Transient Hypertension after Spinal Cord Injury Leads to Cerebrovascular Endothelial Dysfunction and Fibrosis

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Abstract

We aimed to create a clinically relevant pre-clinical model of transient hypertension, and then evaluate the pathophysiological cerebrovascular processes resulting from this novel stimulus, which has recently been epidemiologically linked to cerebrovascular disease. We first developed a clinically relevant model of transient hypertension, secondary to induced autonomic dysreflexia after spinal cord injury and demonstrated that in both patients and rats, this stimulus leads to drastic acute cerebral hyperperfusion. For this, iatrogenic urodynamic filling/penile vibrostimulation was completed while measuring beat-by-beat blood pressure and cerebral blood flow (CBF) in patients. We then developed a rodent model mimicking the clinical reality by performing colorectal distention (to induce autonomic dysreflexia) using pre-clinical beat-by-beat blood pressure and CBF assessments. We then performed colorectal distention in rats for four weeks (6x/day) to evaluate the long-term cerebrovascular consequences of transient hypertension. Outcome measures included middle cerebral artery endothelial function, remodeling, profibrosis and perivascular innervation; measured via pressure myography, immunohistochemistry, molecular biology, and magnetic resonance imaging. Our model demonstrates that chronic repetitive cerebral hyperperfusion secondary to transient hypertension because of autonomic dysreflexia: (1) impairs cerebrovascular endothelial function; (2) leads to profibrotic cerebrovascular stiffening characterized by reduced distensibility and increased collagen deposition; and (3) reduces perivascular sympathetic cerebrovascular innervation. These changes did not occur concurrent to hallmark cerebrovascular changes from chronic steady-state hypertension, such as hypertrophic inward remodeling, or reduced CBF. Chronic exposure to repetitive transient hypertension after spinal cord injury leads to diverse cerebrovascular impairment that appears to be unique pathophysiology compared with steady-state hypertension in non-spinal cord injured models.

Keywords: cerebrovascular disease; transient hypertension; translational health

Introduction

CHRONIC STEADY-STATE HYPERTENSION is the most powerful modifiable risk factor for cerebrovascular diseases¹ and, as such, has received appropriate clinical and research attention. As a clinical community, we have become aware more recently that in addition to chronic hypertension, there is also significantly elevated cerebrovascular disease risk in persons with chronic exposure to labile blood pressure, such as repetitive transient hypertension.² Repetitive bouts of transient hypertension are common in a vast number of prevalent activities including acute caffeine consump-

tion,³ resistance exercise,⁴ high-intensity interval exercise,⁵ defecation, exaggerated blood pressure responses to mental stress,⁶ and drug abuse.⁷ The majority of these stimuli have been linked with stroke^{6,8,9}; however, the direct cerebrovascular consequences of transient hypertension have never been examined.

Repetitive exposure to transient hypertension may exert a unique form of cerebrovascular decline compared with the well-characterized pathophysiology associated with steady-state hypertension, which includes endothelial dysfunction, fibrotic hypertrophic inward remodeling, reduced cerebral blood, and elevated cerebrovascular myogenic tone.^{10,11} Understanding the structural and

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functional changes in the cerebrovasculature resulting from transient hypertension would be invaluable to help understand and potentially manage this condition.

Spinal cord injury (SCI) provides a useful model to study transient hypertension, because injury above the sixth thoracic spinal segment leads to the loss of supraspinal control of autonomic spinal circuitry, resulting in vascular and neuroanatomical changes caudal to the injury, predisposing the spinal sympathetic circuitry to overreact to afferent stimuli, leading to rapid elevations in blood pressure. These episodes of sympathetic overactivation most often result from a full bladder/bowel or sexual stimulation after SCI. Blood pressure can elevate in a matter of seconds up to 300 mm Hg, a phenomena termed autonomic dysreflexia (AD).^{12,13} These transient increases, which occur on average 11 times/day after high-level SCI,¹² can elicit serious clinical consequences. For example, we¹⁴ and others¹⁵ have shown that patients with SCI not only have a staggering 3–4-fold increased odds/risk of stroke (six strokes/1000 person-years), but also from significant cerebrovascular dysfunction.^{16–18}

Here, we first establish the clinical reality of transient hypertension in humans with SCI by characterizing the acute cerebrovascular responses to transient hypertension from AD. Next, to invasively examine the structural alterations and underlying pathophysiology resulting from transient hypertension, we developed a pre-clinical model of transient hypertension in SCI that mimics the clinical scenario. Following this, we employed the model to evaluate whether chronic (four weeks) transient hypertension impairs cerebrovascular function and structure using *in vitro* pressure myography of the middle cerebral artery as well as magnetic resonance imaging (MRI) to evaluate the *in vivo* impact on regional and global cerebral blood flow (CBF). Extrapolating from the chronic steady-state literature,^{19,20} we hypothesized that chronic exposure to transient hypertension would result in declined cerebrovascular health evidenced by impaired endothelial function, profibrotic protein expression, altered constrictive responsiveness, and reduced global CBF.

Methods

Clinical evaluation

We first evaluated the acute effects of transient hypertension on CBF during 12 episodes of AD occurring in six patients undergoing iatrogenic procedures known to elicit AD (i.e., urodynamic filling and penile vibrostimulation for sperm retrieval).^{21,22}

Experimental protocol. Both urodynamic filling and penile vibrostimulation for sperm retrieval are clinical procedures required for persons with SCI, which unfortunately are well documented to elicit AD iatrogenically.^{21,22} Participants included eight male individuals ($n=3$ urodynamics assessments, $n=5$ penile vibrostimulation) with complete cervical chronic SCI (age 37 ± 4 years; $n=4$ American Spinal Injury Association Impairment Scale [AIS]-A; $n=2$ AIS-B; $n=2$ AIS-C; all >2 years post-SCI). The level and completeness of injury was determined by a licensed physician (AVK). Participants had no history of cardiovascular disease. Informed consent was obtained from all subjects, and the protocol was approved by the University of British Columbia Clinical Research Ethics Board, and adhered to the Declaration of Helsinki.

For both urodynamics and penile vibrostimulation, blood pressure was recorded every minute on the right arm using an automated sphygmomanometer (Dinamap V100; GE Medical Systems, Fairfield, CT), using a fitted cuff. Three supine measurements were taken before the procedure and averaged to determine baseline supine blood pressure. Beat-by-beat blood pressure and CBF were measured as described previously.^{17,23}

Urodynamic assessments. Before urodynamics, a urinary dipstick test confirmed the absence of infection. Urodynamics occurred between 0800 and 1200 h in a temperature-controlled room (21°C) according to established standards.²⁴ Cystometry was performed by a double-lumen catheter (6 Fr, Laborie, Canada) with continuous filling of sterile water (37°C) at a fixed rate of 30 mL/min. Abdominal pressure was measured with an intrarectal balloon catheter (10 Fr, Laborie, Canada). Pelvic floor electromyography (EMG) (Aquarius TT, Laborie Model 94-R03-BT, Montreal, Quebec, Canada) was recorded using surface EMG. Filling was stopped if any of the following conditions were observed: (1) reported sensation of fullness; (2) spontaneous urine leakage; (3) intravesical pressure ≥ 40 cm H₂O; (4) infused volume reached 500 mL; or (5) sustained systolic blood pressure ≥ 180 mm Hg or intolerable AD symptoms (e.g., severe goose bumps, sweating, headache, perceived head pressure pulsations that were not present previously, etc.).

Penile vibrostimulation for sperm retrieval. Penile vibrostimulation was applied by a physician experienced in penile vibrostimulation using one or more handheld vibrators (Ferti Care, Multicept, Rungsted, Denmark) placed about the glans penis to induce ejaculation. An amplitude of 1.0–3.5 mm with a frequency of 70–100 Hz was used. Vibration was performed for a maximum of 3 min, followed by a pause of 1 min before the cycle was repeated until ejaculation was achieved.

Clinical cardiovascular and cerebrovascular assessments. The following were sampled at 1000 Hz using an analog-to-digital converter (Powerlab/16SP ML 795; ADInstruments, Colorado Springs, CO) interfaced with data acquisition software (LabChart8; ADInstruments): beat-by-beat blood pressure via finger photoplethysmography (Finometer PRO; Finapres Medicine Systems, Amsterdam, Netherlands), electrocardiogram (ML132; ADInstruments) on the left side, and blood velocity in the left middle cerebral artery (ST3 Transcranial Doppler, Spencer Technologies, Redmond, WA). The middle cerebral artery was insonated using a 2 MHz probe mounted on the temporal bone and a fitted headstrap. The middle cerebral artery (MCA) was insonated at a depth of 45–55 mm and confirmed using ipsilateral common carotid artery compression.

The autonomic dysreflexia was defined by a ≥ 20 mm Hg increase in systolic blood pressure and confirmed by a physician with expertise in this condition (AVK). Mean arterial blood pressure was calculated as $([2 \times \text{diastolic blood pressure}] + [\text{systolic blood pressure}]) / 3$ while mean CBF velocity was calculated by $([2 \times \text{minimum flow velocity}] + [\text{peak flow velocity}]) / 3$. Together a scientist and clinician with expertise in autonomic dysreflexia identified at least three heart beats corresponding to maximum blood pressure (typically three beats) during AD were extracted to calculate the peak change in blood pressure and CBF.

Pre-clinical model

Ethical approval. All procedures were conducted in strict accordance with the Canadian Council for Animal Care. Ethics approval was granted by the University of British Columbia.

Experimental protocol. Surgery and animal care were conducted according to standard procedures in our laboratory to perform a complete spinal transection at the T3 spinal segment.²⁵ Four male rats (age = nine weeks, mass = 250–350 g; Harlan Laboratories, Indianapolis, IN) were used initially to develop the translational model of transient hypertension and ensuing cerebral hyperperfusion. Next, using this model, 50 male rats (identical age, strain, and supplier) were randomly assigned either (Fig. 1d): SCI (T3-SCI; $n=25$), or SCI with AD (T3-SCI+AD; $n=25$). Two

FIG. 1. Developing a clinically relevant pre-clinical model of transient cerebral hyperperfusion secondary to autonomic dysreflexia. Human data: **(a)** Cerebral hyperperfusion during 16 unique episodes of transient hypertension secondary to autonomic dysreflexia occurring in eight patients with chronic spinal cord injury (SCI) above the sixth thoracic spinal cord segment. Animal data: **(b)** Profile of cerebral hyperperfusion during 12 unique episodes of transient hypertension during autonomic dysreflexia induced by colorectal distension in four Wistar rats with a T3 complete spinal cord transection. **(c)** Profile of cerebral hyperperfusion during nine episodes of transient hypertension in three uninjured rats in response to bolus injections of phenylephrine. *Note the similar hemodynamic profile. **(d)** Timeline of experiments. Colorectal distension began 14 days post-SCI. Sample sizes: pressure myography (SCI $n=13$, SCI and colorectal distension $n=15$); immunohistochemistry (SCI $n=6$, SCI and colorectal distension $n=5$); quantitative polymerase chain reaction (qPCR) (SCI $n=6$, SCI and colorectal distension $n=6$); magnetic resonance imaging (MRI) (SCI $n=7$, SCI and colorectal distension $n=5$). Outcome measures took place after four weeks of colorectal distension and included pressure myography, MRI, immunohistochemistry (IHC), and qPCR. MA, middle cerebral artery.

weeks after SCI, repetitive AD induced by colorectal distension (explained below) was initiated for four weeks in the T3-SCI+AD group, which is analogous to two years of human life.²¹ Six weeks after SCI, both groups underwent MRI assessment and were then sacrificed for a range of vascular assessments spanning pressure myography, histology, and molecular biology. All outcome assessments and analysis were performed by blinded personnel.

Pre-clinical spinal cord transection. Animals received prophylactic enrofloxacin (Baytril; 10 mg kg⁻¹, subcutaneously [SC], Associated Veterinary Purchasing [AVP], Langley, Canada) for three days before the SCI surgical procedure. On the day of operation, enrofloxacin (10 mg kg⁻¹, SC), buprenorphine (Temgesic; 0.02 mg kg⁻¹, SC, McGill University), and ketoprofen (Anafen,

5 mg kg⁻¹, SC, AVP) were administered pre-operatively. Rats were anesthetized with ketamine hydrochloride (70 mg kg⁻¹, intraperitoneally [IP]; Vetalar; AVP) and dexmedetomidine (0.25 mg kg⁻¹, IP; Domitor; AVP).

A dorsal midline incision was made in the superficial muscle overlying the C8–T3 vertebrae. The dura was opened at the T2–T3 intervertebral gap, and the spinal cord was completely transected using microscissors. Complete transection was confirmed by two surgeons via visual separation of the rostral and caudal spinal cord stumps, and Gelfoam (Pharmacia & Upjohn Company, Pfizer, New York) was placed between the stumps to achieve hemostasis. The muscle and skin were closed with 4–0 Vicryl and 4–0 Prolene sutures, respectively.

Animals received warmed lactated Ringer solution (5 mL, SC) and recovered in a temperature-controlled environment (Animal

Intensive Care Unit, Los Angeles, CA, USA), after being administered atipamezole (1 mg kg^{-1} , SC, AVP) for reversal of dexmedetomidine. Enrofloxacin (10 mg kg^{-1} , SC), buprenorphine (0.02 mg kg^{-1} , SC) and ketoprofen (5 mg kg^{-1} , SC) were administered once a day for three days post-operatively. The bladder was expressed manually three to four times daily until spontaneous voiding returned (about 10 days post-injury). All assessments and analyses were completed blinded of each condition/group using a secret animal coding system known only to the project lead. Five animals did not survive the SCI transection procedure. Male animals were used because they represent the majority of SCI.²⁶

Ultrasound evaluation of middle cerebral artery blood flow with concurrent blood pressure during acute AD/transient hypertension induced by colorectal distension. After being anesthetized with urethane ($1.5\text{--}2.5\text{ g/kg}$), a calibrated blood pressure catheter (SPR-869, Millar), was implanted in the right femoral artery and was advanced into the right common iliac artery. Further, a $20\text{ mm} \times 10\text{ mm}$ section of the crown of the skull was removed, with care to not pierce the dura. Blood pressure recordings were collected and recorded concurrent to the ultrasound recordings by being input through analog to the ultrasound device (Vevo 3100; Visual Sonics, Inc., a subsidiary of Sonosite, Bothell, WA).

A balloon tip of a Swan-Ganz catheter (10 mm in length) was inserted 2 cm rectally, according to standard procedures to induce transient hypertension secondary to AD.²⁷ For this, the balloon was infused with $1.0\text{--}2.0\text{ mL}$ of air for 60 sec . Three uninjured rats of the same age had transient hypertension induced by bolus injections of $0.05\text{--}0.2\text{ mL}$ ($50\text{ }\mu\text{g/mL}$) phenylephrine (because colorectal distension does not lead to transient hypertension in uninjured rats) through the tail vein, which was cannulated with a 26-gauge butterfly needle.

During scanning, the rat was fixed to a customized scanning platform (Visual Sonics), which was heated to maintain body temperature, while the ultrasound probe (MX250, 24 MHz , Visual Sonics) was aligned to the frontal plane and moved rostrally and caudally; color Doppler was applied to aid in localization of the middle cerebral artery during B-mode imaging (inset Fig. 1b) as described previously.¹⁷ The ultrasound frequency was 16 MHz , while the pulsed repetition frequency was set to 4 kHz . The Doppler sample volume was placed at a depth of $8\text{--}11\text{ mm}$, with Doppler angle set to less than 60 degrees. If we deviated from this for optimization, then these settings were kept consistent throughout the hyperemia. Baseline values and maximum increase in CBF were extracted, along with the corresponding blood pressures. Mean arterial pressure was calculated by $=(2 \times \text{diastolic blood pressure}) + [\text{systolic blood pressure}] / 3$ while mean CBF velocity was calculated by $=(2 \times \text{minimum flow velocity}) + [\text{peak flow velocity}] / 3$.

Repetitive colorectal distension to induce AD. At 14 days post-SCI, the T3-SCI+AD group underwent six successive bouts of AD that we have demonstrated leads to transient hypertension, five days/week for four weeks, each of 10 min duration. In short, the balloon tip catheter was inserted identically as described above; however, in this case inflation was maintained for 10 min .²⁷

Pressure myography. Seven weeks after injury, T3-SCI and T3-SCI+AD rats were anesthetized with 3% isoflurane and euthanized by decapitation after exsanguinations for *ex vivo* pressure myography studies. The MCA function and structure were assessed by pressure myography (Living Systems, St. Albans, VT) as described previously.²⁵ Briefly, we analyzed MCA spontaneous myogenic tone generation and structural and mechanical properties.

A $5 \times 3\text{ mm}$ section of brain tissue containing the MCA was removed and placed in Ca^{2+} -free physiological salt solution (PSS) at 4°C , with 1% bovine serum albumin. A branch-free segment of the MCA was mounted on two glass micropipettes in a pressure myograph (Danish Myo Technology, Aarhus, Denmark). The

MCAs were equilibrated at 80 mm Hg in PSS containing (in mmol) 141.9 NaCl , 4.7 KCl , $1.12\text{ KH}_2\text{PO}_4$, $1.7\text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.8 CaCl_2 , 10 HEPES , 5 dextrose , and 0.5 EDTA ($\text{pH } 7.4$) until development of spontaneous myogenic tone, which was calculated using the following formula: $\% \text{tone} = (1 - [\text{active lumen diameter/passive lumen diameter}]) \times 100$. Passive structure was assessed in calcium-free PSS containing 2 mmol/L EGTA , and intraluminal pressure was increased from 3 to 180 mmHg in 20-mm Hg increments. The wall-to-lumen ratio and circumferential wall stress were calculated.²⁵ Passive distensibility was calculated as described previously.²⁵ The elastic modulus (β -coefficient) was calculated from the stress/strain curves using an exponential model ($y = ae^{\beta x}$), where β is the slope of the curve and is directly correlated to vascular stiffness.

Reactivity of the MCA. Endothelial function in the MCA was assessed by extraluminal perfusion of carbachol in a pressure myograph. A branch-free segment of the MCA was isolated and mounted between two glass cannulas in a pressure myograph chamber, which was then placed in an inverted microscope coupled to a camera. The MCA was then bathed in PSS, pressurized at 80 mm Hg to generate spontaneous myogenic tone. Increasing concentrations of carbachol (10^{-10} M to 10^{-5} M) were then added to the bath. After thorough wash with calcium PSS, a single dose of 5-HT (Sigma: H9523) was added (10^{-5} M) to the bath. Changes in MCA diameter were recorded using the MyoView software (Danish Myo Technology, Aarhus, Denmark).

Immunohistochemical analyses. Briefly, the MCAs sections were thoroughly washed in phosphate buffer saline (PBS) and 0.01 M PBS containing 0.3% Triton X-100 (PBST), followed by a 1 h incubation in 10% normal donkey serum in PBST. Sections were then incubated with the primary antibodies listed in Table 1 overnight at room temperature. Subsequently, sections were washed in PBST and incubated accordingly with AlexaTM-fluor 488 donkey [L_AAP1] anti-mouse ($1:1000$; Molecular Probes[®]) or Alexa Fluor 594-conjugated donkey anti-rabbit ($1:1000$; Jackson ImmunoResearch) for 1 h . Finally, all the sections were washed, mounted with Vectashield mounting medium with DAPI (Vector Laboratories Inc.) and observed in laser confocal AxioImager.Z2 Zeiss[®] LSM 800 microscope using identical exposure times, scanning speeds, gains, and pinhole values. The intensity of immunoreactivity of morphological assessment of MCAs was assessed using the Fiji Software (based on ImageJ, <http://fiji.sc/Downloads#Fiji>).

Tyrosine hydroxylase (TH) analysis of MCAs whole mounts. A small segment of the MCA vessel was used to determine TH expression ($1:1000$; anti-rabbit; Millipore; ref: AB152) by immunofluorescence. For this, the vessel was placed in a small Eppendorf tube and the immunoreaction proceeded as described above. Finally, the vessel was placed in a glass microscope slide,

TABLE 1. LIST OF PRIMARY ANTIBODIES USED FOR MORPHOLOGICAL ASSESSMENT OF MIDDLE CEREBRAL ARTERIES

Primary antibody	Antibody concentration	Reference
Rabbit α Collagen I	1:500	Abcam, (ab34710); USA
Mouse α Collagen III	1:300	Abcam, (ab6310); USA
Rabbit α Elastin	1:200	Millipore, (AB2039); USA
Rabbit α SMA	1:400	Abcam, (ab5694); USA

SMA, smooth muscle actin.

mounted with Prolong Gold[®] mounting medium (Molecular Probes[®]) and observed in the confocal microscope. The intensity of immunoreactivity of morphological assessment of MCAs was assessed using the Fiji Software (based on ImageJ). Staining intensity for each animal was determined from the middle third of the MCA.

Morphological assessment of MCA. A segment of the MCA from each animal of different experimental groups was embedded in optimal cutting temperature (OCT) freezing medium. Transverse 10 μ m sections were cut on a cryostat microtome, collected in Superfrost Plus slides, and stored at -80°C until further processing. The morphological assessment of MCAs was determined by immunofluorescence using the primary antibodies listed in Table 1.

Molecular biology assessment of the MCA. Total RNA was extracted from the MCA using the ribonucleic acid (RNA) Micro Kit (Purelink; Carlsbad, CA) following the manufacturer's instructions. For the MCA tissue, the Rotor-Stator Protocol was used, and the TRIzol[®] Reagent Protocol was used for the aorta tissue. The concentration and purity of the extracted RNA were checked by measuring the optical densities at 260 and 280 nm. Total RNA (2.5 μ g) was reverse transcribed into cDNA using Invitrogen[™] SuperScript[™] VILO[™] mastermix (Invitrogen; Carlsbad, CA), and following the manufacturer's guidelines. Template cDNA was mixed with this mastermix, and the expression of genes involved in proinflammatory and profibrosis was studied using 96-well PCR array plates (MicroAMP[®] Fast Optical 96-Well Reaction Plate with Barcode (0.1 mL), 4346906, Applied Biosystems). Quantitative PCR (qPCR) was performed on an Applied Biosystems ViiATM 7 Real-Time PCR System machine using the following PCR cycling conditions: 50°C for 2 min, and 95°C for 10 min, and 40 cycles at 95°C for 15 sec and 50°C for 1 min. The gene expression fold change relative to the control was calculated using the $2^{-\Delta\Delta\text{CT}}$ method.

CBF. MRI was used to estimate CBF with the arterial spin labeling (ASL) technique. In brief, ASL tags the magnetization of inflowing blood by spin inversion, which modifies the overall signal of a downstream brain slice by an amount proportional to the amount of incoming labeled blood. The resultant CBF describes the amount of arterial blood delivered to the imaging slice within the transit time of the experiment. Experiments were performed on a pre-clinical 7 Tesla MRI scanner (Bruker Biospec 70/30 USR, Bruker, Ettlingen, Germany) using a quadrature volume transmit coil and four-channel receive phased-array. Body temperature was maintained by heated air and rectal temperature probe. The animal was maintained under 2% isoflurane anesthesia on a ventilator (Harvard Apparatus Inspira ASV) at 60–80 breaths per minute with expired CO_2 measured with a Physiosuite capnograph (Kent Scientific) and respiration rate measured by a pressure sensor on the chest.

Multiple inversion recovery echo-planar imaging (EPI) scans were acquired to provide calibration data (tissue T1, inversion efficiency, and proton density) for ASL scans (recovery time = 10 sec, echo time (TE) = 11.47 ms, 22 inversion times between 30–8000 ms spaced geometrically, field of view = 40×40 mm, matrix = 64×64 , slice thickness = 2 mm, nine slices, single shot). FAIR-EPI with a pre-saturation preparation was used to acquire the ASL data at three inversion times to allow correction for variable transit times (inversion times = 600, 800, 1000 ms, single-shot readout, TE = 10.5 ms, recovery time after pre-saturation = 1.1 sec, same geometry as calibration scans, GRAPPA factor = 2, partial-FT acceleration = 1.5, imaging time = 10 min 6 sec).

Statistical analyses. The relationship between blood pressure and CBF in humans and rats was evaluated using the Pearson linear correlation. Spontaneous myogenic tone generation, 5-HT

constriction, passive structure at 80 mm Hg, and immunohistochemistry data were analyzed by the Student *t* test or a nonparametric alternative. Concentration-response curves for carbachol were analyzed further by nonlinear regression (Hill equation) to calculate $\log\text{EC}_{50}$ and $\log\text{EC}_{\text{max}}$. All statistical analyses were performed using GraphPad Prism 6.0 software (GraphPad, San Diego, CA). The difference between means was considered statistically significant when $p < 0.05$. Data are shown as mean $\pm$ standard error of the mean.

Results

Clinical evaluation during transient hypertension

The average resting supine baseline blood pressure for all episodes was 126 ± 5 mm Hg. The elevations in mean arterial pressure (penile vibrostimulation = 43 ± 5 vs. urodynamic filling = 46 ± 6 mm Hg; $p = 0.77$) and CBF (penile vibrostimulation = 21 ± 4 vs. urodynamic filling = 23 ± 4 cm/sec; $p = 0.38$) were not different between penile vibrostimulation and urodynamics. We observed that increases in brain perfusion pressure (i.e., blood pressure) because of transient hypertension are not buffered by cerebral autoregulation (i.e., the capacity to regulate and maintain CBF constant in the face of changing blood pressure), but instead lead to cerebral hyperperfusion, as evidenced by 2–3-fold increases in CBF ($+23 \pm 4$ cm/sec; Fig. 1a), and a strong significant positive correlation between increasing blood pressure and CBF ($R^2 = 0.76$, $p < 0.0001$).

Pre-clinical model of transient hypertension

Our clinically relevant model elicits similar relative increases in CBF during autonomic dysreflexia as those observed during iatrogenic procedures in humans ($+22 \pm 3$ mm/sec; see Fig. 1b), and resulted in a similarly and significant positive correlation between increasing blood pressure and CBF ($R^2 = 0.65$, $p = 0.001$). Further, uninjured animals had very similar blood pressure and CBF responses to transient hypertension secondary to phenylephrine ($R^2 = 0.61$, $p = 0.01$; Fig. 1c).

Cerebrovascular consequences of chronic transient hypertension

Our data demonstrate that repetitive exposure to cerebral hyperperfusion secondary to transient hypertension dramatically impairs endothelial function (Fig. 2a). Specifically, chronic exposure to repetitive autonomic dysreflexia led to reduced maximal dilation to repetitive autonomic dysreflexia led to reduced maximal dilation to carbachol ($\log\text{EC}_{\text{max}}$; but not sensitivity [$\log\text{EC}_{50}$]) (Fig. 2a). Chronic exposure to repetitive autonomic dysreflexia also resulted in stiffening the middle cerebral artery, such that distensibility was reduced (Fig. 2d), while strain (Fig. 2c) and beta stiffness (Fig. 2d) also trended to be impaired compared with animals with SCI. Chronic exposure to repetitive autonomic dysreflexia did not lead to remodeling, such that lumen diameter, wall thickness, and wall-to-lumen ratio were similar between groups (Fig. 2c); however, the middle cerebral artery constricted 37% more in response to 10^{-5}M serotonin (5-HT) (Fig. 2e). Percent tone generation of the middle cerebral artery was not different between groups at 80 mm Hg ($28.5 \pm 2.1\%$ vs. $31.4 \pm 2.1\%$). These changes were accompanied by cerebrovascular profibrotic expression characterized by increased expression of collagen (Fig. 3a).

These findings, along with our *in vivo* data (Fig. 1b), demonstrate that repetitive cerebral hyperperfusion secondary to transient hypertension leads to functional and structural changes in both the

FIG. 2. Chronic exposure to repetitive transient cerebral hyperperfusion impaired middle cerebral artery endothelial function, exacerbated stiffening, and led to vasoconstrictive hypersensitivity in spinal cord injury (SCI). (a) Endothelial dilation in response to increasing cumulative doses of carbachol, (b) as well as distensibility; (c) passive structure and remodeling; (d) beta stiffness as well as passive structure at physiological pressure; (e) constriction in response to serotonin (5-HT). (a,b,c) Repeated measures analysis of variance (main effect) comparisons were made using physiological pressure 80–180 mm Hg. (d,e) unpaired *t* test. T3-SCI, group of animals with spinal cord transection at the third thoracic spinal segment; T3-SCI+AD, T3-SCI animals having also undergone four weeks of repetitive transient hypertension and cerebral hyperperfusion induced by colorectal distension. *Significantly different from T3-SCI ($p < 0.05$). Values are mean $\pm$ standard error of the mean. AD, autonomic dysreflexia; CRD, colorectal distension.

cerebrovascular endothelium (reduced dilatory responsiveness) and smooth muscle (increased reactivity/increased collagen deposition). We also demonstrated a 40% reduction in sympathetic innervation density of the cerebrovasculature when chronically exposed to repetitive autonomic dysreflexia (Fig. 3b).

Discussion

From both a functional and structural perspective, the cerebrovasculature is deleteriously impacted by chronic exposure to repetitive transient hypertension after SCI and the resulting cerebral hyperperfusion. The data showing that cerebral hyperperfusion occurs during transient hypertension in uninjured animals supports the contention that chronic exposure to transient hypertension may also be deleterious in those with SCI. The data showing that cerebral hyperperfusion occurs during transient hypertension in uninjured animals raises the concern that chronic exposure to transient hypertension may also be deleterious to the cerebrovasculature in uninjured models and populations, which is supported by epidemiological evidence,^{6,8,9} but not tested in the present study. Similar

transient elevations in blood pressure as those demonstrated in the present study occur in response to chronic caffeine consumption, high-intensity interval exercise,⁵ defecation, exaggerated blood pressure responses to mental stress, and drug abuse.^{3,7,28,29}

The observed endothelial dysfunction and profibrotic stiffening plays a key role in the development of cerebrovascular complications and therefore likely underpins the association between the majority of the mentioned behaviors/conditions and cerebrovascular diseases. Repetitive transient hypertension is clearly a unique form of cerebrovascular decline compared with that resulting from chronic steady-state hypertension, as demonstrated by the lack of cerebrovascular inward remodeling (Fig. 2c, 2d) and no ensuing reduction in CBF (Fig. 3d), as well as the absence of elevated cerebrovascular myogenic tone.¹⁰

The present data, demonstrating impaired endothelial function, inappropriate vasoconstrictive reactivity, as well as profibrotic stiffening of the cerebrovasculature, because of transient hypertension, undoubtedly contributes to our previous observations of increased risk of stroke after SCI.¹⁴ Further, the pathophysiological changes observed because of autonomic dysreflexia

FIG. 3. Cerebrovascular profibrosis and reduced sympathetic perivascular innervation density in response to chronic exposure to repetitive transient hypertension. **(a)** The immunofluorescence intensity of collagen III was elevated after repetitive transient hypertension; mRNA levels within the middle cerebral artery. **(b)** Repetitive autonomic dysreflexia (AD) led to reduced sympathetic innervation density, indicating sympathetic withdrawal from the cerebrovasculature. **(c)** Representative IHC images for collagen I, III, elastin, smooth muscle actin (SMA) and tyrosine hydroxylase (TH). The top region of interest (ROI; SMA used for example) was used for collagen I, collagen III, elastin, SMA analysis of the media layer of the middle cerebral artery. **(d)** No regional or global differences in cerebral blood flow (CBF) (magnetic resonance imaging) occurred. **(e)** Schematic illustrating chronic exposure to cerebral hyperperfusion secondary to transient hypertension leads to increased collagen expression in the vascular smooth muscle and endothelial dysfunction, as well as vasoconstrictive hypersensitivity, and loss of sympathetic fiber density. T3-spinal cord injury (SCI), animals with spinal cord transection at the third thoracic spinal segment, T3-SCI+AD, T3-SCI animals having also undergone four weeks of repetitive transient hypertension. **Significantly different from T3-SCI ($p < 0.01$). *Significantly different from T3-SCI ($p < 0.05$). Unpaired t test. Values are mean $\pm$ standard error of the mean. CBF, cerebral blood flow; TGF- β , transforming growth factor-beta; MMP, matrix metalloproteinase.

in this study provide a physiological rationale for the impairment of both neurovascular coupling (i.e., hyperemic response to neuronal activation) and autoregulation we have shown after SCI, because appropriate dilation, constriction, and distensibility of the cerebrovasculature are crucial for both of these cerebrovascular regulatory mechanisms.^{16,17,28,30,31} In support of this, very recently we showed that autonomic dysfunction was associated with the development of vascular-cognitive impairment after SCI.¹⁸

Our previous work has also demonstrated that SCI results in an array of cerebrovascular consequences including profibrotic remodeling²⁵ as well as loss of sympathetic regulation,¹⁸ which may make this population more susceptible to cerebrovascular trauma

during transient hypertension because of impaired capacity to regulate CBF. Clearly, it would be beneficial in the clinical setting to prevent the cerebrovascular consequences of repetitive transient hypertension after SCI by reducing the burden of autonomic dysreflexia. We have previously demonstrated that some candidate prevention strategies may include early initiation of passive lower-limb exercise after SCI²⁶ or prophylactic antihypertensive administration with nifedipine or prazosin.²⁶

Chronic exposure to transient hypertension also led to profibrotic stiffening of the cerebrovasculature, which is well described in chronic steady-state hypertension but novel to the presently studied model.^{10,11} The vascular stiffening (and resistance to vasomotion) will also contribute to the observed impaired endothelial dilation,

but also likely masks even more severe vasoconstrictive hypersensitivity. Intriguingly, transient hypertension, as opposed to chronic hypertension, did not lead to inward remodeling of the vasculature, or the resulting reduction in CBF (Fig. 3C) because of increased vascular resistance, demonstrating that transient hypertension may represent a unique form of cerebrovascular decline not yet identified.

The large reduction in sympathetic innervation density of the cerebrovasculature from chronic exposure to transient hypertension (Fig. 3b), taken together with our previous work showing intact sympathetic pathways from pre-ganglionic neurons leading to the cerebrovasculature after T3 spinal cord transection,²⁵ suggests that baroreceptor-mediated sympathetic withdrawal because of elevations in blood pressure is leading to reduced sympathetic control over the cerebrovasculature. Because the sympathetic nervous system functions primarily to increase end-organ vascular resistance, reduced sympathetic control will contribute to the loss of capacity to autoregulate CBF after SCI, and paradoxically impair the brain's ability to protect itself (through increased vascular resistance) from increases in brain perfusion pressure.³² These data provide insight into the mechanisms underlying our previous clinical research demonstrating that cerebral autoregulation is impaired in spinal cord injured patients with autonomic dysreflexia,^{14,16} and the increased risk of stroke in this population.¹⁴

The CBF passively increased during transient hypertension in uninjured and injured animals, as well as humans with SCI, which supports the recently established contention that cerebral autoregulation is not as effective as thought previously.³¹ Spinal cord injured animals had slightly greater elevations in blood flow for a given increase in blood pressure, findings that suggest an impairment of cerebral autoregulation in the spinal cord injured animals (Fig. 1a,1b,1c), and concur our previous clinical research.^{16,18}

This study provided important clinically relevant insight into the association between chronic exposure to transient hypertensive episodes because of autonomic dysreflexia, and cerebrovascular decline after SCI. Some perceived limitations may be that: (1) This study utilized transcranial Doppler to evaluate CBF, which measures velocity of red blood cells and assumes a constant diameter of the vessels being insonated. Other options, such as duplex extracranial ultrasound and MRI were not conducive to be used in a clinical setting such as that of the present study, because they require more personnel to operate as well as significant offline analysis and specialized equipment.³³ (2) We did not assess females in the present study, because they are far less common to have a SCI.²⁶ (3) We insonated the middle cerebral artery and not the posterior cerebral artery, so our findings do not extend to the posterior brain circulation, which may exhibit differential regulation as to the middle cerebral artery under some scenarios.^{16–18}

Clinical Perspectives

Stroke is 300–400% more likely to occur after SCI and is a leading cause of death. Autonomic dysreflexia, which leads to transient hypertension, affects the majority of persons with SCI up to 40 times per day. Acutely, we know that severe episodes of hypertension secondary to autonomic dysreflexia can lead to stroke; however, we do not understand the long-term consequences of this stimulus on the brain and its vasculature. The present translational study first demonstrated that our model of transient hypertension, secondary to autonomic dysreflexia, leads to substantial acute increases in CBF that were mimicking the responses in patients with SCI.

Next, we showed that chronic exposure to this stimuli leads to broad deleterious consequences to the brain vasculature, including endothelial dysfunction and profibrotic remodeling, and provided insight into the molecular mechanisms underpinning these changes (Fig. 3e). Although our findings were in a SCI model, these results may also provide insight into the association between stroke and a number of conditions where there is long-term exposure to transient hypertension, such as exaggerated mental stress, white coat syndrome, and stimulant drug abuse.

It appears that repeated transient elevations in blood pressure are not innocuous to the cerebrovasculature; this link has recently been highlighted in the popular media and questioned in the scientific literature.^{2,5} It may be that exposure to repetitive transient hypertension over time is a critical risk factor for cerebrovascular diseases, and certainly future research into this is warranted, because it has also been suggested that blood pressure oscillations may exert neuroprotective effects during ischemic episodes.³⁴ It should be considered that the human brain has been shown to demonstrate a form of hysteresis, where cerebral autoregulation is more effective when faced with acute elevations in blood pressure compared with acute decreases.^{35,36} As such, it would be prudent to also explore the cardiovascular effects of chronic exposure to orthostatic hypotension, which is widespread after SCI and is epidemiologically linked to cardiovascular morbidity and death in nonspinal cord injured populations,^{37–39} and as such may be exerting even more severe cerebrovascular consequences as transient hypertension. Finally, this study provides evidence that autonomic dysreflexia needs to be aggressively managed after SCI.

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