Microvascular Research 99 (2015) 96–101

Contents lists available at ScienceDirect

Microvascular Research

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

The evaluation of penile microvascular endothelial function using laser speckle contrast imaging in healthy volunteers

Valéria Verri [a], Andréa Brandão [c], Eduardo Tibirica [a,b,1]

[a] Department of Clinical Research, National Institute of Cardiology, Rio de Janeiro, Brazil
[b] Laboratory of Cardiovascular Investigation, Oswaldo Cruz Institute, Rio de Janeiro, Brazil
[c] State University of Rio de Janeiro, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Accepted 26 March 2015
Available online 2 April 2015

Keywords:
Microvascular dysfunction
Laser speckle contrast imaging
Penile microcirculation
Erectile dysfunction
Endothelial function

ABSTRACT

Objective: The primary aim of this study was to evaluate the effectiveness of laser speckle contrast imaging (LSCI) coupled with transdermal iontophoretic delivery of acetylcholine (ACh) for the assessment of penile microvascular function. Additionally, we tested systemic microvascular function using both LSCI and ACh iontophoresis on the forearm.

Methods: We assessed cutaneous, endothelium-dependent microvascular reactivity on the penis and forearm of healthy volunteers (aged 56.6 ± 1.0 years, n = 26) at rest and 60 min following the oral administration of the phosphodiesterase type 5 inhibitor (sildenafil: SIL, 100 mg). LSCI was coupled with the iontophoresis of ACh using increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA during 10-second intervals spaced 1 min apart.

Results: Basal skin microvascular flow in the penis increased significantly following SIL administration. The endothelium-dependent skin microvascular vasodilator responses induced by ACh were also significantly enhanced following SIL administration for each of the following parameters: peak values of cutaneous vascular conductance (CVC); increases in CVC; and the area under the curve for ACh-induced vasodilation. ACh-induced microvascular vasodilation in the forearm was not modified by SIL. Finally, the administration of electric current alone did not affect penile microvascular flow.

Conclusion: LSCI appears to be a promising non-invasive technique for the evaluation of penile microvascular endothelial function. This methodology may be valuable for the evaluation of penile microvascular reactivity among patients with cardiovascular and metabolic diseases and to test the effectiveness of drugs used to treat vasculogenic erectile dysfunction.

Introduction

Erectile dysfunction (ED), a consequence of vascular endothelial dysfunction, is a very common disorder among patients with cardiovascular and metabolic diseases (Vlachopoulos et al., 2013). Moreover, vasculogenic ED is associated with many risk factors for cardiovascular disease, including age, hypertension, dyslipidemia, diabetes, physical inactivity, obesity and cigarette smoking (Javaroni and Neves, 2012; Miner et al., 2014; Vlachopoulos et al., 2013). ED may also be an early marker of both systemic endothelial dysfunction and premature cardiovascular disease (Gandaglia et al., 2014; Miner et al., 2014; Shin et al., 2011). ED is strongly associated with age which affects 39% of men 40 years of age and 67% of men 70 years of age (Bortolotti et al., 1997). A key epidemiological study, the Massachusetts Male Aging Study, reported that the prevalence of ED is 52% among men between 40 and 70 years of age (Feldman et al., 1994). Seftel and colleagues (Seftel et al., 2004) studied a representative managed care claims database in the United States of America that included 272,325 men with ED and noted high prevalence rates of hypertension (41.6%), dyslipidemia (42.4%), diabetes (20.2%) and depression (11.1%).

Ultrasound has been used to estimate penile vascular function via Doppler sonography, which investigates the cavernosal and dorsal penile arteries or the penile macrocirculation (Lue et al., 1985, 1989; Lue, 2000). A modified ultrasound assessment of the flow-mediated dilation of the cavernosal arteries following penile arterial occlusion, a phenomenon known as post-occlusive reactive hyperemia, has also been used for the evaluation of penile endothelial function (Mazo et al., 2006a,b; Virag et al., 2004). Nevertheless, no studies have evaluated penile endothelial microvascular function among patients with cardiovascular disease and ED.

Laser speckle contrast imaging (LSCI) represents an innovative approach in the non-invasive evaluation of skin microvascular endothelial

Abbreviations: ACh, acetylcholine; APUs, arbitrary perfusion units; AUC, area under the curve; CVC, cutaneous vascular conductance; ED, erectile dysfunction; LSCI, laser speckle contrast imaging.

E-mail address: etibi@ioc.fiocruz.br (E. Tibirica).
[1] Avenue Brazil 4365, 21045-900, Rio de Janeiro, Brazil.

http://dx.doi.org/10.1016/j.mvr.2015.03.006
0026-2862/

function (Cordovil et al., 2012; Millet et al., 2011; Roustit and Cracowski, 2013). A major advantage of this technique is that the reproducibility of LSCI is superior to earlier procedures, such as laser Doppler flowmetry and laser Doppler imaging (Roustit et al., 2010; Tew et al., 2011). Moreover, LSCI has already proven to be an effective non-invasive technique for the evaluation of systemic microvascular reactivity among patients with cardiovascular and metabolic diseases (Cordovil et al., 2012; Humeau-Heurtier et al., 2013). LSCI may be coupled with both physiological and pharmacological tests to further characterize microvascular reactivity. The primary techniques used to study systemic microvascular endothelial function are post-occlusive reactive hyperemia and cutaneous iontophoresis of vasodilators, such as acetylcholine (Roustit and Cracowski, 2012, 2013). The methodology of non-invasive transdermal drug delivery using low-intensity electric current (iontophoresis) has previously been established (Cordovil et al., 2012; Kaiser et al., 2013; Roustit et al., 2014; Roustit and Cracowski, 2012, 2013; Souza et al., 2014).

Therefore, the primary objective of the present methodological, cross-sectional study was to test the effectiveness of LSCI coupled with the cutaneous iontophoresis of acetylcholine for the evaluation of penile microvascular reactivity. We tested penile acetylcholine-induced microvascular vasodilator responses in healthy volunteers before and after the oral administration of sildenafil—an inhibitor of the phosphodiesterase type 5 enzyme (PDE5), which increases nitric oxide levels and improves the vasodilation of the penile vasculature (Nguyen and Amanullah, 2014). In the present work, sildenafil was used as a pharmacological tool due to its well-known property of inducing microcirculation dilation in the penis. We also evaluated systemic microvascular endothelial function in the forearm using cutaneous acetylcholine iontophoresis.

Methods

Study design

The present study was performed in accordance with the Helsinki Declaration of 1975 as revised in 2000. The study was approved by the Institutional Review Board (IRB) of the National Institute of Cardiology, Rio de Janeiro, Brazil, under protocol number # CAAE 0257.6812.0.0000.5272. Once eligible, all subjects read and signed an informed consent document approved by the IRB.

This cross-sectional, observational study included twenty-six healthy adult male volunteers free of cardiovascular and metabolic diseases who were university hospital staff members and hospital employees from 50–70 years of age. The absence of clinically relevant sexual dysfunction among the subjects in the study was confirmed via each patient's medical history and the erectile function domain of the Simplified International Index of Erectile Function — IIEF-5 (Rosen et al., 1999). The clinical characteristics of the volunteers are presented in Table 1. All subjects underwent a complete medical examination, and arterial pressure was evaluated using a clinically validated Omron Intellisense M7 upper arm blood pressure monitor (Omron Healthcare Europe B.V., Hoofddorp, The Netherlands) according to the following parameters. Following a 10-minute rest period in a cool and quiet environment, each patient was placed in the supine position. Each patient's blood pressure was measured in both arms, and the arm presenting with the higher pressure was used for subsequent evaluations. Then, the measurements were repeated in both the upright (orthostatic) and seated positions with 1-min interval between each measurement. The mean values of the final two measurements were used in this study. The study subjects did not receive phosphodiesterase-5 inhibitors within 30 days of the beginning of the study. Moreover, none of the volunteers received either acute or chronic pharmacological treatment at the times of their recruitment and microvascular examinations.

All evaluations were performed in the morning between 8 A.M. and 12 P.M. following a 12-hour fast. Blood specimens were collected, and the subjects were asked to lie down in a cool, quiet environment for the microcirculatory tests. All examinations followed the same sequence beginning with the measurements of microvascular reactivity via LSCI in the skin of the forearm followed by measurements in the skin of the penis. The microvascular tests were performed both before and 60 min after the oral administration of 100 mg of sildenafil. The iontophoresis of ACh in the forearm was performed 60 min after oral sildenafil administration. Iontophoresis of ACh in the penis was performed approximately 15 min later. (It is technically impossible to perform both procedures simultaneously).

Table 1
The clinical characteristics of the healthy volunteers. The values of mean arterial pressure during microcirculatory flowmetry (MAP-F) obtained before (PRE) and after (POST) the oral administration of a single dose of sildenafil (100 mg).

Characteristics	Healthy volunteers (n = 26)
Age (years)	56.6 ± 1.0
Body mass index (kg/m^2)	26.6 ± 0.6
Office blood pressure	
SAP (mmHg)	127 ± 3
DAP (mmHg)	82 ± 2
MAP-F PRE (mmHg)	97 ± 2
MAP-F POST (mmHg)	93 ± 1.5*
Creatinine (mg/dl)	0.93 ± 0.03
Urea (mg/dl)	33.4 ± 1.9
Total cholesterol (mg/dl)	218.0 ± 4.5
HDL-C (mg/dl)	42.5 (36.0–46.5)
LDL-C (mg/dl)	140.0 (125.8–153.5)
Triglycerides (mg/dl)	157.0 ± 13.7
Glucose (mg/dl)	99.9 ± 1.6

The results are presented as the means ± SEMs. For values that did not follow a Gaussian distribution, the medians (25th–75th percentile) are presented (Shapiro–Wilk normality test).
DAP, diastolic arterial pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; SAP, systolic arterial pressure.
P values were estimated using two-tailed paired Student's t tests.
* $P = 0.0096$, compared with MAP-F PRE.

The evaluation of microcirculatory reactivity

The microcirculatory tests were performed in an undisturbed quiet room with a defined stable temperature (23 ± 1 °C) following a 20-minute rest period in the supine position. The room temperature was monitored and adjusted if necessary using air conditioning. The outside temperature was usually >25 °C. The acclimatization period lasted until each patient's skin temperature stabilized (Shore, 2000). We demonstrated previously that following 15–20 min of acclimatization, the skin temperature stabilizes at approximately 29 °C (Tibirica et al., 2007).

The evaluation of skin microvascular flow and reactivity

The microvascular reactivity was evaluated using an LSCI system with a laser wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden) in combination with the iontophoresis of acetylcholine (ACh) for the non-invasive, continuous measurement of cutaneous microvascular perfusion changes as measured in arbitrary perfusion units (APUs). The images were analyzed using the manufacturer's software (PIMSoft, Perimed, Järfälla, Sweden). One skin site on the ventral surface of the forearm was randomly chosen for the recordings. Hair, broken skin, areas of skin pigmentation and visible veins were avoided, and a single drug-delivery electrode was installed using adhesive discs (LI 611, Perimed, Järfälla, Sweden). A vacuum cushion (AB Germa, Kristianstad, Sweden) was used to reduce the recording artifacts generated by arm movements. The iontophoresis of ACh at a concentration of 0.14 mol/L (Sigma Chemical CO, USA) was performed using a micropharmacology system (PF 751 PeriIont USB Power Supply, Perimed, Sweden) with increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA for 10 s intervals spaced 1 min apart, and the total