Evaluation of systemic microvascular endothelial function using laser speckle contrast imaging

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Abstract

Objective: The aim of this study was to compare cutaneous microvascular function in young healthy subjects (n = 50) with that of cardiometabolic diseased patients (n = 50) using laser speckle contrast imaging (LSCI) coupled with transdermal iontophoretic delivery of acetylcholine (ACh) and post-occlusive reactive hyperemia (PORH).

Methods: Cutaneous blood flow was assessed in the forearm using LSCI at rest, during PORH and during iontophoresis of ACh with increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA during 10-second intervals spaced 1 min apart.

Results: Endothelium-dependent skin microvascular vasodilator responses induced by both ACh and PORH were significantly reduced in cardiometabolic diseased patients compared to healthy subjects. Vasodilator responses induced by ACh were significantly higher in young women than in young men. Iontophoresis charges up to 1.5 mC do not induce nonspecific effects on skin microvascular flux.

Conclusion: LSCI appears to be a promising noninvasive technique for evaluating systemic microvascular endothelial function.

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ACh iontophoresis in control subjects induced marked and current-dependent increases in CVC (with a maximum of 271.0±24.8%; P<0.001) relative to the mean basal value of 0.20±0.01 APU/mm Hg. Iontophoresis using only the solvent induced mild, but significant, effects at the highest current (13.3±3.3%; P<0.05) relative to the basal values of 0.20±0.01 APU/mm Hg. PORH induced a marked increase in CVC in control subjects, reaching a maximum of 241.3±25.7% (P<0.001) compared to the basal values of 0.31±0.02 APU/mm Hg. Additionally, CVC values measured during iontophoresis of ACh were significantly higher in female (P<0.05) than male control subjects in the range of iontophoresis currents from 90 to 180 μA (Fig. 2). However, PORH responses expressed as the peak CVC minus the baseline CVC were not significantly different between males and females (0.53±0.04 and 0.57±0.04 respectively, P>0.05). Patients’ microvascular responses to both ACh and PORH were significantly reduced compared to healthy control subjects (Fig. 2). The maximum increases in CVC induced by ACh and PORH in patients were 143.7±14.5% and 123.9±7.4%, respectively (P<0.001 for both responses). As expected, the measured values of arterial pressure, blood glucose level, plasma lipid levels (total and LDL-cholesterol) and body mass index were significantly higher in arterial hypertension and dyslipidemia patients than control subjects. Plasma levels of HDL-cholesterol were lower in patients than in control subjects (Table 1).

Conclusions

The main findings of this study are as follows: (i) endothelium-dependent skin microvascular vasodilator responses investigated using LSCI coupled with ACh iontophoresis and PORH are significantly reduced in patients with cardiometabolic diseases compared to healthy subjects; (ii) skin vasodilator responses induced by ACh are significantly higher in young women than in young men; (iii) iontophoresis charges up to 1.5 mC do not induce nonspecific effects on skin microvascular flux.

Using the newly-developed LSCI technique, the present study confirmed that endothelium-dependent skin microvascular reactivity is reduced in patients with cardiometabolic diseases, as described previously with LDF (de Jongh et al., 2007). In addition, the obviously higher age of the patients, when compared to young healthy volunteers, undoubtedly contributed to the observed endothelial dysfunction. In fact, aging is well-known to be associated with progressive deterioration in endothelial function (Rajagopalan et al., 2002; Taddei et al., 2006). LSCI provides a microvascular perfusion measurement in real time that is proportional to the average velocity of red blood cells (Boas and Dunn, 2010). An advantage of LSCI compared to LDF is that the blood flow response is measured over the whole area of drug delivery, thus reducing the variability of the measurements due to the spatial heterogeneity of the skin microvasculature (Koustit et al., 2010). In our experimental protocol of transdermal iontophoresis, a current-induced response was observed only at the highest charge of 1.8 mC. This nonspecific increase in blood flow has previously been attributed to membrane hyperpolarization, and pH changes in the iontophoretic chamber induced by the ions present in the vehicle (Ferrell et al., 2002). The responses of skin microcirculation to pharmacological interventions can also be assessed using microdialysis fiber insertion coupled either with LDF (Hodges et al., 2009) or LSCI (Cracowski et al., 2011). The main advantages of this procedure are that it is reproducible, provides two-dimensional...
Fig. 1. Microvascular blood flux recorded with laser speckle contrast imaging during iontophoresis of ACh 2% w/v using increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA during 10-second intervals spaced 1 min apart (A) and during post-occlusive reactive hyperemia (B).
In conclusion, laser speckle contrast imaging appears to be a promising noninvasive technique in the evaluation of systemic microvascular endothelial function. Nevertheless, several technical aspects related to the physiological and pharmacological reactivity tests require further investigation. Actually, the gender-related differences in microvascular reactivity were observed using transdermal iontophoretic delivery of acetylcholine but not during post-occlusive reactive hyperemia, suggesting the involvement of dissimilar physiological mechanisms in these different vasodilator stimuli.

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**Fig. 2.** (A) A comparison of the effects of cutaneous iontophoresis of acetylcholine (ACh) or distilled water (H2O) on cutaneous microvascular conductance (CVC, expressed in arbitrary perfusion units, APU, divided by mean arterial pressure in mm Hg) of young healthy volunteers (CTL) or dyslipidemic patients (DYS) and (B) a comparison between healthy volunteers of both sexes; (C) a comparison of peak microvascular responses to post-occlusive reactive hyperemia (PORH) in healthy volunteers and patients. The amplitudes of PORH responses are expressed as peak CVC minus baseline CVC. The values are mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 compared to basal values; #P<0.01 and ##P<0.001 compared to ACh-CTL; &P<0.05 compared to MALE subjects.

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female subjects’ menstrual phases, as most of them were taking oral contraceptives, we observed a gender-specific difference in the microvascular endothelial function of young healthy subjects. These results confirm the well-known beneficial effects of estrogen on cardiovascular functions, mainly through the enhancement of nitric oxide and endothelium-derived hyperpolarizing factor production (Huang and Kaley, 2004). In conclusion, laser speckle contrast imaging appears to be a promising noninvasive technique in the evaluation of systemic microvascular endothelial function. Nevertheless, several technical aspects related to the physiological and pharmacological reactivity tests require further investigation. Actually, the gender-related differences in microvascular reactivity were observed using transdermal iontophoretic delivery of acetylcholine but not during post-occlusive reactive hyperemia, suggesting the involvement of dissimilar physiological mechanisms in these different vasodilator stimuli.

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**References**