Microvascular Research 76 (2008) 61–65

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Regular Article

Impaired flow-mediated vasodilation in type 2 diabetes: Lack of relation to microvascular dysfunction

M.F. Meyer [a,*], D. Lieps [b], H. Schatz [a], M. Pfohl [c]

[a] Department of Internal Medicine, University Clinic Bergmannsheil, Ruhr-University Bochum, Germany
[b] Department of Dermatology and Venereology, Vivantes Klinikum Neukölln, Academic Teaching Hospital of Charité University Medicine Berlin, Germany
[c] Department of Internal Medicine, Bethesda Hospital, Duisburg, Germany

ARTICLE INFO

Article history:
Received 5 December 2007
Revised 25 February 2008
Accepted 6 March 2008
Available online 20 March 2008

Keywords:
Capillary blood cell velocity
Endothelial dysfunction
Endothelium-dependent vasodilation
Endothelium-independent vasodilation
Flow-mediated dilation
Laser Doppler anemometry
Type 2 diabetes

ABSTRACT

A reduced availability of nitric oxide (NO) is an important feature of endothelial dysfunction occurring early in the course of type 2 diabetes. The measurement of flow-mediated dilation (FMD) of the brachial artery after forearm ischemia is supposed to be a non-invasive method to assess endothelial production and release of NO. The impairment of reactive hyperemia due to microvascular dysfunction in diabetes might cause an insufficient increase in shear stress stimulating the endothelial NO release, thus leading to an underestimation of FMD. Therefore, the aim of the present study was to investigate the relationship between microcirculatory disturbances and the impairment of FMD in type 2 diabetic patients. 63 type 2 diabetic patients and 44 non-diabetic control subjects were investigated. Capillary blood cell velocity (CBV) was assessed at the dorsal middle phalangeal area of the left ring finger. Lumen diameter of the brachial artery was measured by high-resolution ultrasound. Patients were investigated at rest and after 5-min suprasystolic arterial compression. Percentage change of CBV during reactive hyperemia (CBV%) and flow-mediated dilation (FMD%) of the brachial artery relative to the baseline measurement were calculated. CBV% (63.4±10.7% vs. 124.0±18.5%; $p<0.01$) and FMD% (3.8±0.8% vs. 6.9±0.9%; $p<0.01$) were reduced in the diabetic patients compared to their control subjects. FMD% was not related to CBV% ($r=0.14$; $p=0.139$). The lack of an association between the reduction of endothelium-dependent vasodilation of the brachial artery and the impairment of postocclusive microvascular hyperemia observed in the present study contradicts the assumption that a reduced FMD is only the consequence of an impaired reactive hyperemia due to microvascular dysfunction. It also lends support to the suggestion that endothelial dysfunction in conduit vessels and impaired cutaneous microvascular responses to reactive hyperemia might at least partly develop independently due to several differences in their pathogenesis.

Introduction

Endothelial dysfunction has been suggested to be an early event in diabetic macrovascular disease and precedes the development of morphologic vascular damage detectable by sonographic or angiographic examination (Lüscher and Barton, 1997). The dysfunction of the endothelium comprises disturbances of vasodilatory, anti-thrombogenic, anti-inflammatory, and anti-proliferative capacities (Loscalzo, 2001). These defects are mainly due to alterations in the release and/or the availability of nitric oxide (NO) which are often associated with reduced production of prostacyclin. Tests for endothelial dysfunction might identify presymptomatic diabetic patients at high risk of atherosclerotic complications. The measurement of flow-mediated dilation (FMD) of the brachial artery during reactive hyperemia is supposed to be a non-invasive method to assess endothelial dysfunction. In this test for the endothelium-dependent vasodilation, the increase in the mechanical force of blood flow on the vascular wall, referred to as shear stress, after release of a suprasystolic arterial occlusion of the forearm stimulates the endothelial release of NO that plays a role primarily in the maintenance and not in the initial phase of reactive hyperemia (Joannides et al., 1995).

It might be supposed that the impairment of the initial phase of reactive hyperemia due to disturbances in microvascular blood flow, also occurring early in the course of diabetes, causes an insufficient stimulus for the endothelium to release NO leading to an underestimation of FMD. Therefore, the first aim of the present study was to investigate the relationship between microcirculatory disturbances causing a reduced and delayed reactive hyperemia observed in the skin and the impairment of FMD in type 2 diabetic patients.

Later in the course of diabetes, the endothelium-independent vasodilation also declines due to a reduced NO sensitivity of the vascular smooth muscle cells and a decreased vasodilation capacity resulting from morphological vessel wall damage. To answer the

* Corresponding author. Department of Internal Medicine, University Clinic Bergmannsheil, Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany. Fax: +49 234 302 6415.
E-mail address: martin.meyer-2@rub.de (M.F. Meyer).

0026-2862/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.mvr.2008.03.001

question whether micro- and macrovascular disturbances develop independently in type 2 diabetes, the association between the impaired reactive hyperemia in microcirculation and the reduced endothelium-independent vasodilation in conduit arteries was also studied.

Materials and methods

Subjects

A total of 63 consecutive type 2 diabetic patients aged 48–83 years was recruited for this study from the Department of Internal Medicine at the University Clinic Bergmannsheil, Ruhr-University Bochum. Diabetes duration varied from 0 to 42 years. The clinical characteristics are presented in Table 1. Subjects suffering from diseases known to influence microcirculation of the skin such as atopic dermatitis, psoriasis, collagen-vascular diseases, primary systemic vasculitides, and Raynaud's syndrome were excluded. A separate group of 44 non-diabetic subjects (26 men, 18 women) with a mean age of 57.2±2.5 years and a body mass index of 25.7±0.6 kg/m^2 served as controls.

Assessment of capillary blood cell velocity

All subjects were asked to avoid consuming coffee, tea, and alcohol and to refrain from smoking and strenuous physical activity for 2 h before the measurement. All rings were removed from their fingers 1 day before the measurement. Antihypertensive medication was paused for at least 15 h before the investigation to minimize the interference of vasoactive drugs with the measurements. All subjects were examined between 8:00 and 10:00 a.m. to reduce the effects of circadian fluctuations. Before measurement, the subjects were acclimatized to the conditions of a temperature controlled room (21–24 °C) for a minimum of 20 min. No movement or conversation was allowed during the measurement. All unnecessary activity in the examination room was minimized and noise levels were kept low throughout this period. The study was approved by the clinical research ethics committee at the Ruhr-University Bochum. Written informed consent was obtained from each participant. The reported investigation has been performed in accordance with the principles of the Declaration of Helsinki.

Cutaneous CBV was measured at the dorsal middle phalangeal area of the left ring finger. The subjects were examined in a sitting position with the hand at the heart level. A drop of paraffin oil was placed onto the skin 10 min before each CBV measurement to make the skin transparent and to further reduce surface reflections. The liquid paraffin was at skin temperature to avoid any unwanted physiological disturbances to blood flow. The finger was illuminated by a 100 W halogen cold light source with a fiber-optic light guide (Euromex EK 1, Arnhem, The Netherlands). The light passed through a green filter to enhance the visualization of the capillaries. CBV was measured by means of the laser Doppler anemometer CAM 1 (KK Technology, Braeside, Axminster, Devon, UK; Lawrenz Medizinelektronik, Sulzbach, Germany). This capillaroscopy system includes a microscope objective lens and a CCD camera (Model XC-75CE, Sony, Tokyo, Japan) providing a ~220× magnified image of the column of red blood cells within the capillary on a monitor. CAM1 uses a 780 nm 7 mW near-infrared laser diode with a total laser output <1.5 mW. Actual sample depth depends on the tissue but is typically less than 100 µm. The laser beam was focussed by the microscope objective lens to a spot size of ~10 µm in diameter so that blood cell velocity could be measured in a single capillary. The velocity of the blood cells was directly proportional to the Doppler shift of the frequency of the laser beam which was reflected by blood cells moving perpendicular to the skin surface. Further technical details of the laser Doppler anemometer have been described previously (Meyer and Schatz, 1998). After recording resting CBV for at least 1 min, peak CBV was measured following release of a suprasystolic arterial occlusion for 5 min. The increase in CBV was expressed as percentage change related to the baseline measurement (CBV%).

Table 1
Clinical characteristics and therapy of the diabetic patients

	Type 2 diabetic patients (n=63)
Male:female ratio	37:26
Age (years)	65.3±1.0
Skin temperature (°C)	29.1±0.3
BMI (kg/m^2)	29.0±0.7
Essential hypertension (n)	53
Blood pressure (mm Hg)	
Systolic	147±3
Diastolic	79±1
Dyslipoproteinemia (n)	51
Smokers/ex-smokers (n)	10/20
Packyears (years)	17.7±3.6
Creatinine (mg/dl)	1.09±0.05
HbA_{1c} (%)	8.0±0.2
Plasma glucose (mg/dl)	179.7±8.5
Diabetes duration (years)	11.6±1.2
Diet (n)	12
Oral hypoglycaemic medication (n)	13
Conventional insulin therapy (n)	22
Intensified conventional insulin therapy (n)	16
Neuropathy (n)	42
Retinopathy (n)	19
Nephropathy (n)	26
Peripheral vascular disease (n)	7
Coronary artery disease (n)	29
Myocardial infarction (n)	15
Stroke (n)	2

Results expressed as number (n) or mean±SEM.
BMI, body mass index.

Measurement of flow-mediated dilatation of the brachial artery

After a recovery time of 20 min, endothelium-dependent vasodilation was measured using a technique introduced by Celermajer et al. (1992). Guidelines for this technique have been previously reported (Corretti et al., 2002). The diameter of the brachial artery was assessed using a high-resolution ultrasound device (Toshiba Corevision, SSA-350A, Zoetermeer, The Netherlands), equipped with a 7.5 MHz linear array transducer and an integrated electrocardiography package. The ultrasound procedures were performed with the subject resting quietly in supine position for at least 10 min. All measurements were made at end-diastole triggered by electrocardiogram. First, diameter of the right brachial artery was searched in a cross-sectional view and then scanned over a longitudinal section 5 to 10 cm proximal to the right elbow. The diameter of the brachial artery was measured from the anterior to the posterior intima/lumen interface at a fixed distance. The mean diameter was calculated from 4 cardiac cycles.

After that, a pneumatic tourniquet placed around the right forearm was rapidly inflated to at least 50 mm Hg above the systolic blood pressure for 5 min. A sudden release of the cuff induced an increase in blood flow in the brachial artery located proximal to the tourniquet. During reactive hyperemia, there was an increase in shear stress, which causes endothelium-dependent vasodilation mainly due to endothelial release of nitric oxide (Joannides et al., 1995). This secondary dilation enhances and prolongs the reactive hyperemic phase. FMD of the brachial artery was measured 45–60 s after cuff release. The change in diameter caused by the increased flow was calculated as the percentage change relative to the baseline measurement (FMD%). The brachial artery dilator response to shear stress has been shown to be reproducible (Sorensen et al., 1995). The intraobserver variability for this ultrasound determination of FMD previously showed a coefficient of variation of 1.2–4.2% and the interobserver variability was evaluated as a mean difference±SD of a maximum 0.18±0.10 mm, meaning a difference of <0.21 mm in 95% of all measurements (Enderle et al., 1998).

Measurement of glycerol trinitrate-induced dilatation of the brachial artery

Twenty minutes after the assessment of FMD, a further resting scan was recorded to confirm the vessel recovery. Endothelium-independent responsiveness was evaluated with 400 µg of glycerol trinitrate (GTN) administered sublingually. GTN-induced dilatation of the brachial artery was measured 4 min after nitroglycerin administration. The diameter change caused by GTN was expressed as the percentage change relative to the recovery scan (GTN%). The coefficient of variation for the measurements of GTN-induced dilatation was 3.97±0.24% and that for reproducibility was 7.24±0.49% in a previous study (Hashimoto et al., 2003).

Assessment of neuropathy

All patients filled in a questionnaire asking for clinical symptoms of sensory neuropathy. Tactile sensation was investigated using Semmes–Weinstein monofilament. Quantitative sensory tests included measurements of heat pain, vibration and thermal sensory thresholds (Path-Tester MPI 100, PHYWE, Germany). Seven determinations were made at the dorsum of the left foot to test the warm and 7 to test the cold thresholds. The mean values were calculated. The measurements of the thermal sensory thresholds started at a temperature of 32.0 °C, which was increased or decreased, respectively, at the rate of 0.7 °C/s. The heat pain thresholds were taken as a mean of eight recordings measured on the dorsum of the left foot. The test temperature started at 40.0 °C with an increase of 0.7 °C/s. Vibratory perception thresholds were measured at the external malleolus of the left foot using a vibration frequency of 100 Hz. The intensity of the vibration was three times increased in the first part of the measurement and in the second part three times decreased at the rate of 0.50 µm/s. Abnormal heat pain, vibration and thermal sensory thresholds have been defined as those more than 2 standard deviations above the mean value in normal subjects. Autonomic cardiac neuropathy was assessed by measuring the heart rate variation during rest and its responses to deep breathing and Valsalva manoeuvre (Pro Sci Card, Pro Science, Germany).

Assessment of retinopathy

The eyes were examined in mydriasis by an ophthalmologist with ophthalmoscopy and also by non-mydriatic retinal camera (CR4-45NM, Canon, Japan). Retinopathy was graded into absence or presence of fundus changes indicative of diabetic retinopathy.