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Interaction between uric acid and endothelial dysfunction predicts new onset of diabetes in hypertensive patients

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ABSTRACT

Background: Both uric acid and endothelial dysfunction are associated with new occurrence of type-2 diabetes but, at this moment, there is no evidence about a possible interaction between them.
We tested, in untreated hypertensive patients, without clinical evidence of vascular damage, the hypothesis that serum uric acid and endothelial dysfunction may interact in predicting new diabetes.
Methods: In 500 uncomplicated hypertensive non diabetic (ADA criteria) patients we evaluated endothelial function, by strain-gauge plethysmography, and uric acid.
Results: During the follow-up (median 87.1 months), there were 54 new cases of diabetes (1.8%/year). On univariate analysis, incident diabetes was inversely related with ACh-stimulated FBF (HR = 0.65, 95%CI = 0.52–0.82; $P<0.001$) and directly with serum CRP (HR = 1.22, 95%CI = 1.09–1.37; $P<0.001$), HOMA-index (HR = 1.20, 95%CI = 1.05–1.37; $P=0.007$), fasting insulin (HR = 1.05, 95%CI = 1.01–1.09; $P=0.006$) and age (HR = 1.03, 95%CI = 1.00–1.05; $P=0.014$). At multiple regression analysis, the interaction between ACh-stimulated FBF and uric acid resulted statistically significant. Similar results were observed for the interaction between FBF and CRP.
Conclusions: Our data clearly demonstrate that the coexistence of both hyperuricemia and reduced endothelium-dependent vasodilation increases the risk to develop new diabetes in hypertensive patients. In addition, mild-inflammation seems to be the mediator of the interaction between endothelial dysfunction and uric acid.

1. Introduction

Uric acid (UA), the final oxidation product of purine catabolism, has been associated with diabetes, hypertension, atherosclerotic disease and other clinical conditions [1–10]. Various mechanisms have been suggested to explain these associations, even if UA contribution to these clinical conditions remains still controversial. UA acts as a pro-oxidant and may be a marker of oxidative stress, but it may also have a therapeutic role as an antioxidant substance [11], sustaining the debate about its true role: are high serum UA levels a protective response to injury or a primary cause of disease?

Recognition of high serum UA as a risk factor for diabetes has been a matter of debate for a few decades, since hyperuricemia has been presumed to be a consequence of insulin resistance rather than its precursor [1,11]. However, recent findings suggest that UA could be related to the development of diabetes [10–12]. Serum UA has been shown to be associated with oxidative stress [11] and production of tumor necrosis factor-α [13], which are both related to the development of diabetes. In addition, a recent study in rats showed that fructose-induced hyperuricemia plays a pathogenic role in the metabolic syndrome [14]. These findings support high serum UA as a precursor of type-2 diabetes.

Physiologically, endothelial cells regulate some biological processes related to the vascular wall, such as the regulation of prothrombotic and antithrombotic factors, platelet aggregation, adhesion of leukocytes and monocytes, migration and proliferation of vascular smooth muscle cells [15–17], playing a critical role in vascular homeostasis. Thus, a dysfunctioning endothelium plays a key pathophysiologic role in the development and progression of atherosclerosis since it loses the ability to protect the vascular system by reducing its antiatherosclerotic and antithrombotic actions. On the other hand, previously published findings have demonstrated that endothelial dysfunction, in both coronary [18–21] and forearm [22] vasculature, is an independent prognostic factor for future clinical events.

Finally, we recently demonstrated that hyperuricemia is also associated with impaired endothelium-dependent vasodilation [23] and, clinically relevant, that endothelial dysfunction, in turn, is an independent predictor of new onset of type-2 diabetes in hypertensive

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patients [24]. Taken together, we designed this study to test, in a group of newly diagnosed hypertensive patients, without clinical evidence of vascular damage, the hypothesis that serum UA and endothelial dysfunction may interact in predicting new diabetes.

2. Materials and methods

2.1. Study population

A total of 500 uncomplicated hypertensive patients [systolic blood pressure (BP) ≥ 140 mm Hg and/or diastolic BP ≥ 90 mm Hg] participated in this study. All patients had newly diagnosed, never-treated essential hypertension without previous cardiovascular events or target organ damage investigated by: echocardiography to evaluate cardiac mass, high resolution B-mode ultrasound to measure intima-media thickness, or ankle brachial index to exclude peripheral atherosclerosis. None of the patients had a history of gout, was treated with allopurinol and had a history or clinical evidence of angina, myocardial infarction, valvular heart disease, diabetes hypercholesterolemia, peripheral vascular disease, coagulopathy, vasculitis or Raynaud's phenomenon. In addition, all patients had to be proteinuria-free on the dipstick test. According with the American Diabetes Association diagnostic criteria [25], all patients were non diabetic at the enrolment and did not take any drug known to affect glucose metabolism. All patients underwent simultaneous measurements of endothelial function, C-reactive protein (CRP), insulin resistance, and the full record of classical risk factors. Causes of secondary hypertension were excluded by appropriate investigations, including measurement of plasma renin activity and aldosterone, Doppler studies of the renal arteries, and/or renal scintigraphy or renal angiography.

New cases of diabetes were confirmed on the basis of the following criteria: 1) presence of more than one classic symptom of hyperglycemia plus either a fasting plasma glucose ≥7.0 mmol/l or random plasma glucose ≥11.1 mmol/l, 2) two or more elevated plasma glucose concentrations (fasting plasma glucose ≥7.0 mmol/l, random plasma glucose ≥11.1 mmol/l, or 2-h plasma glucose ≥11.1 mmol/l during oral glucose tolerance testing), and 3) use of an oral hypoglycemic drug or insulin. Follow-up included periodic control visits in the outpatient clinic for most patients. To improve long-term follow-up, a questionnaire was also mailed to family physicians, and patients were contacted by phone every 4 months. Vascular function assessments were performed at the first observation. The local ethics committee approved the study. All participants gave written informed consent for all procedures and use of all data for successive evaluations.

2.2. Renal function

Creatinine measurements were carried out at baseline and at the end of follow up. Values of e-GFR (ml/min/1.73 m^2) were calculated by using the new equation proposed by investigators in the chronic kidney disease epidemiology (CKD-EPI) collaboration. This equation was developed from a much larger cohort of patients, including both normal and CKD individuals, than the MDRD study. We preferred this equation because it is more accurate in subjects with GFR > 60 ml/min/1.73 m^2, as our patients were supposed to have considering the creatinine value <1.5 mg/dl [26].

2.3. Forearm blood flow measurement

All studies were performed at 9:00 AM after overnight fasting, with the subjects lying supine in a quiet, air-conditioned room (22–24 °C). The subjects were instructed to continue their regular diet, and were asked to refrain from alcohol and smoking for 24 h before the test. Forearm volume was determined by water displacement. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter (Vasculon 2, Baxter Healthcare Corp., Deerfield, Illinois) was inserted into the brachial artery of the non-dominant arm of each subject for evaluation of BP (Baxter Healthcare Corp.) and for drug infusion. This arm was slightly elevated above the right atrium, and a mercury-filled silastic strain-gauge was placed on the widest part of the forearm. The strain-gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson, Issaquah, Washington) calibrated to measure the percent change in volume; this was connected to a chart recorder to obtain the forearm blood flow (FBF) measurements. A cuff placed on the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E-10, D.E. Hokanson) to exclude venous outflow from the extremity. The antecubital vein of the opposite arm was cannulated. The FBF was measured as the slope of the change in the forearm volume; the mean of at least three measurements was obtained at each time point.

2.4. Vascular function

We used the protocol described by Panza [27] and subsequently employed by us [22–24]. We measured FBF and BP during intra-arterial infusion of saline, acetylcholine (ACh), and sodium nitroprusside (SNP) at increasing doses. All participants rested 30 min after artery cannulation to reach a stable baseline before data collection; measurements of FBF and vascular resistance (VR), expressed in units (U), were repeated every 5 min until stable. Endothelium-dependent and -independent vasodilation was assessed by a dose–response curve to intra-arterial ACh infusions (7.5, 15, and 30 $\mu g \cdot ml^{-1} \cdot min^{-1}$, each for 5 min) and SNP infusions (0.8, 1.6, and 3.2 $\mu g \cdot ml^{-1} \cdot min^{-1}$, each for 5 min), respectively. The sequence of administration of ACh and SNP was randomized to avoid any bias related to the order of drug infusion. ACh (Sigma, Milan, Italy) was diluted with saline immediately before infusion. SNP (Malesci, Florence, Italy) was diluted in 5% glucose solution immediately before each infusion and protected from light with aluminum foil.

2.5. Laboratory methods

Serum creatinine and UA were measured in the routine laboratory by an automated technique based on the measurement of Jaffe chromogen and by the URICASE/POD (Boehringer Mannheim, Mannheim, Germany) method implemented in an autoanalyzer. CRP was measured by a high-sensitivity turbidimetric immunoassay (Behring, Marburg, Germany). Insulin resistance was estimated by the homeostasis model assessment (HOMA-index) from the fasting glucose and insulin concentrations according to the equation HOMA = [insulin (μU/ml × glucose (mmol/l)]/22.5 [28].

2.6. Statistical analysis

Data are expressed as mean ± SD or as percent frequency, and comparisons between groups were made by 1-way ANOVA, Student's t test or the χ^2 test, as appropriate. Events rate is reported as the number of events per 100 patient-year based on the ratio of the number of events observed to the total number of patient-years of exposure up to the terminating event or censor. For the patients without events, the date of censor was that of the last contact with the patient.

The association between UA and incidence risk of diabetes was analyzed by univariate and multiple Cox regression analyses. Tested covariates included UA, maximal vasodilatory response to ACh as well as traditional [age, sex, smoking, serum cholesterol, systolic BP, body mass index (BMI)] and emerging cardiovascular risk factors (HOMA-index and serum CRP). The multiple Cox regression model was constructed by including all variables that resulted to be associated with the incident risk of diabetes ($P<0.05$) at univariate Cox regression analysis. By this strategy we constructed a Cox model of adequate statistical power (at least 10 events for each variable into the final model). Data are expressed as hazard ratio (HR), 95% confidence interval (CI) and P value.

The analysis of the effect modification of endothelial function (maximal vasodilatory response to ACh) on the hazard ratio of UA (1 mg/dl of increase) or CRP (1 mg/dl of increase) on the incidence rate of diabetes was investigated as suggested by Altman [29], by simultaneously including uric acid, ACh and ACh*UA into the same multivariate model 1, and CRP, ACh and ACh*CRP into model 2. The hazard ratio of UA and CRP across a series of pre-defined values of maximal vasodilatory response to ACh was calculated by the standard linear combination method.

In 2-tailed tests, a value of $P<0.05$ was considered statistically significant. All comparisons were performed using the statistical package SPSS 10.0 for Windows.

3. Results

In the study population, mean baseline serum UA was 5.03 ± 1.63 mg/dl, with a range of 2.10 to 8.50 mg/dl. Mean baseline fasting glucose and insulin were 95 ± 11 (range 67 to 112 mg/dl) and 14.1 ± 6.4 U/l (range 2 to 36 U/l), respectively. Baseline demographic and clinical characteristics of patients who progressed toward type 2 diabetes (progressors) and those remaining free of type 2 diabetes (non-progressors) are reported in Table 1. There were no statistically significant differences between groups in age, gender, BMI, smoking habit, lipid profile, fasting glucose, systolic and diastolic BP, or basal FBF. On the contrary, progressors were older and had higher baseline fasting insulin, HOMA-index and CRP mean values. Similarly, mean serum UA was significantly higher in progressors than in the control group (5.1 ± 1.8 vs 4.9 ± 1.5 mg/dl; $P<0.001$) (Table 1). In addition, the highest response in ACh-stimulated FBF was significantly lower in progressors compared with that in non-progressors (218 ± 143 vs 313 ± 181%; $P<0.0001$) (Table 1); in contrast, no significant differences were observed in maximal vasodilation induced by sodium nitroprusside (307 ± 118 vs 318 ± 109%; $P=0.488$). At the first eligibility visit, none of the patients had been treated with antihypertensive or other medications known to interfere with insulin sensitivity or UA metabolism. Baseline BP values were 149/91 ± 17/12 mm Hg and did not differ between groups. All patients were treated to reduce clinical BP ≤ 140/90 mm Hg using standard lifestyle and pharmacological treatment. Diuretics, ß-blockers, ACE inhibitors, calcium channel blockers, angiotensin II receptor antagonists, and α1-blockers were used alone or in various associations without significant differences among groups (Table 1). During the follow-up (median 87.1 months,