Age-related impairment of neurovascular coupling responses: a dynamic vessel analysis (DVA)-based approach to measure decreased flicker light stimulus-induced retinal arteriolar dilation in healthy older adults

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Abstract Aging is a major risk factor for vascular cognitive impairment and dementia (VCID). Recent studies demonstrate that cerebromicrovascular dysfunction plays a causal role in the development of age-related cognitive impairment, in part via disruption of neurovascular coupling (NVC) responses. NVC (functional hyperemia) is responsible for adjusting cerebral blood flow to the increased energetic demands of activated neurons, and in preclinical animal models of

aging, pharmacological restoration of NVC is associated with improved cognitive performance. To translate these findings, there is an increasing need to develop novel and sensitive tools to assess cerebromicrovascular function and NVC to assess risk for VCID and evaluate treatment efficacy. Due to shared developmental origins, anatomical features, and physiology, assessment of retinal vessel function may serve as an important surrogate outcome measure to study neurovascular dysfunction.

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The present study was designed to compare NVC responses in young (<45 years of age; $n = 18$) and aged (>65 years of age; $n = 11$) healthy human subjects by assessing flicker light-induced changes in the diameter of retinal arterioles using a dynamic vessel analyzer (DVA)-based approach. We found that NVC responses in retinal arterioles were significantly decreased in older adults as compared with younger subjects. We propose that the DVA-based approach can be used to assess NVC, as a surrogate cerebromicrovascular outcome measure, to evaluate the effects of therapeutic interventions in older individuals.

Keywords Aging · Neurovascular coupling · Dynamic retinal vessel analysis · Endothelial dysfunction · Vascular cognitive impairment and dementia

Introduction

The central nervous system (CNS) is the most metabolically active organ system in the human body. It requires 20–25% of the body's resting energy consumption and utilizes over 20% of the total oxygen consumption reflecting the high metabolic demand of neural processing. Constant and adequate provision of nutrients and oxygen to the CNS is essential to its health and normal function, since energy stores in the CNS are scarce. The CNS relies on the tightly regulated dense microcirculatory network for continuous supply of glucose and oxygen and for efficient washout of metabolic waste products. Oxygen and energy demand of the CNS show significant spatial and temporal variation with rapid changes in neuronal activity, which necessitates prompt adjustments of blood flow to maintain cellular homeostasis and ensure normal neural function (Enager et al. 2009; Mathiesen et al. 1998; Tarantini et al. 2017c). Moment-to-moment adjustment of blood flow to the metabolic demands of active neurons is accomplished through a process termed neurovascular coupling (or functional hyperemia) (Tarantini et al. 2017c). Active neurons in each regions of the CNS, including the brain (Attwell et al. 2010), the spinal cord (Piche et al. 2017), and the retina (Albanna et al. 2018; Lasta et al. 2013; Newman 2013; Noonan et al. 2015; Querques et al. 2019; Ravi Teja et al. 2017; Riva et al. 2005), depend on neurovascular coupling responses for the preservation of normal CNS function. Neurovascular coupling responses are orchestrated by an inter-cellular signaling

network that is comprised of active neurons, astrocytes, and arteriolar vascular smooth muscle cells and endothelial cells (Chen et al. 2014; Petzold and Murthy 2011; Stobart et al. 2013; Wells et al. 2015).

There is strong evidence obtained both in older adults and laboratory rodent models of aging that advanced age results in significant impairment of neurovascular coupling responses (Balbi et al. 2015; Fabiani et al. 2013; Park et al. 2007; Sorond et al. 2013; Tong et al. 2012; Toth et al. 2014; Zaletel et al. 2005). Recent preclinical studies demonstrate that neurovascular dysfunction is an important factor contributing to the pathogenesis of cognitive decline in aging and pathological conditions associated with accelerated microvascular aging including hypertension, metabolic diseases, and Alzheimer's disease (Girouard and Iadecola 2006; Tarantini et al. 2017b, 2018; Toth et al. 2015a, b; Tucsek et al. 2014). Selective pharmacological disruption of neurovascular coupling responses in young animals was shown to induce cognitive impairment mimicking the aging phenotype (Tarantini et al. 2015, 2017d). Importantly, rescue of functional hyperemia in aged animals by experimental anti-aging pharmacological interventions leads to improved cognitive performance (Tarantini et al. 2018; Toth et al. 2014). These preclinical observations show the feasibility of developing translationally relevant intervention strategies to improve neurovascular coupling responses and CNS blood flow in older individuals at risk for vascular cognitive impairment.

Translational studies to evaluate efficacy of novel interventions in older adults require standardized assessment of neurovascular outcome measures with high sensitivity and repeatability. Several methodologies have been adapted to study neurovascular coupling responses in humans including functional MRI (fMRI) (Duarte et al. 2015; Fabiani et al. 2013), functional near-infrared spectroscopy (fNIRS), and transcranial Doppler ultrasonography (Sorond et al. 2010, 2008). However, many of these methodologies have important disadvantages and technical constraints, which limit their use in follow-up studies in older individuals to assess treatment efficiency. These include limited sensitivity, artifacts (e.g., physiological and motion artifacts), the inherently highly complex relationship between neural activity and the blood oxygen level-dependent (BOLD) contrast, issues with patient compliance (e.g., claustrophobia) and contraindications to being in a magnetic field (e.g., metal in the body from a previous

surgery in case of fMRI), high costs, and the frequent lack of an acoustic window in older adults. Due to these practical limitations, there is an urgent need to develop/adapt new sensitive, fast, and easy-to-use methodologies to aid in investigations of neurovascular coupling responses in older adults.

Due to shared developmental, anatomical, and physiological origins, retinal vessels may serve as an important surrogate vascular bed to study cerebromicrovascular structure and function in elderly human subjects. Clinical studies demonstrate that structural alterations of retinal vessels predict vascular pathologies affecting the brain. For example, abnormal retinal arteriovenous ratios (AVRs) predict the risk of stroke and vascular dementia (de Jong et al. 2011; Heitmar et al. 2015; McGrory et al. 2017). Similar to neurovascular coupling responses in the brain, which rely on the close interaction of neurons, glial cells, and cerebromicrovascular endothelial cells, a causal relationship between retinal neuronal activity, activation of glial cells, and endothelium-dependent vasodilation has been described in the retinal microcirculation (Metea and Newman 2006, 2007; Montero-Odasso et al. 2012). Recent developments also enable the assessment of neurovascular coupling responses in the retina (Kneser et al. 2009; Lim et al. 2013; Seshadri et al. 2016). The present study was designed to assess age-related changes in neurovascular coupling responses in healthy human subjects by assessing flicker light-induced changes in the diameter of retinal arterioles using a dynamic vessel analyzer (DVA)-based approach.

Materials and methods

Subjects

In this study, we have evaluated a subset of human subjects from an on-going clinical cohort study of vascular aging at the University of Oklahoma Health Sciences Center. Protocols were approved by the Institutional Review Board. Prior to participation in the study, participants were familiarized with study procedures and signed written consent forms which were collected. Eighteen healthy young subjects aged 21–45 (12 males and 6 females) and eleven clinically healthy elderly subjects above 65 years of age (6 male and 5 females) were selected. Exclusion criteria consisted of diabetes, active cancer, recent or active cardiovascular disease (angina, myocardial infarction, heart failure, stroke),

presence of peripheral artery disease, active infection, and hormone replacement therapy. Subjects with blood pressure above 139/89 mmHg were excluded from this study. Patients did not consume caffeine nor use nicotine-containing products before the measurements.

Visual acuity, slit-lamp, and intraocular pressure examination

All subjects were evaluated for visual acuity using a Snellen chart in a well-lit room. After taking the extensive history, a slit-lamp examination was done to rule out any relevant ocular pathologies. Intraocular pressure was measured using a Goldmann applanation tonometer. Measurements were conducted in the right eye.

Dynamic retinal vessel analysis

The measurements were performed in a quiet, dimly lit, temperature-controlled room (22 °C). The pupil of the right eye was dilated using topical application of tropicamide (1% Tropicamide Ophthalmic Solution USP, AKORN, Lake Forest, IL), followed by a period of rest to achieve stable hemodynamic conditions. The patient's fixation was adjusted using a fixation target so that the site of interest was located in the middle of the fundus picture. Evaluation of retinal arteriolar and venular diameters was performed using the dynamic vessel analyzer (DVA, IMEDOS Systems, Jena, Germany) according to published protocols (Garhofer et al. 2010; Gugleta et al. 2006). The DVA device allows for noninvasive and continuous assessment of retinal vessel diameters along a selected vessel segment before, during, and after flicker light stimulation. To allow these measurements, DVA is equipped with a mydriatic retinal camera (450 FF; Carl Zeiss, Jena, Germany), a charge-coupled device camera for electronic online imaging and a personal computer for system control, analysis, and recording. Each participant was evaluated for mean maximal arteriolar dilation and mean maximal venular dilation in response to flicker light stimulation (Kotliar et al. 2011). The flicker light was of the same wavelength as the illumination light. The flicker frequency was 12.5 Hz and its duration was 20 s. Before starting the flicker stimulation, a baseline recording for a minimum period of ~100 s was performed. After the flicker, 80 s of steady illumination was applied to allow the vessel diameter to return to baseline. The statistical mean of 3 consecutive examinations was calculated for

each subject and each evaluated parameter. Vessel segments of approximately 1 mm in length, located in the upper temporal quadrant 1–3 optic disc diameters away from the optic disc edge, were assessed in the arteriolar and venular branches (Kotliar et al. 2011). Sites where two vessels were very close to each other were avoided.

Statistical analysis

All statistical tests were performed in Graphpad Prism 7.0e (Graphpad Software, La Jolla, CA, USA). Following the D'Agostino-Pearson normality test, measured parameters were compared using nonparametric Mann-Whitney *U* test or χ^2 when appropriate. A *p* value of < 0.05 was considered significant.

Results

Participants

Table 1 shows the baseline characteristics of young (< 45 years old) and aged (> 65 years old) study participants. The average age for aged individuals was 74.5 ± 7.2 vs 32.7 ± 6.3 for young. There were no medications reported in the young subjects, while 7 out of 11 received prescribed anti-hypertensive medications, 3 received diuretics, and 3 received lipid-lowering medications. Mean arterial pressure for aged group was 91.1 ± 8.2 mmHg vs 91.3 ± 8.8 mmHg in young group (*p* = NS), average systolic pressure for aged group was 128.9 ± 8.2 vs 117.9 ± 10.6 in young (*p* = 0.006),

average diastolic pressure for aged group was 75.3 ± 8.7 vs 78.1 ± 8.7 in young (*p* = NS). Although average systolic pressure was significantly higher in aged individuals as compared with young ones, individual levels were never above the 139 mmHg threshold.

Neurovascular coupling responses are impaired in retinal arterioles of aged individuals

All subjects had a best-corrected distance visual acuity (VA) > 20/25 and an intraocular pressure below 21 mmHg. Exclusion criteria for both groups were photosensitive epilepsy, glaucoma, distinct cataract, and known intolerance of tetracaine and tropicamide. Aged individuals showed a 51.78% reduction in the mean maximal arteriolar dilation in response to flicker light stimulation (*p* = 0.0129, Fig. 1b and c). No statistical difference in the mean venular dilation between young and aged individuals was observed (*p* = 0.16).

Discussion

The major finding of our study is that advanced age is associated with impaired neurovascular coupling responses in retinal arterioles in otherwise healthy subjects. Our study extends the findings of previous investigations demonstrating age-related impairment of neurovascular coupling responses in the brain of older adults using other methodologies including fMRI (West et al. 2018).

Table 1 Baseline characteristics of young (< 45 years old) and aged (> 65 years old) study participants

	Young (<i>n</i> = 18)	Aged (<i>n</i> = 11)	<i>p</i> value (<i>t</i> test or χ^2 test, where applicable)
Sex			
Male	12	6	0.67
Female	6	5	
Age (years)	32.7 ± 6.3	74.5 ± 7.2	< 0.001*
Mean arterial pressure (mmHg)	91.3 ± 8.8	93.1 ± 7.6	0.57
Systolic blood pressure (mmHg)	117.9 ± 10.6	128.9 ± 8.2	0.006*
Diastolic blood pressure (mmHg)	78.1 ± 8.7	75.3 ± 8.7	0.41
BMI (kg/m ²)	24.0 ± 3.2	25.6 ± 3.7	0.22
Antihypertensive drugs	0/18	7/11	0.99
Diuretics	0/18	3/11	0.99
Lipid-lowering medications	0/18	3/11	0.99

BMI, body mass index

*Significant difference by *t* test or χ^2 test

Fig. 1 Aging impairs neurovascular coupling in healthy individuals. **(a)** Representative fundus image showing a retinal arteriole (red arrow) and a retinal venule (blue arrow) in which flicker light stimulus-induced diameter changes were recorded using the dynamic vessel analysis (DVA) approach. **(b)** Time course of flicker light stimulation-induced changes in diameter of retinal arterioles

in young (black, $n = 18$) and aged (red, $n = 11$) individuals. **(c)** Summary data showing that older individuals exhibit reduced mean maximal arteriolar dilation (% to baseline) in response to flicker light stimulation as compared to young controls. Data are mean $\pm$ SEM

Our study demonstrates the applicability of the DVA-based approach to assess microvascular reactivity in retinal vessels as a tool to evaluate age-related impairment of neurovascular coupling responses in older adults. Preclinical studies confirm the connection between neurovascular coupling and DVA microvascular reactivity by demonstrating selective increase in metabolic activity in inner retinal layers (Ames 3rd et al. 1992) and changes in oxygen tension in the retinal microcirculation in response to flicker light stimulation (Albanna et al. 2018; Shakoob et al. 2006). Human studies demonstrated the change in retinal blood flow, vessel diameter, and oxygen saturation coupled with retinal neural activity during flicker light stimulation (Falsini et al. 2002; Hammer et al. 2011) using DVA and laser Doppler velocimetry. The reproducibility and sensitivity of the DVA in healthy subjects is excellent: the coefficient of variation for the day-to-day variability of the instrument is $\sim 5.2\%$ for retinal arteries (Garhofer et al. 2010; Polak et al. 2000). Unlike fMRI, fNIRS, and transcranial Doppler (Huneau et al. 2015; Huppert et al. 2009; Sorond et al. 2011), which are based on indirect assessment of cerebral blood flow changes estimated from hemoglobin distribution in cortex regions or operator-dependent assessment of blood flow velocity, the DVA-based method provides direct assessment of microvascular reactivity during functional hyperemia. The non-invasive DVA-based method is also quick and its operational costs are significantly lower as compared with other methods (e.g., fMRI). Thus, the DVA-

based approach (Kneser et al. 2009; Nagel et al. 2004; Seshadri et al. 2016) can be used for longitudinal studies to evaluate efficiency of anti-aging treatments on neurovascular coupling responses in older adults. As neurovascular coupling responses are also compromised in Alzheimer's disease (Tarantini et al. 2017c), the DVA-based approach to assess retinal vascular reactivity may also be useful to monitor progression of the disease and/or to evaluate the effects of vasoprotective treatments in Alzheimer's patients (Querques et al. 2019).

The cellular and molecular mechanisms by which aging impairs cerebrovascular function and compromise neurovascular coupling responses are multifaceted (Tarantini et al. 2017c). Neurovascular coupling responses in the brain involve, at least in part, release of NO from microvascular endothelial cells (Toth et al. 2015b). Similarly, microvascular responses measured by the DVA in the retina are also dependent on the activation of endothelial nitric oxide synthase (Lim et al. 2013). Aging is associated with generalized endothelial dysfunction due to an age-related increase in cellular oxidative stress and consequential decreases in bioavailability of nitric oxide (Ungvari et al. 2018). The mechanisms contributing to age-related increased oxidative stress and endothelial dysfunction include deficiency of vasoprotective IGF-1, heightened inflammatory status, and mitochondrial alterations (Pearson et al. 2008; Tarantini et al. 2018; Toth et al. 2015a, 2014; Ungvari et al. 2018). Novel experimental therapeutic interventions that reduce oxidative stress (Gioscia-

Ryan et al. 2014; Pearson et al. 2008; Tarantini et al. 2018; Toth et al. 2014) were reported to significantly improve endothelial function in the cerebral circulation and rescue both neurovascular coupling responses and cognitive function in aged animals (Oomen et al. 2009; Tarantini et al. 2018). The DVA-based approach appears to be ideal for the design of studies translating the results of the aforementioned preclinical investigations.

This study has several limitations, including the fact that studied population had minor or no comorbidities. Therefore, further investigations are warranted to study the effect of age-related diseases and medication used on neurovascular coupling responses in the retina.

In conclusion, the DVA-based approach to assess neurovascular coupling in the retina represents an adjunct and clinically relevant cerebro-microvascular outcome measure that can be used to evaluate therapeutic interventions targeting cellular mechanisms of aging (Ashpole et al. 2017; Carlson et al. 2018; Csipo et al. 2018; Csizsar et al. 2017; Fulop et al. 2018; Lee et al. 2018; Nacarelli et al. 2018; Sarker and Franks 2018; Scerbak et al. 2018; Tarantini et al. 2017a; Ungvari et al. 2017) in older individuals. This method potentially also has the capacity to impact management strategies for the prevention of vascular cognitive impairment and dementia in older patients.

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Compliance with ethical guidelines Protocols were approved by the Institutional Review Board.

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