ELSEVIER

International Journal of Cardiology 100 (2005) 207–212

International Journal of Cardiology

www.elsevier.com/locate/ijcard

Soluble vascular endothelial growth factor, soluble VEGF receptor Flt-1 and endothelial function in healthy smokers

Caroline Schmidt-Lucke[a], Funmi Belgore[b], Dirk Reinhold[c], Siegfried Ansorge[d], Helmut U. Klein[a], J. André Schmidt-Lucke[a], Gregory Y.H. Lip[b,*]

[a]*Department of Internal Medicine, Division of Cardiology, Angiology and Pneumology, Otto-von-Guericke University, Magdeburg, Germany*
[b]*Haemostasis Thrombosis and Vascular Biology Unit, University Department of Medicine, City Hospital, Birmingham B18 7QH, UK*
[c]*Institute of Immunology, Otto-von-Guericke University, Magdeburg, Germany*
[d]*Institute of Experimental Internal Medicine, Otto-von-Guericke University, Magdeburg, Germany*

Received 21 January 2004; received in revised form 28 April 2004; accepted 5 May 2004
Available online 8 September 2004

Abstract

Objective: To relate levels of vascular endothelial growth factor (VEGF) and its soluble receptor, sFlt-1, with endothelial function in healthy smokers.
Methods: Plasma levels of VEGF and sFlt-1 were measured by ELISA in 22 healthy smokers and 22 matched healthy non-smoking controls, and compared to flow- (FMD) and acetylcholine-mediated (AMD) vasodilatation (endothelial-dependent) (EDV) and nitroglycerine-mediated (NMD) vasodilatation (endothelial-independent) of lower extremities were measured with plethysmography.
Results: Smokers and controls had similar plasma VEGF levels, but sFlt-1 levels were lower in smokers than in controls ($p<0.01$). AMD was lower in smokers compared to controls ($p<0.05$), but FMD and NMD levels were similar. Smokers and controls with high AMD (>12 ml/100 ml tissue/min) had significantly lower plasma VEGF levels ($p<0.001$). An inverse correlation was found in both groups, between VEGF and AMD (smokers: $r=-0.6$, $p<0.01$; controls: $r=-0.71$, $p<0.005$) and with FMD (smokers: $r=-0.56$, $p<0.05$; controls: $r=-0.58$, $p<0.005$). There were no significant correlations between sFlt-1 with VEGF levels or endothelial-dependent dilatation.
Conclusion: In conclusion, healthy smokers demonstrate abnormal AMD, and an inverse correlation between plasma VEGF levels (but not sFlt-1) with indices of endothelial dysfunction (FMD and AMD) exists. VEGF, and not sFlt-1, may be related to the pathogenesis of endothelial dysfunction in healthy smoking individuals.

Keywords: Endothelial dysfunction; Smoking; Vascular endothelial growth factor; Soluble Flt-1

1. Introduction

Vascular endothelial growth factor (VEGF) is a potent endothelium-specific secreted mitogen which regulates mitogenesis, vascular tone, permeability, and vasodilatation. VEGF interacts with the endothelium via two high-affinity membrane-spanning receptors, fms-like tyrosine kinase (Flt-1) and kinase insert domain-containing receptor (KDR) [1,2]. In contrast to KDR, a naturally occurring soluble form of the Flt-1 (sFlt-1) is produced by endothelial cells by differential splicing of the Flt-1 gene [3,4]. Soluble Flt-1 (sFlt-1) was initially isolated from human umbilical vessel endothelial cell culture media [5], and the presence of sFlt-1

Abbreviations: VEGF, vascular endothelial growth factor; FLT-1, fms-like tyrosine kinase, vascular endothelial growth factor receptor-1; KDR, kinase insert domain-containing receptor, vascular endothelial growth factor receptor-2; FMD, flow-mediated vasodilatation; AMD, acetylcholine-mediated vasodilatation; EDV, endothelial-dependent vasodilatation; NMD, nitroglycerine-mediated vasodilatation; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; NO, nitric oxide; S, smoker; C, control; VOP, venous occlusion plethysmography.

* Corresponding author. Tel.: +44 121 507 5080; fax: +44 121 554 4083.
E-mail address: G.Y.H.LIP@bham.ac.uk (G.Y.H. Lip).

0167-5273/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ijcard.2004.05.046

in serum samples significantly reduces the detectable levels of VEGF ELISA in a dose–response manner [6]. sFlt-1 has been shown to form heterodimers with membrane-bound Flt-1 and KDR, thus abolishing their signal transduction by acting as a dominant negative inhibitor in vitro [3]. Only sFlt-1 acts as a receptor antagonist, as KDR neither competed with the binding of VEGF to human endothelial cells nor blocked VEGF induced mitogenity [7]. In contrast, activation of KDR lead to up-regulation of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in porcine aortic endothelial cells, whilst stimulation of Flt-1 does not generate such a signal [6].

VEGF increases endothelial release of the vasodilator, nitric oxide (NO) by the activation of NO synthase. Elevated and/or abnormal VEGF levels are present in patients with atherosclerosis, proliferative retinopathy, hypertension and inflammatory diseases [8–10]. Healthy smokers have also been reported to have reduced circulating sFlt-1 when compared to non-smokers [11]. As smoking causes endothelial dysfunction, we hypothesised that VEGF and sFlt-1 could be related to endothelial function in healthy smokers, who were compared to matched healthy non-smoking controls.

2. Patients and methods

We studied 22 clinically healthy smokers, who were matched with 22 non-smoking volunteers (aged 20–59, mean 38.4±12 years). Informed consent was obtained from all volunteers, who were all free of symptoms and signs of vascular disease by careful history and examination, and were not taking any medication. Smoking was the only cardiovascular risk factor in both groups of volunteers. All subjects participated in mild to moderate leisure physical activity and females were examined on days 10–14 of their menstrual cycle. We investigated individuals from 20 to 60 years of age. Only individuals with a minimum of 6 pack-years (mean 21±19) and smoking at least 10 cigarettes per day in the last year were defined as smokers, whilst former smokers and individuals with passive exposure were not included [12]. Smokers over 32 had a minimum of 8 pack-years and those aged over 40 years at least 16 pack-years. The study was approved by the local institutional ethics board.

2.1. Measurements of vascular function

Normal perfusion was confirmed by routine tests (Doppler ankle–arm ratio at rest >0.9, normal resting mechanical oscillogramme) in all subjects in our vascular laboratory. Both studies were performed in the early afternoon. Measurements of vascular function were performed essentially as previously described [13]. In brief, calf blood flow was assessed by extent and course of post-occlusive reactive hyperaemia of resistance vessels, after 3 min hypersystolic occlusion, measured by a mercury-in-silastic strain gauge venous occlusion plethysmography (VOP) (Periquant 815, Gutman Electronics, Eurasburg, Germany). We obtained the following measurements at baseline: (i) flow-mediated dilation (FMD); (ii) acetylcholine-mediated dilation (AMD) (both as endothelium-dependent parameters), and (iii) nitroglycerine-mediated dilation (NMD), as an endothelium-independent parameter. The inter- and intra-observer variation of these measures in our laboratory was <10%.

After measuring baseline values, a 21-gauge catheter was placed in the right femoral artery using Seldinger's technique. Infusing 1–5 ml/min of saline prevented occlusion of the catheter and served as placebo. Acetylcholine (CIBAvision, Basel, Switzerland) was infused at 1.5 and 3 mg/min, whilst nitroglycerine was infused at infusion rates of 0.085 and 0.17 mg/min. All substances were given intra-arterially for 3 min, each prior, during and 1 min after arterial occlusion, and were administered on separate days double-blinded. A minimum of 20 min of rest was allowed between each measurement. Heart rate and blood pressure were recorded before and after each cycle. Blood samples were taken from the femoral vein with a 23-gauge needle at the end of each cycle.

2.2. Measurements of VEGF and sFlt-1

The sFlt-1 ELISA system is capable of detecting unbound sFlt-1 which is able to bind exogenous recombinant VEGF-165. This ELISA system has inter- and intra-coefficient of variation less than 10% and 5%, respectively [14]. Levels of VEGF were measured from citrate plasma with a commercially available ELISA (R&D Systems, Minneapolis, MN). Careful attention was taken to processing samples without delay within few minutes after collection and transporting on ice. Inter- and intra-assay coefficients of variation for all assays were <5% and <10%, respectively.

2.3. Statistics

Continuous variables were tested for normal distribution by Kolmogorov–Smirnov test. Values are presented as mean±SD or median and interquartile range (IQR)—as applicable; clinical data were compared between smokers and non-smokers by the unpaired Student's *t*-test or Mann–Whitney *U*-test, as appropriate. In the clinical study, data for sFlt-1 are non-parametrically distributed and are presented as median±IQR. Qualitative data were compared by the χ^2 test. Univariate and multivariate multiple regression analyses were performed to determine clinical predictors for our research indices. Statistical analysis was performed with SPSS 10.0®, and a $p<0.05$ was considered as statistically significant.

3. Results

The characteristics of both groups were comparable, as shown in Table 1.