RESEARCH ARTICLE

Phosphodiesterase-5 inhibition potentiates cerebrovascular reactivity in chronic traumatic brain injury

Kimbra Kenney[1], Franck Amyot[1], Carol Moore[1], Margalit Haber[2], L. Christine Turtzo[3], Christian Shenouda[4], Erika Silverman[2], Yunhua Gong[2], Bao- Xi Qu[1], Leah Harburg[1], Eric M. Wassermann[5], Hanzhang Lu[6] & Ramon Diaz-Arrastia[2]

[1]Department of Neurology, Uniformed Services University of the Health Sciences, Bethesda, Maryland
[2]Department of Neurology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania
[3]Radiology and Imaging Sciences, National Institutes of Health, Bethesda, Maryland
[4]Department of Physical Medicine and Rehabilitation, National Institutes of Health Clinical Center, Bethesda, Maryland
[5]Behavioral Neurology Unit, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland
[6]Johns Hopkins University, Baltimore, Maryland

Correspondence
Ramon Diaz-Arrastia, Department of Neurology, University of Pennsylvania Perelman School of Medicine, Penn Presbyterian Medical Center, 51 North 39th St. Philadelphia, PA 19104.
Tel: 215 662 8000; Fax: 215 243 3246; E-mail: Ramon.Diaz-Arrastia@uphs.upenn.edu

Abstract

Background: Traumatic cerebrovascular injury (TCVI), a common consequence of traumatic brain injury (TBI), presents an attractive therapeutic target. Because phosphodiesterase-5 (PDE5) inhibitors potentiate the action of nitric oxide (NO) produced by endothelial cells, they are candidate therapies for TCVI. This study aims to: (1) measure cerebral blood flow (CBF), cerebrovascular reactivity (CVR), and change in CVR after a single dose of sildenafil (ΔCVR) in chronic TBI compared to uninjured controls; (2) examine the safety and tolerability of 8-week sildenafil administration in chronic symptomatic moderate/severe TBI patients; and as an exploratory aim, (3) assess the effect of an 8-week course of sildenafil on chronic TBI symptoms. **Methods**: Forty-six subjects (31 chronic TBI, 15 matched healthy volunteers) were enrolled. Baseline CBF and CVR before and after administration of sildenafil were measured. Symptomatic TBI subjects then completed an 8-week double-blind, placebo-controlled, crossover trial of sildenafil. A neuropsychological battery and neurobehavioral symptom questionnaires were administered at each study visit. **Results**: After a single dose of sildenafil, TBI subjects showed a significant increase in global CVR compared to healthy controls ($P < 0.001$, $d = 0.9$). Post-sildenafil CVR maps showed near-normalization of CVR in many regions where baseline CVR was low, predominantly within areas without structural abnormalities. Sildenafil was well tolerated. Clinical Global Impression (CGI) scale showed a trend toward clinical improvement while on sildenafil treatment. **Findings**: Single-dose sildenafil improves regional CVR deficits in chronic TBI patients. CVR and ΔCVR are potential predictive and pharmacodynamic biomarkers of PDE5 inhibitor therapy for TCVI. Sildenafil is a potential therapy for TCVI.

Introduction

Traumatic brain injury (TBI), a major cause of death and disability, annually accounts for 2.5 million Emergency Department visits, 52,000 deaths and 92,000 survivors with disability in the United States alone.[1] It is estimated that the prevalence of Americans with chronic TBI-related disability is 5.3 million.[2–5] The magnitude of this problem has led to extensive pre-clinical research and clinical trials to improve functional outcomes.[6] Despite success in animal models, all Phase III human clinical trials to date have failed.[7–10] Consensus conferences have concluded that biomarkers will be critical for the development of effective TBI therapies targeted at injury-specific mechanisms.[6,11]

While the mechanisms responsible for functional deficits after TBI are incompletely understood, substantial data indicate that traumatic cerebrovascular injury (TCVI) contributes to TBI-related disability. TCVI is a

near universal pathologic finding in fatal TBI,[12] but has also been detected in mild or moderate TBI cases.[13] Vascular injury is detected on MRI in 25–30% of mild TBI cases imaged soon after injury.[14,15] In animal TBI models, TCVI is universally detected,[16–20] and persistent microcirculatory deficits correlate with behavioral and cognitive deficits.[18] The distribution of TCVI is diffuse and multifocal, and often noted at some distance from visible lesions.[18,20,21]

Cerebrovascular reactivity (CVR) is the change in cerebral blood flow in response to a vasodilatory stimulus and is a measure of vascular reserve and microvascular health.[22] There are established methods to study CVR non-invasively in humans including transcranial Doppler (TCD), near infra-red spectroscopy (NIRS) with hypercapnia,[23] and MRI-Blood Oxygen Level Dependent (MRI-BOLD) with hypercapnia challenge. In professional boxers, CVR is decreased in the first week after a fight, and correlates with cumulative lifetime concussions.[24] A meta-analysis of CVR after sports concussion concluded that CVR is decreased acutely.[25] Our group has found that MRI measures of CVR reliably distinguishes chronic TBI from HC with the greatest effect size in the gray matter.[26] Furthermore, CVR maps show focal areas of decreased CVR in regions with visible encephalomalacia as well as in areas of normal structural parenchyma.

Because of the plasticity of the cerebral vasculature, TCVI is an attractive target for intervention. Multiple pharmacologic and non-pharmacologic therapies with well-established medical indications promote blood vessel repair and may prove beneficial as therapy for TCVI in appropriately selected patients.[27–30] Sildenafil is a potent and specific inhibitor of cGMP-specific phosphodiesterase (Phosphodiesterase 5, PDE5), thereby prolonging the elevation of cGMP resulting from the induction of guanylyl cyclase by nitric oxide (NO). Sildenafil is FDA-approved for the treatment of erectile dysfunction and primary pulmonary hypertension. In patients with microvascular dysfunction, vasodilation is impaired by the inability of damaged endothelial cells to produce sufficient NO. PDE5 inhibitors potentiate NO-dependent vasodilation by prolonging the elevation of cGMP. Sildenafil is under active study in a variety of conditions associated with microvascular dysfunction.[31–34] Sildenafil's endothelial repair properties have been studied in several conditions associated with chronic diabetes.[34] In extensive pre- and post-marketing use, it is safe and generally well-tolerated.

We hypothesize that sildenafil will potentiate CVR in patients with chronic TBI. We further hypothesize that the effect of sildenafil is greater in cortical regions where baseline CVR is low ("CVR holes"). Furthermore we hypothesize that chronic low-dose sildenafil will prove safe and well tolerated in chronic TBI patients. Finally, as an exploratory hypothesis, we propose that sildenafil will prove beneficial for improving chronic post-TBI symptoms.

Methods

Subjects

Adults between 18 and 55 years were consented and enrolled under an IRB-approved Phase IIa clinical trial (NCT01762475). Inclusion criteria for TBI subjects included TBI 6 months to 10 years prior to enrollment with any one of the following at the time of injury: Glasgow Coma Score between 3 and 12; post-traumatic amnesia > 24 h; or TBI-related neuroimaging abnormality (on CT or MRI). Exclusionary criteria included any penetrating TBI, pre-existing disabling neurologic or psychiatric disorder, pregnancy, unstable pulmonary or vascular disorder, allergy to sildenafil or contraindications to taking a selective PDE-5 inhibitor. Gender and age-matched individuals with no history of TBI, pre-existing disabling disorders or pregnancy were enrolled as healthy controls (HC). Each subject underwent a baseline visit which included a history and physical, baseline surveys including headache diagnostic interview (HIT-6) and sildenafil side effect symptom survey, brief neurocognitive testing and neurobehavioral surveys, and MRI with CVR assessments before and 60 min after a single oral dose of sildenafil citrate 50 mg. Symptomatic TBI subjects were defined, according to DSM-IV-TR post-concussion syndrome criteria,[35] as those with cognitive/memory dysfunction and at least 3 of the following symptoms: fatigability, disordered sleep, headache, vertigo/dizziness, irritability/aggression, anxiety/depression/affective lability, and personality change. Symptomatic TBI subjects were entered into an 8-week crossover, double-blind, placebo-controlled trial of sildenafil 25 mg twice daily with a 2-week washout in between the two drug trial phases. After each drug trial phase, TBI subjects underwent the same evaluation as at baseline, including CVR before and after single-dose sildenafil (to assess CVR and ΔCVR in response to PDE5 inhibitor), symptom surveys (with HIT-6) and brief neuropsychological testing. At each study visit, subjects were asked to guess what drug phase they felt they had just completed (active drug or placebo), if they had experienced any adverse advents and if they had any clinical change (improvement or worsening) during the prior 8 weeks. The Clinical Global Impression (CGI) scale was completed based on interview with the examining study investigator (RDA, KK, CS, CM or EW). The pharmacy randomized active drug versus placebo administration. All research staff, including MRI technologists and psychometric technicians were blinded as to group assignment, and the blind was not broken until all subjects had completed the study.

MRI imaging

MRI was performed on a Siemens Biograph mMR, fully integrated 3T MRI/PET. T1 Magnetization Prepared Rapid Gradient Echo (MPRAGE), Susceptibility weighted imaging (SWI), T2- gradient echo sequences (GRE), Diffusion weighted imaging (DWI), Diffusion tensor imaging (DTI), Fluid-attenuated inversion recovery (FLAIR), pulsed Arterial Spin Labeling (ASL) and Magnetic Resonance Imaging–Blood Oxygenation Level Dependent (MRI- BOLD) sequences were acquired on each subject.

FLAIR images were acquired with the following parameters: TR/TE=9090/112 msec, inversion time = 2450 msec, slice thickness = 3.0 mm. ASL images were acquired using a pulsed sequence with the PICORE Q2TIPS labeling scheme. The following parameters were established for the acquisition of 111 2D-EPI volumes: TR/TE =2700/ 15 msec, TI1/TI2 = 700/1800 msec.

Each participant underwent MRI-BOLD sequences with CVR hypercapnia measurements according to the methods developed previously.[22,36] Hypercapnia was induced via a Douglas bag alternating between a flow of room air and 5% carbon dioxide (CO_2) mixed with room air every minute for 7 min total while the MRI-BOLD images were acquired. End-tidal CO_2 ($EtCO_2$) was measured continuously using a capnograph (Smith Medical, Model 9004). These conditions (5% CO_2 for no longer than one minute at a time) did not result in significant changes in mean arterial blood pressure, heart rate, or oxygen saturation.[37] The sequence's parameters were TR/TE = 2000/25 msec, flip angle=80°, field of view=220 × 220 mm^2, matrix= 64 × 64, 36 slices, thickness=3.6 mm, no gap between slices, 210 volumes.

Data processing

ASL

Image data analyses were performed with the Statistical Parametric Mapping software (SPM12) and ASL data processing toolbox. The control and label ASL images were realigned and re-sliced to correct for head motion. They were smoothed using an isotropic Gaussian kernel with a 4 mm full-width at half-maximum. CBF images were reconstructed using the ASL Data Processing Toolbox ASLtbx.

CVR

Images were spatially realigned and re-sliced to correct for head motion with SPM (realign and re-slice toolbox). We monitored six parameters (x, y, z translation and pitch, toll and yaw) to assure correct co-registration. Scans from 2 subjects from whom head motion was not correctable were not analyzed. The $EtCO_2$ measure was individually shifted to achieve the maximum correlation with the BOLD signal due to the travel time between the lungs to the cerebrovascular network. Voxel-by-voxel CVR maps (in the units of % BOLD change/mmHg CO_2 change, %/mmHg) were calculated based on a linear regression between BOLD signal time courses and the vascular input function (time shifted $EtCO_2$), as previously described by Lu et al.[38] Global CVR is calculated as the mean voxel CVR measure for the whole brain, and compartment (white or gray matter) CVR is calculated as the mean voxel CVR measure within the region of interest.

At baseline, the mean and standard deviation (SD) $EtCO_2$ values were 39.9 (1.6) mmHg and rose to 49.0 (1.2) mmHg during hypercapnia. After sildenafil administration, the mean (SD) initial $EtCO_2$ was 40.1 (1.5) and rose to 48.6 (1.8) mm Hg during hypercapnia. There was no difference in baseline $EtCO_2$ or $\Delta EtCO_2$ between the TBI and control groups ($P > 0.2$). The CVR (pre- and post-sildenafil), CBF (pre-sildenafil only) and FLAIR images were each co-registered with the MPRAGE images using SPM12. ΔCVR was calculated as the difference in the global CVR measures before and after a single-dose of oral sildenafil 50 mg and ΔCVR maps were computed as the difference in CVR maps before and after single-dose sildenafil within a single imaging session.

Neurobehavioral assessments

A psychometrist administered a battery of neuropsychological tests and neurobehavioral symptom surveys selected from the TBI Common Data Elements, as follows: learning portions of the California Verbal Learning Test (CVLT), Word Reading subtest of the Wide Range Achievement Test (WRAT), Trail Making Test (TMT) Parts A & B (TMT-A, TMT-B, respectively), Digit Symbol and Symbol Search subsets of the Wechsler Adult Intelligence Scale (WAIS-IV), the Brief Symptom Inventory (BSI), and the Rivermead Post Concussion Symptoms Questionnaire (RPQ). Raw scores on the CVLT, WRAT, TMT, and WAIS-IV were normalized for age and education.

Statistical analysis

The statistical analysis was performed with Matlab statistical toolbox, using the Kolmogorov–Smirnov test for standard normal distribution. If one dataset rejected the null hypothesis at 5% significance level, we performed a Spearman rank order correlation. Otherwise we performed Pearson linear correlation. The correlation coefficient is associated with a *P*-value testing the hypothesis of no correlation. The statistical analysis of independent samples was done with two independent *t*-tests when the samples were normally distributed and with Mann–Whitney U-test otherwise.

Results

Demographics & TBI characteristics

Forty-six (31 TBI and 15 HC) age and gender-matched subjects were consented, enrolled in the study and had data available for analysis (Table 1). TBI subjects had a mean ($\pm$ SD) age of 38 ($\pm$10) years (range 20–54). TBI subjects were enrolled a median of 23 months post-injury (range 6 months to 8.5 years). The majority (58%) of TBIs occurred as a result of road traffic incidents. All but three subjects had TBI-related neuroimaging findings on initial cranial computerized tomography (CT), with hematomas/hemorrhages (subdural, subarachnoid, and intraparenchymal) and contusions the most common abnormality. Five (16%) received neurosurgical intervention (craniotomy or craniectomy) for their injury. All but one TBI subject (whose TBI occurred in a combat setting)

Table 1. Demographics, TBI characteristics and Rivermead test results.

	TBI (*n* = 31)	HC (*n* = 15)	*P*
Age (years), mean $\pm$ SD	37.9 $\pm$ 10.2	37.9 $\pm$ 7.2	0.99
Gender, % male	74	80	0.67
Education (years), mean $\pm$ SD	15.5 $\pm$ 3.1	17.5 $\pm$ 3.5	0.054
Employed- full or part time (at enrollment), %	77	80	
Time since TBI (months), median (IQR)	23 (14, 41)	*	*
Road traffic incident (%)	58	*	*
LOC $\geq$ 30 min (%)	59	*	*
Abnormal neuroimaging, CT or MRI (%)	92	*	*
Days in ICU (86% of TBI), median (IQR)	5 (1, >14)	*	*
Received Rehabilitation (%)	48	*	*
GOS-E, median (IQR)	7 (6,7)	*	*
RIVERMEAD (total), median (IQR)	18 (8, 26)	1 (0, 5)	<0.0001
RIVERMEAD 13, median (IQR)	16 (5, 23)	1 (0, 5)	<0.0001
RIVERMEAD 3, median (IQR)	2 (1,4)	0 (0, 0)	<0.0001
BSI (total), mean $\pm$ STD	2.0 $\pm$ 2.9	4.9 $\pm$ 5.7	0.067
BSI Somatic, mean $\pm$ STD	0.13 $\pm$ 0.35	1.1 $\pm$ 1.4	<0.01
BSI Depression, mean $\pm$ STD	1.1 $\pm$ 1.8	2.2 $\pm$ 3.5	0.23
BSI Anxiety, mean $\pm$ STD	0.8 $\pm$ 1.1	1.8 $\pm$ 2.1	0.08

TBI, traumatic brain injury; HC, healthy control; SD, standard deviation; IQR, interquartile range (25th and 75th); LOC, loss of consciousness; CT, computerized tomography; MRI, magnetic resonance imaging; ICU, intensive care unit; Inpt/Outpt, inpatient or outpatient; GOS-E, Glasgow Outcome Scale-Extended; NSI, Neurobehavioral Symptom Inventory; BSI, Brief Symptom Inventory.*, not applicable.

were hospitalized acutely, stayed a median of 7 days in the intensive care unit (ICU) and nearly half (48%) received rehabilitation services (24% inpatient and 24% outpatient). They had a median Glasgow Outcome Score-Extended (GOS-E) of 7 (range 3–8) at study enrollment, and the median score in the RPQ was 18 (range 7–43). On study baseline MRI, 20 showed focal or multifocal areas of encephalomalacia with T2 hyperintensities with or without focal microhemorrhages, 4 with focal microhemorrhages only, 1 with a chronic subdural and focal microhemorrhages, and 6 were read as normal (one patient's MRI was read as normal at study enrollment, showing resolution of a subdural hematoma previously imaged by CT). Eight subjects had volume loss or global atrophy noted by a clinical neuroradiologist.

ΔCVR after single-dose sildenafil

All subjects were studied with MRI-BOLD with hypercapnia challenge before and after a single-oral dose of sildenafil 50 mg to calculate ΔCVR. The procedure was well tolerated by both TBI and HC subjects without adverse effects. Nearly all TBI subjects (28/31, 90%) experienced a potentiation of CVR after administration of single-dose sildenafil (mean CVR increase in 10.1% $\pm$ 11.2%, see Fig. 1A). We observed increased CVR in response to sildenafil even among TBI patients whose baseline CVR before sildenafil was comparable to measures in healthy volunteers. In contrast, among healthy volunteers (14/15, 93%) single-dose sildenafil administration resulted in a slight CVR decrease (−3.3% $\pm$ 2.8%, see Fig. 1B). Quantitatively, mean baseline global CVR measures were 18.1% lower among TBI subjects pre-sildenafil compared to gender and age-matched controls, while mean post-sildenafil global CVR measures were 8.2% lower among TBI subjects compared to HC (Table 2).

Both baseline global CVR and ΔCVR measures are good discriminators between TBI subjects and healthy controls (Area Under the Curve (AUC) was 0.90 for baseline CVR, and 0.89 for ΔCVR (Table 1). In addition, as shown in Figure 1B, there was a good correlation between the CVR deficit and the increase in CVR with sildenafil (Spearman's correlation = −0.628, *P* < 0.0001).

While the global changes in CVR after single-dose sildenafil are modest, local effects are much more robust. Sildenafil does not potentiate CVR equally throughout the brain. Rather its effect is specific to brain regions with low baseline CVR measures ("CVR holes", Fig. 2), and most commonly normal CBF and structural imaging. We found that within brain regions with co-localized low CBF and CVR measures after pre-sildenafil hypercapnia challenge, we measured CVR increases after sildenafil administration (region 3, Fig. 2B5). The focal areas with
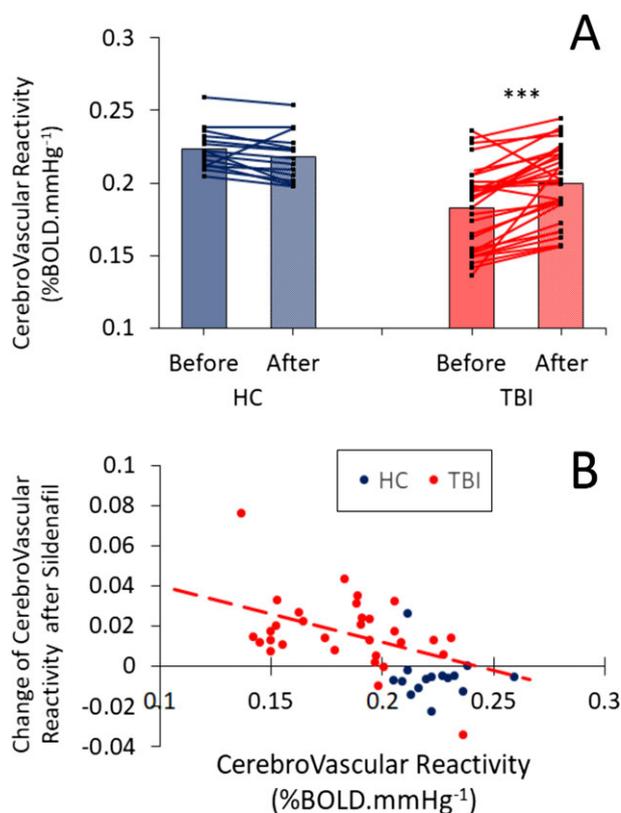
Figure 1. Global CVR measures before and after 50mg of sildenafil (figure 1-A). Each bar illustrates the mean value of global CVR for the HC group (blue) and the TBI group (red). The black dots represent the individual global CVR and each line represents the global CVR change after sildenafil for a single subject. Figure 1-B shows the correlation between the global CVR measure before sildenafil and the increase of global CVR with sildenafil. The correlation (Spearman) between these two measures is -0.628 ($p<10^{-4}$).

positive ΔCVR are associated with either visible encephalomalacia on FLAIR imaging (region 1, Fig. 2B1) or, more frequently, with no visible structural abnormality (region 1, Fig. 2C2). We further observed that sildenafil potentiated CVR primarily in regions of normal appearing brain which had normal resting CBF but low baseline CVR (region 4, Fig. 2B3 and region 2, Fig. 2C3). In areas of focal cystic encephalomalacia or gliosis (region 2, Fig. 2B2), we usually noted low CVR and CBF without a measurable increase in CVR after single-dose sildenafil.

Post-concussive symptoms and cognitive function and relationship with baseline CVR and ΔCVR

Despite having suffered moderate to severe injuries, most subjects had made a good recovery. There was no difference in normalized neuropsychological testing scores

(WRAT, WAIS, TMT-A and TMT-B, $P > 0.1$) between TBI and HC. As expected, there was a significant difference between neurobehavioral symptom surveys (RPQ) and TBI status (Table 1). Among TBI subjects, the relationship between neurobehavioral tests and symptom surveys and ΔCVR measures was assessed (Table 3). There was no correlation between normalized neuropsychological test or symptom survey scores and baseline CVR or ΔCVR measures.

Sildenafil safety and tolerability

Twenty-three symptomatic TBI subjects enrolled in the 18-week double-blind crossover trial and 21 completed. No subject discontinued active drug during the trial. One subject discontinued study drug during the placebo phase because of transient palpitations and subjective visual changes. Another subject never initiated the drug trial because of study logistics. Daily sildenafil was not associated with headache worsening. Headache severity scores increased by 6 or more points on the HIT-6 survey during the sildenafil treatment arm in four subjects (18%), decreased in 7 (31.8%) and remained unchanged in the remaining 11 subjects; conversely headache severity scores increased by ≥ 6 points during the placebo phase in 4 subjects (17%), decreased in 3 (13%) and remained unchanged in 16 subjects (Table 4). There was no difference in headache scores between the sildenafil and placebo-treatment phases ($P = 0.99$).

Neurobehavioral assessments and symptom reports after 8-week sildenafil therapy

There was no statistically significant difference in neuropsychological tests scores or symptom reports assessed after 8 weeks of sildenafil treatment compared to those after 8 weeks of placebo (data not shown). There was no correlation between the change in neuropsychological test scores between baseline and 8 weeks of sildenafil therapy and baseline CVR (Table 5). There was a trend level correlation between improvement in the TMT-A score after sildenafil treatment and ΔCVR (Spearman's $r = 0.55$, $P = 0.028$). There was also a trend toward improvement in the anxiety component of the BSI-13 at the completion of the sildenafil treatment phase and ΔCVR at the baseline visit (Spearman $r = -0.48$, $P = 0.057$). These p values were not corrected for multiple comparisons.

Trend toward clinical benefit

From CGI survey responses, there was a trend toward subjective clinical improvement at the end of 8-week therapy with sildenafil. Among TBI subjects, 6/22 (27%)

Table 2. Measures of global CVR, before and after single dose of sildenafil 50 mg, for the HC and the TBI groups.

	Global CVR, before sildenafil	Global CVR, after sildenafil	ΔCVR
HC (n = 15), mean ± SD	0.223 ± 0.014	0.218 ± 0.017	−0.006 ± 0.010
TBI (n = 31), mean ± SD	0.183 ± 0.028	0.200 ± 0.026	0.017 ± 0.018
P value	<0.0001	0.018	<0.0001
Cohen's d	0.90	0.69	0.86
AUC (95% CI)	0.90 (0.79, 1.0)	0.69 (0.52, 0.86)	0.89 (0.80, 0.98)

ΔCVR is the difference between global CVR measures before and after single-dose sildenafil. The statistical differences between groups are characterized with their P value (t-test), effect size (Cohen's d) and area under the curve (AUC), with 95 confidence interval. HC, healthy control; TBI, traumatic brain injury subject; CVR, cerebrovascular reactivity; C.I., confidence interval.

noted a clinical improvement during the sildenafil treatment phase, and of those 2/22 (9%) noted much or very much improvement. One of the two patients who noted significant improvement during active drug treatment described dramatic subjective improvement in processing speed, memory and cognitive functioning. The other patient reported that he felt mentally clearer, and had improved sleep and decreased headaches. In contrast, only 2/22 (9%) noted any clinical improvement during the placebo phase, and neither rated the effect as being much or very much improved. 11/22 (50%) noted no changes clinically during either active drug or placebo phase. A Fisher test gave a relative risk of benefit of 4.00 (95% confidence interval 0.95–16.8, $P = 0.069$). We did not find that participants who had lower CVR at baseline or those who experienced a larger ΔCVR after sildenafil were more likely to have reported a subjective benefit.

Efficacy of blinding

Two TBI subjects complained of erectile dysfunction at baseline. Neither noted improved sexual function after either 8-week drug phase, either sildenafil or placebo. While on active drug, 14 of 22 (64%) guessed correctly that they were taking sildenafil, but for multiple different reasons: decreased headache (4), flushing and rhinitis (3), improved memory (2), improved sexual function (despite no report of erectile dysfunction at baseline) (1), increased headache (1), improved sleep (1), and nausea (1) or muscle aches (1). The remaining eight either could not distinguish any differences between the two drug phases or their guess was not recorded. During the placebo phase, one participant incorrectly guessed that he was on active drug because of subjective improved sexual function.

Discussion

TCVI, a relatively understudied endophenotype of TBI, is an attractive target for therapeutic intervention as there are multiple well established pharmacologic and non-pharmacologic therapies which improve blood vessel injury and promote vascular health. Candidate pharmacologic therapies targeted at improving cerebrovascular function include PDE-5 inhibitors, 3-hydroxy-3-methyl-glutaryl coenzyme-A (HMG-CoA) reductase inhibitors, high-density lipoprotein (HDL) mimetics, and peroxisome proliferator-activated receptor- gamma (PPAR-γ) agonists, among others.[27,39] Non-pharmacologic therapies targeted at improving vascular function include aerobic exercise, dietary interventions, and nutraceuticals such as omega-3 fatty acids.[28]

While the pathogenesis of vascular injury after TBI has not been completely elucidated, there is evidence of traumatic cerebral microvascular injury by histochemical and ultrastructural studies even in brain regions remote from the focal injuries.[13,21,40] In healthy endothelial cells, NO is the primary endogenous vasodilator and exerts its vasodilatory effects through increased intracellular cyclic guanosine monophosphate (cGMP). PDE5 inhibitors, like sildenafil, extend the action of normally short-lived cGMP and improve microvascular function when endothelial cells fail to produce sufficient NO. Furthermore, in studies on stroke animal models, sildenafil appears to have angiogenic properties in addition to its immediate vasodilatory effects.[41–45] Sildenafil enters the brain readily and is a good candidate therapy for TCVI because of these vasodilatory and microvascular reparative properties.

In this study, we used MRI to investigate an individual's response to a single dose of a selective PDE-5 inhibitor and its potential as a biomarker of TCVI in chronic moderate and severe TBI. Furthermore, we carried out a double-blind crossover trial of sildenafil 25 mg twice daily in TBI patients to assess its safety and tolerability and, in an exploratory fashion, to detect any clinical benefit. Measuring the CVR response to hypercapnia to assess microvascular function allows us to use a stimulus independent of neuronal activity, thus directly measuring microvascular function independent of neuronal metabolism.[26]

At baseline testing, global CVR and ΔCVR measures reliably differentiate chronic TBI from HC subjects. Sildenafil administration in HC did not result in positive ΔCVR (Fig. 1B), rather a paradoxical minor CVR decrease was noted. This is attributable to desensitization of the cerebral microvasculature after administration of a second hypercapnia challenge within a short time period
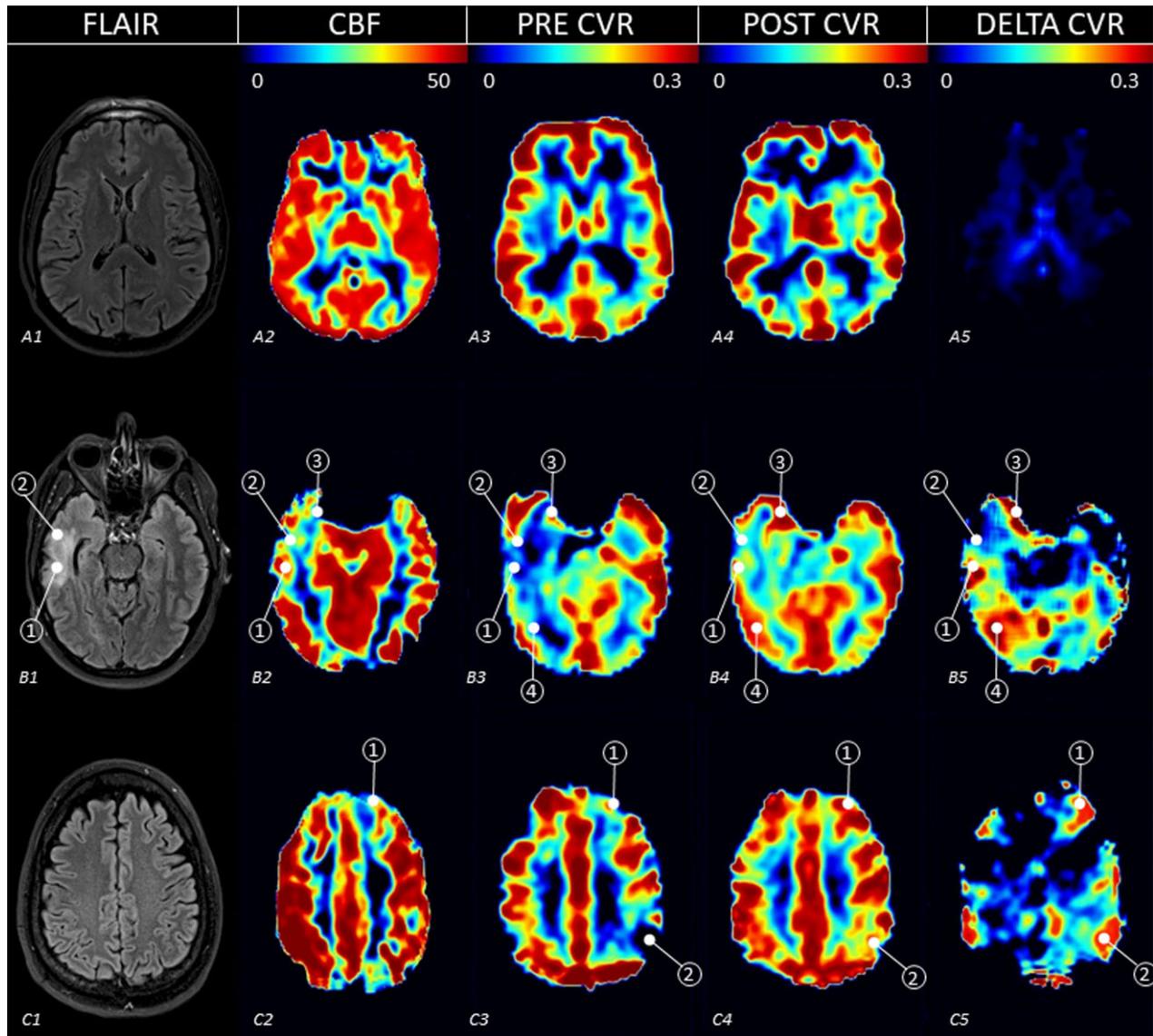
Figure 2. Structural, CBF and CVR maps from 1 HC and 2 TBI patients. Structural (FLAIR), CBF and CVR maps of one healthy control (A1 to A5), one TBI subject with focal gliosis (B1 to B5), and one TBI subject with no structural abnormality (C1 to C5). CBF and pre-sildenafil CVR maps were acquired before sildenafil administration. Post-sildenafil CVR maps were acquired one hour after single dose of sildenafil 50mg. ΔCVR is the difference between the post-sildenafil and the pre-sildenafil CVR maps. All images were co-registered and re-sliced with the structural image (FLAIR). Regions of visible encephalomalacia (region B2) typically showed low CBF, low CVR, and low ΔCVR. We interpret these findings as indicating that in regions with extensive microvasculature damage, there is insufficient NO produced to allow potentiation by sildenafil. More commonly in regions where there is no visible encephalomalacia noted on FLAIR (such as in regions B4, C1, and C2) CBF may be normal or low, but CVR is low, a robust ΔCVR is noted. We interpret these findings as indicating that, in these areas without structural abnormality, some NO is produced by damaged endothelial cells, insufficient to produce normal vasodilation to the hypercapnia stimulus, but enough to produce normal or near-normal vasodilation with the addition of NO enhancement by PDE5 inhibition.

(<1 h in our study experimental design). In contrast, we found a uniform potentiation of CVR (positive ΔCVR) among chronic TBI subjects, reflecting the near universal presence of cerebral microvascular dysfunction (Fig. 1A). We observed this potentiating response even among TBI subjects whose global CVR measures did not differ

significantly from those of the healthy controls. This suggests that TBI patients may benefit from PDE5 inhibitor therapy even when their baseline CVR is normal. Two TBI patients (<10%) failed to show a positive ΔCVR. These may be patients who did not have TCVI and exhibited negative ΔCVR after sildenafil as did the uninjured

Table 3. Spearman's correlations, between global CVR, ΔCVR, neuropsychological test scores and symptom outcomes.

	Global CVR (Before Sildenafil)		ΔCVR	
	Correlation coefficient	Sig. (2-tailed)	Correlation coefficient	Sig. (2-tailed)
WRAT	−0.054	0.774	0.062	0.742
WAIS	0.120	0.522	0.331	**0.069**
TMT A	−0.194	0.295	0.266	0.148
TMT B	−0.084	0.654	0.044	0.815
CVLT	−0.033	0.860	0.193	0.297
GOS-E	−0.011	0.954	−0.022	0.908
RIVERMEAD3	0.075	0.689	0.034	0.857
RIVERMEAD13	0.270	0.142	0.062	0.740
BSI (total)	0.409	**0.025**	−0.282	0.132
Somatic	0.270	0.149	−0.248	0.187
Depression	0.168	0.374	−0.092	0.630
Anxiety	0.305	0.101	−0.293	0.117

WRAT, Wide Range Achievement Test; WAIS-PSI, Wechsler Adult Intelligence Scale-Processing Speed Index; TMT, Trail Making Test; GOS-E, Glasgow Outcome Scale-Extended; CVLT, California Verbal Learning Test; BSI, Brief Symptom Inventory. Sig., Significance (*P* value). Bolded values indicate significance with p less than 0.05 or trend towards significance.

Table 5. Correlation between change in neuropsychological test and symptom survey scores at baseline and after 8 weeks of sildenafil and ΔCVR (change in global CVR) after single dose of sildenafil.

	Spearman	*P* value
WRAT	−0.01	0.98
WAIS	0.20	0.47
TMT-A	0.55	**0.028**
TMT-B	−0.01	0.98
CVLT	0.16	0.56
BSI som	0.06	0.82
BSI dep	−0.19	0.47
BSI anx	−0.48	**0.057**
BSI global	−0.31	0.24
RIVERMEAD 3	0.07	0.80
RIVERMEAD 13	−0.29	0.28
Headache (Rivermead)	−0.16	0.57
Headache (interview)	−0.36	0.17

WRAT, Wide Range Achievement Test; WAIS, Wechsler Adult Intelligence Scale; TMT-A, Trail Making Test Part A; TMT-B, Trail Making Test Part B; CVLT, California Verbal Learning Test; BSI, Brief Symptom Inventory; som, somatic; dep, depression; anx, anxiety. Bolded values indicate significance with p less than 0.05 or trend towards significance.

controls. TBI is a heterogeneous disease, and it is likely that some patients may have no or very low degrees of TCVI. Such patients may not be good candidates for TCVI-directed therapies.

While nearly all TBI subjects had abnormalities detectable on structural imaging (MPRAGE, FLAIR, or SWI), most of the regions of abnormally low CVR or high ΔCVR appeared in normal-appearing brain (Fig. 2). CVR and ΔCVR measures show asymmetry in the chronic TBI population that reliably differentiates them from HC subjects. As noted in Figure 2B1, in cases with focal lesions visible on structural images, ΔCVR increases were prominent around, rather than within, these areas of encephalomalacia. One interpretation of these results is that in areas of focal injury, the local microvasculature has been sufficiently impaired so that it no longer carries sufficient blood flow or produces sufficient NO, resulting in the observed focal deficits in

both CBF and CVR along with the absence of measurable ΔCVR after sildenafil administration. The endothelial cells in these regions are either absent or so physiologically damaged that they fail to produce sufficient NO in response to hypercapnia to allow vasodilation even when NO signaling is potentiated by PDE5 inhibition. In contrast, we identified areas with normal appearing structure and CBF, but with focally decreased CVR and positive ΔCVR. These areas were commonly adjacent to regions of encephalomalacia on structural imaging, but also occurred in areas distant from focal lesions. We interpret these findings as indicating that the microvasculature in these regions has normal or near-normal density, but has been sufficiently damaged so that it cannot vasodilate normally in response to a hypercapnia challenge. After a single dose of sildenafil, its physiologic response is improved by PDE-5 inhibition, because the drug potentiates the endothelial derived NO. While the global ΔCVR is modest (Fig. 1), the ΔCVR produced by

Table 4. Headache severity scores from the Rivermead post-concussion symptoms questionnaire (RPQ) and the Headache Impact Test-6 (HIT-6) for 31 TBI subjects.

	Mean (SD)			Median		
	Baseline	Active drug	Placebo	Baseline	Active drug	Placebo
RPQ headache score	1.9 (1.2)	1.7 (1.3)	1.7 (1.4)	2	2	2
HIT-6 score	48 (12)	48 (11)	50 (13)	45	47	49

The scores were obtained at baseline, after 8 weeks of placebo and after 8 weeks of active drug (sildenafil). Each score is characterized by its mean, standard deviation (in parenthesis) and median.

sildenafil in focal areas of endothelial dysfunction is quite robust, usually >10% and resulting in near-normalization of CVR in those regions.

Among chronic TBI subjects, oral sildenafil 25 mg twice daily was safe and well tolerated. No subject withdrew because of sildenafil adverse effects. Since headache is very common in chronic TBI subjects and is also the most common side effect of sildenafil treatment (occurring in up to 16% of users for other indications),[46] our study was designed to carefully assess headache prevalence and severity before and during sildenafil treatment. Headaches were more common among the TBI subjects than in HC, but headache burden, as measured by the HIT-6, did not increase during the 8-week sildenafil treatment phase. No trial participant dropped out because of headache, but a larger study is necessary to determine its frequency and impact in chronic TBI patients. While almost two-thirds of patients guessed correctly when they were taking active drug, the reasons were generally unrelated to the well-known effect of sildenafil on erectile dysfunction. Since so many patients correctly identified the phase of the study when they were taking active drug, it is difficult to assess a possible placebo effect. Future studies with larger sample sizes will look at the relationship between subjective benefit and global and regional ΔCVR.

There was no significant correlation between neuropsychological test performance or symptom self-report between the TBI subjects and CVR or ΔCVR measures. It is not surprising that the relationship between global CBF, CVR, and ΔCVR with neurobehavioral symptoms and neuropsychological performance is weak or absent. It is likely that analysis of regional abnormalities in cerebrovascular parameters will be needed to identify relationships with particular cognitive functions. Large sample sizes and sophisticated multivariate models will be required to fully assess the relationship between global and regional CBF, CVR, and ΔCVR and outcome after TBI. The study was not powered to detect a clinically significant benefit, but the correlation between ΔCVR and improvement in processing speed (TMT-A) and anxiety symptoms after 8 weeks of sildenafil therapy needs to be studied further.

Our sample size was too small to evaluate the effect of non-TBI related co-morbidities (such as hypertension) on CBF and CVR. We did exclude participants older than 55 years, as CVR is known to decrease in older individuals. Since older individuals are at high risk for TBI, future studies will be needed to explore the relationship between pre-morbid vascular risk factors and TCVI.

In summary, this study shows that ΔCVR in response to a single dose of sildenafil, a dynamic measure of microvascular function, is a sensitive biomarker of TCVI. Further, in this Phase IIa clinical trial, sildenafil 25 mg twice daily was well tolerated, and was not associated with increased headaches. The study was not designed to determine the timing, dose, and length of therapy for maximal efficacy. Finally, while larger studies are required to determine the role of sildenafil as therapy for TCVI, our findings support the use of CBF, CVR, and ΔCVR to screen for patients who may benefit from a vascular reparative therapy, and as a marker of target engagement and response to therapy.

Acknowledgments

Conflict of Interest

The authors have no conflicts of interest to disclose.

References

1. Rutland-Brown W, Langlois JA, Thomas KE, Xi YL. Incidence of traumatic brain injury in the United States, 2003. J Head Trauma Rehabil 2006;21:544–548.
2. Faul MXL, Wald MW, Coronado VG. Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalizations, and Deaths 2002 - 2006. 2010 Centers for Disease Control and Prevention, National Center for Injury Prevention and Control.
3. Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. J Head Trauma Rehabil 2006;21:375–378.
4. National Research Council. Gulf War and Health: Volume 7: Long-Term Consequences of Traumatic Brain Injury. 2008; Washington (DC).
5. Langlois JA, Sattin RW. Traumatic brain injury in the United States: research and programs of the Centers for Disease Control and Prevention (CDC). J Head Trauma Rehabil 2005;20:187–188.
6. Narayan RK, Michel ME, Ansell B, et al. Clinical trials in head injury. J Neurotrauma 2002;19:503–557.
7. McIntosh TK. Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: a review. J Neurotrauma 1993 Fall;10:215–261.
8. McIntosh TK, Juhler M, Wieloch T. Novel pharmacologic strategies in the treatment of experimental traumatic brain injury: 1998. J Neurotrauma 1998;15:731–769.
9. Marklund N, Bakshi A, Castelbuono DJ, et al. Evaluation of pharmacological treatment strategies in traumatic brain injury. Curr Pharm Des 2006;12:1645–1680.

10. Doppenberg EM, Bullock R. Clinical neuro-protection trials in severe traumatic brain injury: lessons from previous studies. J Neurotrauma 1997;14:71–80.

11. Saatman KE, Duhaime AC, Bullock R, et al. Classification of traumatic brain injury for targeted therapies. J Neurotrauma 2008;25:719–738.

12. Graham D, Gennarelli T, McIntosh T. Cellular and molecular consequences of TBI. In: D. I. Graham, L. PL, editors. Greenfield's Neuropathology. pp. 823–898. London: Arnold; 2002.

13. Stein SC, Graham DI, Chen XH, Smith DH. Association between intravascular microthrombosis and cerebral ischemia in traumatic brain injury. Neurosurgery 2004;54:687–691.

14. Yuh EL, Mukherjee P, Lingsma HF, et al. Magnetic resonance imaging improves 3-month outcome prediction in mild traumatic brain injury. Ann Neurol 2013;73:224–235.

15. Chiara Ricciardi M, Bokkers RP, Butman JA, et al. Trauma-specific brain abnormalities in suspected mild traumatic brain injury patients identified in the first 48 hours after injury: a blinded magnetic resonance imaging comparative study including suspected acute minor stroke patients. J Neurotrauma 2017;34:23–30.

16. Baranova AI, Wei EP, Ueda Y, et al. Cerebral vascular responsiveness after experimental traumatic brain injury: the beneficial effects of delayed hypothermia combined with superoxide dismutase administration. J Neurosurg 2008;109:502–509.

17. Gao G, Oda Y, Wei EP, Povlishock JT. The adverse pial arteriolar and axonal consequences of traumatic brain injury complicated by hypoxia and their therapeutic modulation with hypothermia in rat. J Cereb Blood Flow Metab 2010;30:628–637.

18. Wei EP, Hamm RJ, Baranova AI, Povlishock JT. The long-term microvascular and behavioral consequences of experimental traumatic brain injury after hypothermic intervention. J Neurotrauma 2009;26:527–537.

19. Oda Y, Gao G, Wei EP, Povlishock JT. Combinational therapy using hypothermia and the immunophilin ligand FK506 to target altered pial arteriolar reactivity, axonal damage, and blood-brain barrier dysfunction after traumatic brain injury in rat. J Cereb Blood Flow Metab 2011;31:1143–1154.

20. Park E, Bell JD, Siddiq IP, Baker AJ. An analysis of regional microvascular loss and recovery following two grades of fluid percussion trauma: a role for hypoxia-inducible factors in traumatic brain injury. J Cereb Blood Flow Metab 2009;29:575–584.

21. Rodriguez-Baeza A, Reina-de la Torre F, Poca A, et al. Morphological features in human cortical brain microvessels after head injury: a three-dimensional and immunocytochemical study. Anat Rec A Discov Mol Cell Cvol Biol 2003;273:583–593.

22. Yezhuvath US, Lewis-Amezcua K, Varghese R, et al. On the assessment of cerebrovascular reactivity using hypercapnia BOLD MRI. NMR Biomed 2009;22:779–786.

23. Kassner A, Roberts TP. Beyond perfusion: cerebral vascular reactivity and assessment of microvascular permeability. Top Magn Reson Imaging 2004;15:58–65.

24. Bailey DM, Jones DW, Sinnott A, et al. Impaired cerebral haemodynamic function associated with chronic traumatic brain injury in professional boxers. Clin Sci (Lond) 2013;124:177–189.

25. Gardner AJ, Tan CO, Ainslie PN, et al. Cerebrovascular reactivity assessed by transcranial Doppler ultrasound in sport-related concussion: a systematic review. Br J Sports Med 2015;49:1050–1055.

26. Amyot F, Kenney K, Moore C, et al. Imaging of cerebrovascular function in chronic traumatic brain injury. J Neurotrauma 2017; https://doi.org/10.1089/neu.2017.5114. [Epub ahead of print].

27. Nicolakakis N, Aboulkassim T, Aliaga A, et al. Intact memory in TGF-beta1 transgenic mice featuring chronic cerebrovascular deficit: recovery with pioglitazone. J Cereb Blood Flow Metab 2011;31:200–211.

28. Wang J, Shi Y, Zhang L, et al. Omega-3 polyunsaturated fatty acids enhance cerebral angiogenesis and provide long-term protection after stroke. Neurobiol Dis 2014;68:91–103.

29. Inoue M, McHugh M, Pappius HM. The effect of alpha-adrenergic receptor blockers prazosin and yohimbine on cerebral metabolism and biogenic amine content of traumatized brain. J Cereb Blood Flow Metab 1991;11:242–252.

30. Pappius HM, Dadoun R, McHugh M. The effect of p-chlorophenylalanine on cerebral metabolism and biogenic amine content of traumatized brain. J Cereb Blood Flow Metab 1988;8:324–334.

31. Bellando-Randone S, Lepri G, Bruni C, et al. Combination therapy with Bosentan and Sildenafil improves Raynaud's phenomenon and fosters the recovery of microvascular involvement in systemic sclerosis. Clin Rheumatol 2016;35:127–132.

32. Wu J, Li C, Yuan W. Phosphodiesterase-5 inhibition improves macrocirculation and microcirculation during cardiopulmonary resuscitation. Am J Emerg Med 2016;34:162–166.

33. Sperandio PA, Oliveira MF, Rodrigues MK, et al. Sildenafil improves microvascular O2 delivery-to-utilization matching and accelerates exercise O2 uptake kinetics in chronic heart failure. Am J Physiol Heart Circ Physiol 2012;303:H1474–H1480.

34. Mandosi E, Giannetta E, Filardi T, et al. Endothelial dysfunction markers as a therapeutic target for Sildenafil treatment and effects on metabolic control in type 2 diabetes. Expert Opin Ther Targets 2015;19:1617–1622.

35. Post-concussion syndrome. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (4th ed., text rev.). Washington, DC: American Psychiatric Association, 2000. http://dsm.psychiatryonline. org/doi/abs/10.1176/appi.books.9780890420249.dsm-iv-tr

36. Yezhuvath US, Uh J, Cheng Y, et al. Forebrain-dominant deficit in cerebrovascular reactivity in Alzheimer's disease. Neurobiol Aging 2012;33:75–82.

37. Zhu YS, Tarumi T, Tseng BY, et al. Cerebral vasomotor reactivity during hypo- and hypercapnia in sedentary elderly and Masters athletes. J Cereb Blood Flow Metab 2013;33:1190–1196.

38. Lu H, Liu P, Yezhuvath U, et al. MRI mapping of cerebrovascular reactivity via gas inhalation challenges. J Vis Exp 2014;94:1–9.

39. Nicolakakis N, Hamel E. Neurovascular function in Alzheimer's disease patients and experimental models. J Cereb Blood Flow Metab 2011;31:1354–1370.

40. Oppenheimer DR. Microscopic lesions in the brain following head injury. J Neurol Neurosurg Psychiatry 1968;31:299–306.

41. Atalay B, Caner H, Cekinmez M, et al. Systemic administration of phosphodiesterase V inhibitor, sildenafil citrate, for attenuation of cerebral vasospasm after experimental subarachnoid hemorrhage. Neurosurgery 2006;59:1102–1107.

42. Ding G, Jiang Q, Li L, et al. Magnetic resonance imaging investigation of axonal remodeling and angiogenesis after embolic stroke in sildenafil-treated rats. J Cereb Blood Flow Metab 2008;28:1440–1448.

43. Pifarre P, Prado J, Giralt M, et al. Cyclic GMP phosphodiesterase inhibition alters the glial inflammatory response, reduces oxida-tive stress and cell death and increases angiogenesis following focal brain injury. J Neurochem 2010;112:807–817.

44. Puzzo D, Staniszewski A, Deng SX, et al. Phosphodiesterase 5 inhibition improves synaptic function, memory, and amyloid-beta load in an Alzheimer's disease mouse model. J Neurosci 2009;29:8075–8086.

45. Zhang R, Wang Y, Zhang L, et al. Sildenafil (Viagra) induces neurogenesis and promotes functional recovery after stroke in rats. Stroke 2002;33:2675–2680.

46. Package insert, sildenafil citrate, Table 1. 2015. http://med library.org/lib/rx/meds/viagra-1/.