Journal of Diabetes and Its Complications 28 (2014) 353–359

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Clinical correlates of serum pigment epithelium-derived factor in type 2 diabetes patients

Alicia J. Jenkins [a,b,1], Dongxu Fu [a,1], Madona Azar [c,1], Julie A. Stoner [d], Derrick G. Kaufman [e], Sarah Zhang [c,f], Richard L. Klein [g], Maria F. Lopes-Virella [g], Jian-xing Ma [h], Timothy J. Lyons [a,c,*] VADT investigators

[a] Centre for Experimental Medicine, Queen's University of Belfast, Belfast, N. Ireland
[b] University of Sydney, NHMRC Clinical Trials Centre, Camperdown, Sydney, NSW, Australia
[c] Section of Endocrinology and Diabetes, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA
[d] College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA
[e] Hines VA Cooperative Studies Program (CSP) Coordinating Center, Edward Hines Jr. VA Hospital, Hines, IL, USA
[f] Ross Eye Institute, Department of Ophthalmology, State University of New York at Buffalo, Buffalo, NY, USA
[g] Division of Endocrinology, Medical University of South Carolina, Charleston, SC, USA
[h] Department of Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

ARTICLE INFO

Article history:
Received 24 May 2013
Received in revised form 31 December 2013
Accepted 9 January 2014
Available online 17 January 2014

Keywords:
PEDF
Type 2 diabetes
Cardio-vascular risk factors

ABSTRACT

Aim: To determine if serum pigment epithelium-derived factor (PEDF) levels in Type 2 diabetes are related to vascular risk factors and renal function.
Methods: PEDF was quantified by ELISA in a cross-sectional study of 857 male Veterans Affairs Diabetes Trial (VADT) subjects, and associations with cardiovascular risk factors and renal function were determined. In a subset ($n = 246$) in whom serum was obtained early in the VADT (2.0 ± 0.3 years post-randomization), PEDF was related to longitudinal changes in renal function over 3.1 years.
Results: Cross-sectional study: In multivariate regression models, PEDF was positively associated with serum triglycerides, waist-to-hip ratio, serum creatinine, use of ACE inhibitors or angiotensin receptor blockers, and use of lipid-lowering agents; it was negatively associated with HDL-C (all $p < 0.05$).
Longitudinal study: PEDF was not associated with changes in renal function over 3.1 years ($p > 0.09$).
Conclusions: Serum PEDF in Type 2 diabetic men was cross-sectionally associated with dyslipidemia, body habitus, use of common drugs for blood pressure and dyslipidemia, and indices of renal function; however, PEDF was not associated with renal decline over 3.1 years.

1. Introduction

Pigment epithelium-derived factor (PEDF), an adipokine, is a secreted glycoprotein belonging to the superfamily of serine protease inhibitors (serpins). Although first described in the eye (Filleur et al., 2009), the major sources of circulating PEDF are thought to be liver and adipose tissue (Famulla et al., 2011). PEDF has potent anti-angiogenic, anti-inflammatory, anti-oxidant, and neuroprotective properties (Yamagishi & Matsui, 2010; Yamagishi et al., 2010), and has been associated with insulin resistance (Crowe et al., 2009; Gattu et al., 2012; Nakamura et al., 2010; Sabater et al., 2010), diabetes mellitus, and diabetic vascular complications, including nephropathy (Jenkins et al., 2007; Tombran-Tink & Barnstable, 2003; Wang et al., 2005; Yamagishi et al., 2008). PEDF has been shown to inhibit the secretion of angiogenic and pro-fibrotic factors (Wang et al., 2005), and to suppress vascular endothelial cell proliferation (Dawson et al., 1999), microvascular cell apoptosis (Ishibashi et al., 2013) and renal fibrosis (Mao et al., 2011). In relatively small cross-sectional studies, we previously reported elevated serum PEDF levels in Type 2 diabetic vs. non-diabetic subjects (Jenkins et al., 2008), and in Type 1 diabetic subjects with vs. without microvascular complications (Jenkins et al., 2007). We found associations of PEDF with body mass index (BMI), lipid levels, and renal and vascular dysfunction (Jenkins et al., 2007, 2008). Altered levels of PEDF and growth factors such as TGFβ and VEGF have been associated with, and mechanistically implicated in, diabetic nephropathy (Wang et al., 2005), diabetic retinopathy (Mohan et al., 2012), and atherosclerosis (Tahara et al., 2011), and PEDF has been found to be independently associated with coronary

Authors have no relevant conflicts of interest.

* Corresponding author at: Centre for Experimental Medicine, Queen's University of Belfast, ICS-A, Grosvenor Road, Belfast, BT12 6BA, N. Ireland.
E-mail address: t.lyons@qub.ac.uk (T.J. Lyons).

[1] Equal contribution.

1056-8727/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jdiacomp.2014.01.008

artery disease (Wang et al., 2013). In animal and cell culture models of diabetic microvascular damage, PEDF has exhibited protective effects (Liu et al., 2012; Wang et al., 2006, 2008; Zhang et al., 2006, 2008; Zhu & Zou, 2012).

The Veterans Affairs Diabetes Trial (VADT) was a prospective, longitudinal study of 1791 subjects with Type 2 diabetes (T2DM), of whom 97% were male. Participants were randomized to receive either intensive or standard glycemic management, with the purpose of assessing the effect of intensive management on major cardiovascular disease (CVD) events (primary end-point) and microvascular complications (secondary end-points) (Duckworth et al., 2009). Six months after randomization, mean glycated hemoglobin (HbA1c) levels in the intensive and standard groups were 6.9% and 8.4% respectively (Duckworth et al., 2009). As intended, lipid and blood pressure levels, as defined by ADA-recommended targets (Abraira et al., 2003), were well controlled in both treatment groups. The study did not demonstrate any favorable effects of intensive glucose control on CVD events, neuropathy, or retinopathy. Intensive control was however associated with diminished progression of albuminuria (Duckworth et al., 2009), but despite efforts to manage hyperglycemia, hypertension, and dyslipidemia, renal function still declined in 8.8% of VADT participants during the 5-year trial (defined as doubling of serum creatinine level within the study time-frame), regardless of treatment assignment (Duckworth et al., 2009).

Identification of novel biomarkers and mechanisms implicated in diabetic microvascular damage may facilitate early identification and treatment of people at risk. In the present work, we studied subsets of the VADT cohort to assess the significance of serum PEDF levels, defining its cross-sectional associations with clinical factors, including CVD risk factors and renal function. In a smaller and more rigorously defined cohort, we also assessed whether PEDF is associated with subsequent decline in renal function. Renal function was assessed by serum creatinine (sCr), urine albumin-to-creatinine ratio (ACR), and estimated glomerular filtration rate (eGFR).

1.1. Subjects

The VADT was conducted according to the principles of the Declaration of Helsinki and was approved by Human Ethics Committees at all participating institutions. Each participant gave written informed consent. Our sub-study received Ethics Committee approval at the Medical University of South Carolina and the University of Oklahoma Health Sciences Center. Details of design and clinical and routine biochemical procedures of VADT have been reported previously (Abraira et al., 2003; Duckworth et al., 2009). In brief, participants with T2DM ($n = 1791$, of whom 1739 were male) were enrolled and randomized to intensive vs. standard glucose control between December 1, 2000 and May 30, 2003, and followed for five years. The treatment and initial follow-up period ended on May 30, 2008, and an additional observational follow-up is underway.

In our ancillary study of 857 VADT men ('Group A'), serum for quantification of PEDF was collected on a single occasion from each participant. PEDF values were analysed against clinical data based on the nearest annual study visit. For blood pressure and body mass index (BMI), the serum sample was matched to data from the nearest visit within six months. For medication effects, the serum data were matched to data from the nearest visit prior to sample collection to ensure that the data reflected actual medication exposure.

In order to standardize exposure to the VADT treatment assignment, a further subset of 246 men from Group A (referred as 'Group B') was chosen for a longitudinal analysis (and additional cross-sectional analysis). This subset included only those subjects in whom the PEDF sample was obtained during a narrow time frame centred on the two-year post-randomization visit (range 1.51 to 2.49 years post-randomization), and in whom complete PEDF, renal function, and covariate data were available. The sampling criteria for the longitudinal cohort were more restrictive to ensure that, by design, the study participants were standardized in terms of their exposure to the VADT treatment assignment when investigating the association between PEDF and subsequent renal function. Restriction was chosen as the preferred method to adjust for confounding and effect modification due to time on randomized therapy, which may impact both the PEDF measure and renal outcomes, as opposed to post-hoc statistical adjustment through regression modelling.

2. Materials and Methods

2.1. Biochemistry

HbA1c, serum lipid profiles, and renal function tests were performed by VADT laboratories as previously described (Abraira et al., 2003). Renal function was reflected by serum creatinine (sCr; μmol/L), urinary albumin to creatinine ratio (ACR; mg/g creatinine), and estimated glomerular filtration rate (eGFR; MDRD formula; mL/min/1.73 m^2). Renal dysfunction was defined as $sCr \geq 176.8$ μmol/L and/or $eGFR < 60$ ml/min/1.73 m^2. Categories of albuminuria were defined as 'no albuminuria' (ACR from 0–29 mg/g creatinine), microalbuminuria (30–300 mg/g creatinine), and macroalbuminuria (>300 mg/g creatinine).

Serum PEDF was quantified by ELISA (Chemicon Int., Inc., Temecula, CA) as previously described (Jenkins et al., 2008), with intra- and inter-assay coefficients of variation of 3.4% and 12.0% respectively. The mean of duplicate measures was used in data analyses.

2.2. Statistics

Data analysis was limited to male subjects, because the number of females in VADT was so low. Spearman's rank correlation coefficient was used to quantify the strength of the linear association between pairs of continuous measures. Linear regression modelling was used to investigate the association between PEDF levels (independent factor of interest) and renal outcome measures (dependent variables) with and without adjustment for age, T2DM duration, race/ethnicity, lipid-lowering therapy, VADT treatment assignment, hypertension, BMI, and waist-to-hip ratio (WHR). For the longitudinal analyses, a mixed effects modelling approach was used to account for the correlation among repeated annual renal function measures (outcome variable) for each subject. The regression models included a random intercept for each subject and utilized a linear link for continuous outcome measures and a cumulative logistic link for ordered categorical measures. The interaction between study time point and the PEDF measure was investigated to indicate whether the changes in renal function over the course of the follow-up, considered as repeated continuous or categorical measures, were significantly associated with the 2-year PEDF measure (Group B). Cox proportional hazards regression modelling was used to model the association between the hazard of renal disease progression, based on a categorical definition of a clinical disease progression event, and PEDF measures. Data were analysed using SAS (SAS System for Windows, ver. 9.1, SAS Institute Inc., Cary, NC) and statistical significance was defined as $p < 0.05$.

3. Results

3.1. Subject characteristics

Clinical characteristics at the time of serum collection for PEDF measurement are shown in Table 1: there were no significant differences between Groups A and B. As shown in Supplemental Table 1, compared to all non-participating VADT subjects ($n = 882$), Group A subjects were more likely to be non-Hispanic White, and to use angiotensin receptor blockers (ARB) and aspirin. They also had higher systolic blood pressures and lower LDL-cholesterol (LDL-C)