Miran A. Jaffa,[1] Deirdre Luttrell,[2] Alvin H. Schmaier,[3] Richard L. Klein,[2,4] Maria Lopes-Virella,[2] Louis M. Luttrell,[2] Ayad A. Jaffa,[2,5] and the DCCT/EDIC Research Group

Plasma Prekallikrein Is Associated With Carotid Intima-Media Thickness in Type 1 Diabetes

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COMPLICATIONS

The hypothesis that plasma prekallikrein (PK) is a risk factor for the development of vascular complications was assessed in a study using the Diabetes Control and Complications Trial (DCCT)/Epidemiology and Diabetes Interventions and Complications (EDIC) cohort of subjects with type 1 diabetes. The circulating levels of plasma PK activity were measured in the plasma of 636 subjects with type 1 diabetes (EDIC years 3–5). Common and internal carotid intima-media thickness (IMT) were measured by B-mode ultrasonography in EDIC years 1 and 6. Plasma PK levels were positively and significantly associated with BMI, hemoglobin A_{1c}, systolic blood pressure, total cholesterol, LDL cholesterol, and triglycerides but not with age, sex, duration of diabetes, or HDL cholesterol. Univariate and multivariable statistical models after controlling for other risk factors consistently demonstrated a positive association between plasma PK and progression of internal carotid IMT. Multivariate analysis using a general linear model showed plasma PK to be significantly associated with progression of both internal and combined IMT (Wilks Λ *P* value of 0.005). In addition, the mean internal carotid IMT levels were higher in subjects with plasma PK levels in the highest 10th percentile compared with subjects with plasma PK levels in the lower 10th percentile (*P* = 0.048). These novel findings implicate plasma PK as a risk factor for vascular disease in type 1 diabetes.

Atherosclerosis is a leading cause of morbidity and mortality in diabetes, but the accelerated vascular pathology associated with diabetes is not fully explained by the coexistence of traditional cardiovascular risk factors such as hypertension, hyperlipidemia, smoking, and a positive family history of cardiovascular disease. Early atherosclerotic lesions are characterized by endothelial dysfunction, accumulation of inflammatory cells, vascular smooth muscle cell (VSMC) proliferation and migration, and extracellular matrix deposition in the vessel wall (1,2).

The localization of all the components of the kallikrein-kinin system (KKS) within the vessel wall suggests a role for this system in the regulation of ultrastructure and vascular tone (3–7). Moreover, plasma prekallikrein (PK) has been implicated as a modulator of diabetic microvascular complications (nephropathy and retinopathy), with higher plasma PK activity associated with higher blood pressure and greater albumin excretion rates in patients with type 1 diabetes (8,9). Additionally, plasma PK has a role in vascular remodeling by promoting growth of vascular smooth muscle cells through transactivation of epidermal growth factor receptors (10). Finally, *Klkb1*$^{-/-}$ mice (PK deficient) have delayed arterial thrombosis by increasing protective vascular transcription factors Sirt1 and Kruppel-like factor 4 to reduce vessel wall tissue factor and inflammation, a forerunner of vessel atherothrombosis (11).

The contribution of plasma PK to vascular disease in subjects with diabetes has been incompletely explored. Increased circulating levels of KKS components in subjects at risk for vascular disease would provide evidence for heightened system activity and support their potential role in vascular disease. In the current study, we evaluated whether elevated plasma PK levels are associated with vascular disease in the Diabetes Control and Complications

[1]Epidemiology and Population Health Department, Faculty of Health Sciences, American University of Beirut, Beirut, Lebanon
[2]Department of Medicine, Medical University of South Carolina, Charleston, SC
[3]Department of Medicine, Case Western Reserve University, Cleveland, OH
[4]Research Service, Ralph H. Johnson Department of Veterans Affairs Medical Center, Charleston, SC
[5]Department of Biochemistry and Molecular Genetics, Faculty of Medicine, American University of Beirut, Beirut, Lebanon

Corresponding author: Ayad A. Jaffa, aj24@aub.edu.lb.

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Trial (DCCT)/Epidemiology and Diabetes Interventions and Complications (EDIC) cohort of subjects with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Study Population

The study was conducted on a subset of 636 subjects from the DCCT/EDIC cohort. The DCCT cohort included 1,441 subjects and consisted of men and women between the ages of 13 and 40 years with 1–15 years of diabetes at study entry (12). The patients enrolled in the DCCT between 1983 and 1989, and the randomized clinical trial ran for ~6.5 years. Half of the subject population was randomly assigned to conventional diabetes treatment, and the other half was assigned to intensive diabetes treatment. In 1993, the DCCT was stopped 1 year ahead of its scheduled end, when intensive treatment was clearly shown to reduce the risks of retinopathy, nephropathy, and neuropathy (13). The subjects were invited to enroll in EDIC, a multicenter longitudinal observational study of the development of macrovascular complications and further progression of microvascular complications (14). The DCCT and EDIC were approved by all institutional review board of all participating DCCT/EDIC centers, and all participants provided written informed consent.

Assessment of Carotid Intima-Media Thickness

Carotid intima-media thickness (IMT) (common and internal) was measured by B-mode ultrasound 1–2 years after the start of EDIC (year 1) and repeated 5 years later (EDIC year 6) as previously described in detail (15).

Assessment of Components of the KKS

Plasma PK, factor XII coagulant (FXII), and high–molecular weight kininogen (HK) coagulant were measured as previously reported (8). KKS components were measured in 636 subjects (EDIC years 3–5) out of a total of 905 EDIC subjects who participated in our ancillary longitudinal study. They were selected sequentially as they appeared at study sites for scheduled visits. The clinical characteristics of the subjects in whom KKS components were measured were compared with those of the remaining EDIC subjects. No differences in age, sex, hemoglobin A_{1c} (HbA_{1c}), systolic blood pressure (SBP), DCCT treatment group, duration of diabetes, or BMI were observed between the two groups (8).

Assessment of Coagulation/Fibrinolysis Factors

Plasma concentrations of fibrinogen and plasminogen activator inhibitor (PAI)-1 activity levels were determined as previously described (16,17).

Statistical Analysis

Plasma PK was hypothesized to be associated with carotid IMT in patients with type 1 diabetes. For examination of this hypothesis, univariate and multivariable regression analysis as well as a general linear model multivariate analysis was utilized. Linear regression analyses with plasma PK as the dependent variable were initially performed to assess whether changes in the levels of plasma PK (upregulated or downregulated) are influenced by changes in the levels of cardiovascular risk factors such as HbA_{1c}, BMI, lipids, and blood pressure, in addition to associations with other components of the KKS (FXII, HK, and plasma tissue kallikrein). The association between plasma PK and progression of internal carotid IMT (year 6 − year 1) was assessed using multivariable regression adjusted for all covariates listed in Table 2. To assess the multivariate effect of plasma PK on carotid IMTs (internal and combined), the general linear model multivariate analysis was performed and the significance of the Wilks Λ P value pertaining to plasma PK was determined. The effect of each component of the KKS (FXII and tissue kallikrein) was evaluated in the model to determine whether it had a direct significant association with carotid IMTs or whether these covariates modified the relationship between plasma PK and carotid IMTs. Risk factors that had a P value ≤ 0.2 in the simple linear regression were included in the multivariable linear analysis in addition to other covariates that we considered of clinical relevance such as the components of the KKS, treatment, sex, age, lipoproteins, and blood pressure (Table 2).

RESULTS

Plasma PK Levels in DCCT/EDIC Cohort of Subjects With Type 1 Diabetes

The circulating levels of plasma PK were measured in 636 subjects with type 1 diabetes from plasma collected in years 1997–1999 (EDIC years 3–5). The PK levels were symmetrically distributed and ranged from 0.2 to 3.0 units/mL, with a mean value of 1.29 units/mL. The univariate analysis of PK with vascular disease risk factors in the cohort is shown in Table 1. The cross-sectional data showed a positive and significant association between PK levels and BMI, with HbA_{1c}, a marker of metabolic control, and with SBP. A positive and significant association was also observed between PK levels and total cholesterol, LDL cholesterol, and triglycerides. With respect to components of the KKS and coagulation factors, a positive and significant association was also detected with FXII, HK, fibrinogen, and PAI-1 activity (Table 1). No association was observed between PK and age, sex, duration of diabetes, ACE inhibitor use, DCCT treatment group, HDL cholesterol, or tissue kallikrein (Table 1).

Plasma PK and Carotid IMT

We evaluated whether PK levels were associated with subclinical macrovascular disease by examining the relationship between plasma PK activity and common, internal, and/or combined carotid IMT. The combined IMT was defined as the sum of the intima-media measurements of the common and internal carotid arteries (18). Plasma PK levels were positively and significantly associated with the internal ($P < 0.001$) and combined ($P = 0.011$) carotid IMT but not with the common ($P = 0.384$) carotid IMT (Table 1).

We next determined whether plasma PK levels were associated with progression of carotid IMT by evaluating the association of plasma PK with changes in carotid IMT

from EDIC year 1 to EDIC year 6. Variations in internal carotid IMT were assessed by fitting the model with the difference (Δ change) between internal carotid IMT measurements at year 6 and year 1 as the outcome of interest. The effect of plasma PK on the Δ change in IMT was determined using multivariable analysis adjusted for FXII, tissue kallikrein, duration of diabetes, age, sex, DCCT treatment group, SBP, log albumin excretion rate (AER), current smoking, BMI, HbA_{1c}, ACE inhibitors, total cholesterol, LDL cholesterol, triglycerides, fibrinogen, PAI-1 activity, and ultrasonography equipment used at EDIC year 6 (Table 2). Our results demonstrated a significant positive association between plasma PK on the Δ difference in internal IMT (year 6 − year 1), $P = 0.017$. Furthermore, the mean internal carotid IMT levels were highest in subjects with plasma PK levels in the upper 10th percentile (0.87 ± 0.07 mm) compared with subjects with plasma PK levels in the lower 10th percentile (0.72 ± 0.04 mm) ($P = 0.048$) (Fig. 1).

A multivariate regression model was implemented using a general linear model with internal IMT and combined year 6 both as multivariate outcomes and plasma PK as the key risk factor, with adjustment for combined IMT year 1 along with other covariates listed in Table 3. Specifically, the global association of plasma PK and progression of carotid IMT attained by accounting for year 1 IMT in the model were evaluated simultaneously using year 6 internal and combined IMT as multivariate outcomes. Here, plasma PK was shown to be significantly associated with both internal and combined carotid IMT concurrently with a global test effect of Wilks Λ P value of 0.005. Statistical tests between individual subject effects revealed a significant and positive association between plasma PK and internal and combined carotid IMT with P values of 0.001 and 0.042, respectively.

Table 1—Univariate linear regression with plasma PK as dependent variable and risk markers and clinical parameters as covariates

Variable	Estimate	SE	*P*
FXII (units/mL)	0.040	0.019	0.033
HK (units/mL)	0.118	0.022	0.001
Tissue kallikrein (ng/mL)	0.006	0.015	0.676
Duration of diabetes (years)	0.002	0.004	0.612
HbA_{1c} (%)	0.049	0.013	0.001
Age (years)	0.001	0.003	0.875
BMI (kg/m^2)	0.112	0.004	0.006
SBP (mmHg)	0.005	0.001	<0.001
Total cholesterol (mg/dL)	0.002	0.001	<0.001
LDL cholesterol (mg/dL)	0.002	0.001	<0.001
HDL cholesterol (mg/dL)	0.000	0.001	0.466
Triglycerides (mg/dL)	0.001	0.000	<0.001
ACE inhibitor	0.005	0.050	0.090
Sex (male)	0.005	0.034	0.879
DCCT treatment group	−0.001	0.033	0.965
Log AER	0.046	0.012	<0.001
Fibrinogen (mg/dL)	0.001	0.000	<0.001
PAI-1 activity (units/mL)	0.008	0.003	0.004
Internal IMT (mm)	0.111	0.035	0.001
Common IMT (mm)	0.011	0.012	0.384
Combined IMT (mm)	0.450	0.176	0.011

Table 2—Multiple linear regression with outcome Δ change in internal carotid IMT from EDIC year 1 to EDIC year 6 and risk markers and clinical parameters as covariates

Variable	Estimate	SE	*P*
Plasma PK (units/mL)	0.146	0.060	0.017
FXII (units/mL)	0.019	0.018	0.312
Tissue kallikrein (ng/mL)	0.004	0.015	0.796
Duration of diabetes (years)	0.004	0.005	0.415
Age (years)	0.006	0.004	0.094
Sex (male)	−0.022	0.049	0.655
DCCT treatment group	0.023	0.045	0.615
SBP (mmHg)	0.003	0.002	0.132
Log AER (mg/24 h)	0.029	0.023	0.220
Current smoker	0.013	0.065	0.846
BMI (kg/m^2)	0.003	0.006	0.578
HbA_{1c} (%)	0.009	0.018	0.622
ACE inhibitor	0.038	0.061	0.529
Total cholesterol (mg/dL)	0.000	0.002	0.871
LDL cholesterol (mg/dL)	0.000	0.002	0.898
Triglycerides (mg/dL)	0.001	0.001	0.240
Fibrinogen (mg/dL)	0.000	0.000	0.218
PAI-1 activity (units/mL)	0.007	0.005	0.213
Ultrasonography equipment used	0.001	0.001	0.428

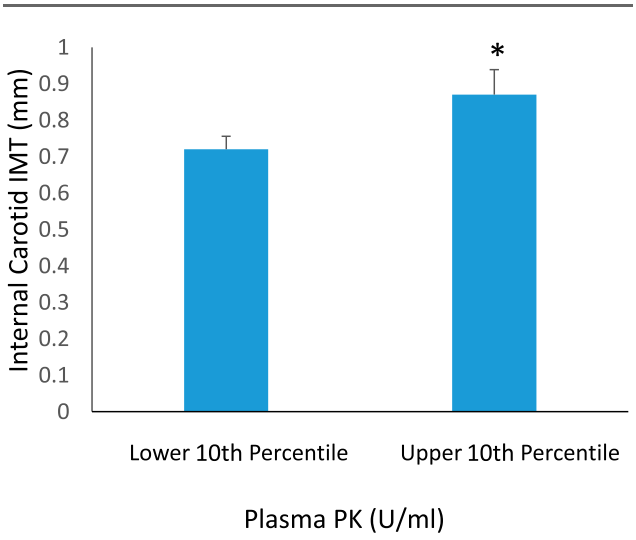

Figure 1—Internal carotid IMT (mean ± SE) stratified by plasma PK levels (lower 10th percentile and upper 10th percentile). *$P = 0.048$.

Table 3—Multivariate regression using general linear model with internal and combined carotid IMT for EDIC year 6 as multivariate outcomes and risk markers and clinical parameters as covariates

Variable	Wilks Λ *P* value
Plasma PK (units/mL)	0.005
FXII (units/mL)	0.727
Tissue kallikrein (ng/mL)	0.909
Duration of diabetes (years)	0.175
Age (years)	0.000
Sex (male)	0.403
DCCT treatment group	0.425
Current smoker	0.964
Hypertension#	0.603
Total cholesterol (mg/dL)	0.940
Triglycerides (mg/dL)	0.390
Combined IMT (year 1)	0.000
Ultrasonography equipment used	0.525

#Blood pressure ≥140/90 mmHg or antihypertension medications used.

DISCUSSION

Data in the current study demonstrate that circulating levels of plasma PK are associated with carotid IMT and its progression in subjects with type 1 diabetes. Progression of internal and combined carotid IMT is increased in subjects with higher levels of plasma PK, and this is consistently demonstrated in the univariate and multivariable analysis as well as in the multivariate model after controlling for other risk factors. The association of plasma PK with the combined carotid IMT is driven by the strength of the association between plasma PK and internal carotid IMT. These findings support plasma PK as an independent risk factor for cardiovascular complications in subjects with type 1 diabetes.

Plasma PK is predominately synthesized in the liver and secreted into the circulation where it circulates as a bimolecular complex bound to its substrate HK (19). Under physiological conditions, the assembly of the plasma PK-HK complex on the surfaces of endothelial cells is facilitated by the binding of domains 3, 4, and 5 of HK to a multiprotein receptor complex that consists of cytokeratin 1, the receptor for globular head of the C1q, and the urokinase plasminogen activator receptor (20–22). Endothelial cell prolylcarboxypeptidase, which is bound to the complex, activates plasma PK to activate kallikrein, which in turn cleaves HK to release bradykinin (BK). The generated BK acts on its B2 receptors in an autocrine and/or paracrine manner to initiate a multitude of cellular signals that influence vascular structure and tone (23).

However, when endothelial injury and dysfunction occur under pathologic conditions such as atherosclerosis or diabetes, circulating plasma PK is in constant contact with VSMC leading to its direct activation by VSMC through a novel yet unidentified putative plasma PK activator (24). Once activated on the surface of exposed VSMC, plasma PK not only generates BK but also activates protease activated receptors 1 and 2, leading to transactivation of epidermal growth factor receptors, release of proinflammatory cytokines, and proliferation of VSMCs that comprise the main cellular component in atherosclerotic lesions, contributing to the thickness of the intima (10). Moreover, our results demonstrate that plasma PK positively associates with fibrinogen and PAI-1 activity, factors that promote thrombogenesis and increase vascular disease risk. In this regard, it is important to point out that *Klkb1*$^{-/-}$ mice are protected against thrombosis by both *1*) reduced contact activation and *2*) reduced vessel wall tissue factor (11).

Our findings point to a role for plasma PK as an independent risk factor in vascular disease, but they do not demonstrate whether the increase in the levels of plasma PK that we observed are the result of or the root of vascular disease. For investigation of a causal role for plasma PK in vascular disease, longitudinal studies should focus on determining whether prior increases in the levels of plasma PK are predictive of future increases in measures of subclinical vascular disease as well as developments of future cardiovascular event rates in type 1 diabetes. Furthermore, the human population studies should be translated into animal models for type 1 diabetes that will evaluate the role of plasma PK as a pathogenic risk factor for vascular disease progression by using plasma PK inhibitors as a therapeutic approach to ameliorate vascular disease.

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