease, although mechanisms that might be involved have not been established. This view has been supported by the observation that the administration of allopurinol improves endothelium-dependent vascular responses in patients with CHF [23,24]. Specifically, however, inhibition of xanthine oxidase by allopurinol is likely to reduce ROS production, reducing the oxidative stress, independently of its effects on uric acid. In fact, administration of probenecid, which reduces serum uric acid levels, has no effect on endothelial function in CHF as described by George et al. [24]. Beneficial cardiovascular effects of allopurinol require careful interpretation and do not specifically address the question of a biological link between uric acid and mechanisms of endothelial dysfunction in CHF.

In this study, uric acid levels were within normal limits in the majority of the CHF patients, despite the use of loop diuretics. These findings are consistent with previously published data from other authors [23,24]. Of interest, ecSOD levels in both control and CHF patients in our group are several times smaller than previously published elsewhere[15]. Since ecSOD activity is measured as the change in plasma SOD activity after heparin bolus injection over a period of time, values largely depend on the method chosen for determination of plasma SOD activity, which can explain the observed divergence and limits direct comparison with data published by other groups.

Our results show a correlation between serum uric acid and FDD in patients with CHF. This observation can be explained by several mechanisms. Peroxynitrite, the main product between superoxide and nitric oxide reaction is a potentially harmful oxidant that provides a source of free radicals and may contribute to vascular dysfunction in CHF [12–15]. Uric acid is able to directly scavenge peroxynitrite, resulting in the formation of a stable nitric oxide donor *in vitro* [25]. However, whether this activity is relevant *in vivo* is not known. Recently, Hink et al. demonstrated that uric acid effectively prevents inactivation of ecSOD by hydrogen peroxide at concentrations close to physiological levels in humans [17]. These findings support the observation of a beneficial impact of uric acid on endothelial function as stated in our study. The seemingly paradoxical effect of allopurinol could be explained by its ability to reduce vascular oxidative stress through xanthine oxidase inhibition and not by urate reduction, as shown by George et al. [24]. In fact, medical therapy with both allopurinol and probenecid is expected to induce only a modest reduction in uric acid levels. Methods that directly reduce uric acid concentration, such as urate oxidase (rasburicase) should allow a clearer interpretation of the role of uric acid in CHF. However, clinical experience with rasburicase is scarce, precluding its use in this group of patients.

The salutary effect of uric acid on the cardiovascular system has been observed in large trials. In the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT), which included over 30,000 patients, the positive

effects of chlorthalidone-based antihypertensive treatment on cardiovascular outcomes gave rise to the theory that the reduction in renal excretion and accompanying increase in the serum level of the proven antioxidant uric acid, induced by diuretics, may contribute to the improvement in cardiovascular prognosis brought about by therapy with these agents in hypertensive patients, as proposed by Reyes et al. [1]. Whether this effect is related to an improved endothelial function in this group of patients remains unknown.

Finally, we hypothesize that during the early stages of CHF, endothelial dysfunction is triggered by ROS produced by NADPH oxidase and xanthine oxidase (12,16). This event could be responsible not only for the oxidative inactivation of ecSOD, but also for the increase in serum uric acid levels in CHF patients without renal dysfunction (12,15). However, in the later stages of CHF, the accumulation of serum uric acid could exert antioxidant effects on endothelial cells, protecting ecSOD from a ROS-dependent inactivation (5–7). Therefore, in the early stages of CHF increased serum uric acid levels, as an indicator of xanthine oxidase activation, could be used as a risk marker. However, in the later phases, uric acid could act *per se* as an antioxidant, having a protective action on ecSOD and improving endothelial function.

There are several limitations to these findings. This small observational study included mostly male subjects; however, in observational studies, the relationship between high uric acid levels and cardiovascular risk seems to be more apparent in females [26]. Furthermore, the inclusion criteria specified a FDD < 8% in order to be eligible to participate in the study. Even though this restriction was aimed to increase the sensitivity of the study by selecting a population with demonstrated endothelial dysfunction, it could impair the generalization of the results to a less severe CHF population.

To our knowledge, this is the first study to show a positive correlation between uric acid levels, FDD and ecSOD activity in CHF. Although these findings do not support a causal relationship between uric acid and endothelial function, they suggest that uric acid could be part of an adaptive response to the increased oxidative stress present in CHF. Further experimental trials should be conducted to clarify the real impact of uric acid in the physiology of cardiovascular disease.

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