Published in final edited form as:

Transl Stroke Res. 2016 April ; 7(2): 156–165. doi:10.1007/s12975-016-0449-7.

Effects of Acute Stroke Serum on Non-ischemic Cerebral and Mesenteric Vascular Function

Isabella Canavero, MD¹, Helene A. Sherburne¹, Sarah M. Tremble, BS¹, Wayne M. Clark, MD², and Marilyn J. Cipolla, PhD^{1,3,4}

¹Department of Neurological Sciences, University of Vermont College of Medicine, Burlington, VT

²Department of Neurology, Oregon Stroke Center, Oregon Health and Science University, Portland, OR

³Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Vermont College of Medicine, Burlington, VT

⁴Department of Pharmacology, University of Vermont College of Medicine, Burlington, VT

Abstract

We investigated the effects of circulating factors in serum obtained from patients in the acute phase of different subtypes of ischemic stroke on non-ischemic cerebral and mesenteric arteries, as a potential mechanism involved in influencing regional perfusion and thus clinical evolution. Posterior cerebral arteries (PCAs) and mesentery arteries (MAs) isolated from Wistar Kyoto rats were perfused with serum from acute stroke patients with large vessel disease without (LVD) or with hypertension (LVD+HTN), cardioembolism with hypertension (CE+HTN) or physiologic saline as controls. Myogenic activity and nitric oxide-dependent vasorelaxation were assessed after 2 hours of intraluminal exposure to serum. Vascular function was differentially affected by sera. Exposure to LVD serum increased myogenic tone and produced endothelial dysfunction in both PCAs and MAs. However, CE+HTN serum increased tone and decreased smooth muscle sensitivity to NO in vessels from both vascular beds. LVD+HTN serum was associated with reduced smooth muscle sensitivity to NO in vessels from both vascular beds but increased tone only in PCAs. Inflammation and oxidative stress, determined by measurement of high sensitivity C-reactive protein, uric acid and free 8-isoprostane, were enhanced in all the serum groups. These results demonstrate vasoactive properties of acute stroke serum related to stroke subtypes that could potentially contribute to the pathogenesis of early hemodynamic-based clinical events.

Corresponding author: Marilyn J. Cipolla, PhD, Department of Neurological Sciences, University of Vermont, 149 Beaumont Ave., HSRF 416A, Burlington, VT 05405, USA, Phone: +1-802-656-9714; Fax: +1-802-656-8704, Marilyn.Cipolla@uvm.edu.

Compliance with Ethics Guidelines

Ethical Approval

All procedures involving human patients were approved by the OHSU Institutional Review Board (study #6333), in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and in accordance with the Helsinki Declaration of 1964, as revised in 2008.

All procedures involving animals were approved by the University of Vermont Institutional Animal Care and Use Committee and complied with the NIH guidelines for the Care and Use of Laboratory Animals.

Informed Consent

Informed consent was obtained from all patients or their families included in the study.

Conflict of Interest

None to declare.

Keywords

Circulating factors; acute stroke; stroke subtypes; inflammation; oxidative stress; free 8-isoprostane

Introduction

Acute ischemic stroke triggers an inflammatory response and enhances oxidative stress with release of cytokines, chemokines, adhesion molecules, proteolytic enzymes and oxidative stress biomarkers into the blood [1–3]. Furthermore, the various patterns of circulating biomarkers that have been reported in patients' blood within the first hours from stroke onset may relate to the etiopathological heterogeneity of stroke [4–5]. For example, large vessel disease and hypertension are currently thought to share a common pathophysiological background characterized by chronic low-grade systemic inflammation, endothelial dysfunction and oxidative stress, consistent with raised levels of high sensitivity C-reactive protein (hsCRP), pro-inflammatory cytokines, endothelin-1 (ET-1), vascular endothelial growth factor and oxidative stress biomarkers found in patients with these diagnosis [2–9]. In patients with cardioembolic stroke, the key role of intracardiac activation of coagulation is supported instead by a “thrombogenic profile” of biomarkers [7]. Circulating factors are usually considered in diagnostics as pathophysiological markers, but many of them also possess biological activity with documented vasoactive properties [9–10].

In the acute phase of stroke, the presence of vasoactive circulating factors may be of fundamental importance by potentially influencing vascular function outside of the ischemic area, with repercussions on hemodynamics, regional blood perfusion and potentially clinical management and outcome. Cerebral hemodynamics are often impaired in the acute phase post stroke [11]. Cerebral autoregulation and hemodynamics have been found altered after stroke by several techniques (transcranial Doppler ultrasound, positron emission tomography, magnetic resonance imaging) in both hemispheres [12–15]. These changes can occur as attempts to restore adequate perfusion in the penumbra or to enhance unaffected hemisphere function for compensation, but are often involved in determining early complications responsible for neurological deterioration [14–17]. Peri-ictal alterations of blood pressure, crucial for clinical management, have been described in both directions and express altered peripheral vascular resistance, mainly determined by changes in the state of constriction of systemic resistance vessels [18–19].

The etiology of these early hemodynamic-based clinical events post-stroke is likely multifactorial but at present poorly understood [12, 15, 17]. A contribution from vasoactive circulating factors has been postulated but to our knowledge only a few studies have explored the potential “systemic” (i.e., blood-borne) effect of stroke on non-ischemic vessels in animal models and human patients [12, 20–22]. The aim of the present study was to investigate the effect of circulating factors originated during the acute phase of stroke on non-ischemic cerebral and systemic vascular function. We used a combination of animal model and human serum, by perfusing rat cerebral (posterior cerebral artery, PCA) and systemic (mesentery artery, MA) vessels with human serum from acute stroke patients with

different etiologic subtypes and comorbidities. We hypothesized that the serum-induced vascular dysfunction would be different according to the serum subtype and vascular bed. The contribution of inflammation and oxidative stress was investigated by assessing the levels of hsCRP, uric acid and free 8-isoprostane in the different sera.

Materials and Methods

Patients and Serum Samples

After acquisition of informed consent, blood samples were collected from patients hospitalized for acute ischemic stroke and enrolled in an ongoing Institutional Review Board approved study at the Oregon Health and Science University (OHSU; Portland, OR, USA). Clinical variables were assessed and recorded by the hospital physicians and nurses. Stroke subtypes were classified according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [23]; stroke severity at admission was assessed by National Institute of Health Stroke Scale (NIHSS) [24] and pre-stroke disability by modified Rankin scale [25]. Comorbidities and previous pharmacological treatments were identified if formally reported in patients' medical history. Venous blood was drawn 24±4 hours from the onset of symptoms and, after centrifugation, was immediately stored in polypropylene tubes at -80 °C. We considered sera from patients with acute stroke due to large vessel disease or cardioembolism [23] with or without medical history of hypertension. To reduce confounding factors, patients with a diagnosis of current acute infection, chronic inflammatory diseases, cancer, or with an acute event classified as "stroke of uncertain or multiple etiologies" were excluded. Sample selection was performed ensuring adequate amount and quality with no visual hemolysis. Along these lines, samples from 12 patients were identified and divided into 3 groups of 4 patients each: large vessel disease with hypertension (LVD+HTN), large vessel disease without hypertension (LVD), and cardioembolism with hypertension (CE+HTN). Sera were pooled within each group and stored at -80 °C as aliquots until experimentation.

Measurement of Circulating Markers of Inflammation and Oxidative Stress

For each patient, standard clinical stroke assessments were done and hsCRP and uric acid levels were obtained at the same time as sera using routine testing by the OHSU hospital laboratory.

Free 8-isoprostane (8-iso-PGF_{2α}), used as an index of oxidative stress [3], was measured in our laboratory for each patient group on pooled samples by a monoclonal antibody-based ELISA method (Cayman Chemical, Ann Arbor, Michigan US). Serum samples were diluted 1:2. All samples were measured in triplicate.

Animals

Male Wistar Kyoto rats (~300 g) were used for all experiments. Animals were either single or group-housed in the Animal Care Facility, an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility. Animals had access to food and water *ad libitum*, and followed a 12-hour light/dark cycle.

Preparation of Arterial Segments and Arteriograph System

Animals were anesthetized with 3% isoflurane prior to being euthanized. The brain and a section of gut with mesentery and intact vasculature were removed and placed into cold, oxygenated physiologic saline solution (PSS). Second order PCAs and third order MAs were carefully isolated and excised. Vessels were mounted on glass cannulas in a pressurized arteriograph system, and perfused intraluminally through the proximal cannula. To evaluate the effect of circulating factors in acute stroke serum on vascular function, arteries were perfused with human serum from the different stroke subtypes (diluted to 20% volume/volume with PSS). Because of the unavailability of serum from age-matched healthy subjects (without stroke but also without hypertension and atherosclerosis), we perfused arteries for the control group with PSS. A total of 36 experiments were performed for each vessel type, divided in groups according to the perfusate: PSS (Control, n=8), LVD+HTN serum (n=10), LVD serum (n=10) or CE+HTN serum (n=8). A pressure transducer connected to a pressure servo controller (Living Systems Instrumentation Inc., Burlington, VT, USA) was attached to the proximal cannula to control and maintain intravascular pressure. There was no intraluminal flow to avoid flow-induced responses. Vessels were visualized using an inverted microscope, with attached videocamera. Lumen diameter and wall thickness were measured by a video dimension analyzer. WINDAQ data-acquisition software was used to continuously record changes in intraluminal pressure and inner diameter.

Experimental Protocol

Vessels were allowed to equilibrate at 50 mmHg for 2 hours prior to experimentation in the presence of intraluminal human serum or PSS. Intraluminal pressure was increased to 175 mmHg in 25 mmHg increments, and lumen diameter and wall thickness recorded to measure active myogenic responses and calculate percent tone. The pressure was then reduced to 75 mmHg, where it was maintained throughout the experiment. MAs and PCAs were precontracted to ~50% of the baseline using the thromboxane agonist U46619 ($\sim 10^{-7}$ and $\sim 10^{-8}$ M, respectively). In the presence of U46619, both vessels were given increasing concentrations of acetylcholine (10^{-5} to 10^{-8} M) to evaluate receptor-mediated endothelium-dependent vasorelaxation. The vessels were then washed with PSS. N^o-Nitro-L-arginine methyl ester (L-NAME, 10^{-3} M) was given to inhibit nitric oxide synthase (NOS), and constriction in response to the NOS inhibitor recorded. Since L-NAME did not produce sufficient constriction in MAs, U46619 was added to produce a constriction of ~50% of the baseline diameter. Increasing concentrations of sodium nitroprusside (SNP, 10^{-5} to 10^{-8} M) were then given to both vessels to determine vascular smooth muscle sensitivity to NO. The vessels were washed with calcium-free PSS and given papaverine (10^{-4} M) and diltiazem (10^{-5} M) to ensure passivity of the smooth muscle. Pressure was increased to 200 mmHg, and passive lumen diameter and wall thickness recorded at pressures from 200 to 5 mmHg. During the entire experiment pH and temperature of the bath were constantly measured and kept stable at 7.4 ± 0.05 and 37 ± 0.5 °C, respectively.

Solutions and Drugs

PSS was made weekly with the following ionic composition (mM): 119 NaCl, 4.7 KCl, 1.17 MgSO₄, 0.026 EDTA, 3.4 CaCl₂, 24 NaHCO₃, 1.18 KH₂PO₄. Calcium-free PSS was also made weekly, using the same ingredients as above, excluding CaCl₂. Both solutions were stored at 4 °C without glucose; 5.5 mM dextrose was added before each experiment. During the experiments, solutions were aerated with 5% CO₂, 10% O₂, and 85% N₂ to maintain pH at 7.4. Acetylcholine, L-NAME, SNP, diltiazem and papaverine were purchased from Sigma-Aldrich (St Louis, MO, USA) and U46619 from Enzo Life Sciences (Farmingdale, NY, USA). Drug stocks were prepared weekly and stored at 4°C.

Data Calculations

Myogenic tone was calculated at each pressure using the equation $[1 - (\phi_{active}/\phi_{passive})] \times 100\%$, where ϕ_{active} is vessel active diameter and $\phi_{passive}$ is the diameter in calcium-free PSS in the presence of diltiazem and papaverine. Percent constriction to L-NAME was calculated as a percentage decrease of vessel diameter from the baseline at 75 mmHg after addition of the vasoconstrictor. Percent reactivity to acetylcholine and SNP were calculated using the equation $[(\phi_{dose} - \phi_{start})/(\phi_{passive} - \phi_{start})] \times 100\%$, where ϕ_{start} is baseline vessel diameter, ϕ_{dose} is the diameter after each addition of vasodilator, and $\phi_{passive}$ is the diameter in calcium-free PSS in the presence of diltiazem and papaverine at 75 mmHg.

Statistical Analysis

Patients' demographics and clinical features are reported as mean±SEM for continuous variables, median [IQR] for skewed continuous variables and frequencies (percentages) for categorical variables; differences were evaluated with one-way ANOVA and χ^2 test, respectively. Free 8-isoprostane levels are reported as mean±SD of triplicate measurements on pooled samples and thus statistical analyses were not performed. Experimental data are presented as mean±SEM. Differences between the groups were assessed using one-way ANOVA with a post-hoc analysis for multiple comparisons (Tukey's or Dunnett's correction test, where appropriate). Differences at various pressures within a serum group were determined by repeated-measures ANOVA with a post-hoc analysis for multiple comparisons (Dunnett's correction test). Differences between PCAs and MAs were determined using unpaired t-test. Differences were considered statistically significant at $p < 0.05$.

Results

Clinical Features of the Groups

Demographics and clinical features of the patients are shown in details in Table 1. As expected, the rate of atrial fibrillation was significantly higher in the CE+HTN group compared to other groups. Also, the incidence of previous treatment with antihypertensives was higher in the same group. Interestingly, none of the patients included in the LVD+HTN group reported an ongoing antihypertensive therapy despite the diagnosis of hypertension. LVD+HTN group had the highest NIHSS at admission, although without significant difference compared to the other groups. Mean serum hsCRP in all groups was higher than

normal (<3 mg/L). LVD+HTN group had substantially higher mean hsCRP compared to other groups, although it was not statistically significant ($p=0.09$). Mean uric acid concentrations were within reported normal range (3.7–8.0 mg/dL) and similar within the groups. Table 1 also reports the levels of free 8-isoprostane in each group. LVD+HTN serum was characterized by the highest concentration of 8-isoprostane, followed in order by LVD and CE+HTN sera.

Effects of Stroke Serum on Myogenic Reactivity and Tone in Non-ischemic Vessels

We investigated the effect of intraluminal exposure to sera on myogenic reactivity in response to stepwise increases in intraluminal pressure. Three PCA experiments from LVD+HTN, one from LVD and two from CE+HTN group and one MA experiment from the LVD+HTN group were excluded due to technical difficulties.

Changes in inner diameter of PCAs in response to increasing pressure are shown in Figure 1A. The presence of serum did not alter inner diameters of PCAs at pressures from 50 to 100 mmHg. However, when pressure was increased to 125 mmHg, PCAs perfused with CE+HTN serum constricted demonstrating myogenic reactivity. PCAs perfused with LVD and LVD+HTN serum did not significantly change diameters with increasing pressure. Figure 1B shows changes in inner diameter of MA groups in response to pressure. The presence of LVD+HTN serum caused MA vessels to be relatively passive and diameters increased with increasing pressures, suggesting diminished myogenic activity. In contrast, inner diameter of MAs from LVD and CE+HTN groups were smaller and did not change appreciably with increased pressure. These differences in diameters were not due to vessel size of MAs as passive diameters were similar among the groups ($p>0.05$, data not shown).

To determine if serum caused differences in myogenic tone, we calculated the amount of myogenic tone at 75 mmHg, in both PCAs and MAs (Figure 2). The presence of serum in PCAs caused an increase in tone compared to controls. In addition, CE+HTN serum caused the greatest vasoconstriction and the highest amount of tone, with a significant difference compared to LVD+HTN group. MAs had significantly lower tone than PCAs in all groups, as shown previously [26]. However, the presence of both LVD and CE+HTN serum caused an increase in tone in MAs compared to control PSS and LVD+HTN serum.

Effects of Stroke Serum on NO contribution to tone and NO-dependent vasodilation

Figure 3 shows percent constriction to the NOS inhibitor L-NAME of PCAs and MAs from all groups. Control PCAs constricted ~30% compared to only ~5% of control MAs ($p<0.01$), demonstrating a larger contribution of NO to inhibiting tone in cerebral vessels. Interestingly, serum from all groups reduced constriction to L-NAME in PCAs compared to control. However, the presence of serum in MAs did not affect constriction to L-NAME, which was significantly lower than PCAs of the same groups.

Percent reactivity in response to increasing concentrations of acetylcholine and SNP (10^{-8} M to 10^{-5} M) in PCAs and MAs from all groups are shown in Figure 4. The presence of serum perfused in PCAs caused reduced reactivity to acetylcholine, although this was statistically significant only for LVD serum at 10^{-7} M and 3×10^{-7} M (Figure 4A). As shown in Figure 4B, PCAs perfused with CE+HTN serum exhibited decreased reactivity to SNP

compared to control at concentrations 3×10^{-7} M. In addition, PCAs perfused with serum from the CE+HTN group had reduced reactivity to SNP at the highest concentration (10^{-5} M) compared to the other serum groups. All serum-perfused MAs had decreased reactivity to acetylcholine at 10^{-8} M and 3×10^{-8} M compared to control (Figure 4C). Of note, reactivity to acetylcholine in MAs was significantly higher than in PCAs (maximum response at 10^{-5} M in controls: $96 \pm 1\%$ in MAs vs. $45 \pm 2\%$ in PCAs, $p < 0.01$) regardless of the presence of serum. Figure 4D shows percent reactivity to SNP of MAs perfused with all types of serum. All serum-perfused MAs had reduced reactivity to SNP. However, this was statistically significant only for LVD+HTN group from 10^{-7} to 10^{-6} M.

Discussion

Inflammation is a pathophysiological hallmark in acute cerebral ischemia. The serum from all groups of acute stroke patients was indeed characterized by notably raised hsCRP levels than normal, widely recognized as index of ongoing inflammation. HsCRP is often raised after stroke as part of a non-specific acute phase reaction, and greater values have been associated with higher stroke severity and larger infarcts [5, 27]. Moreover, each group displayed a diagnosis of large vessel disease and/or hypertension, which have been reported to be associated with higher hsCRP levels [4, 5, 7, 27, 28]. However, in some cohorts hsCRP was elevated also in acute cardioembolic stroke, possibly biased by coexisting higher stroke severity [4, 28]. Other than a marker, CRP is known to be vasoactive, leading to vasoconstriction through various mechanisms. In endothelial cells, CRP facilitates the release of ET-1 and superoxide production by NADPH oxidase [29, 30]. In vascular smooth muscle cells CRP stimulates reactive oxygen species (ROS) formation, increases angiotensin type 1 and reduces angiotensin type 2 receptor number [31, 32]. In serum from all groups of patients with acute stroke, it is reasonable to expect an enhanced expression of pro-inflammatory circulating factors, such as cytokines and chemokines, which have been shown to correlate with hsCRP levels and have vasoactive properties [9, 10, 33], although we did not perform specific measurements. In addition, correlation between CRP and other biomarkers with vessel reactivity was not performed because the serum samples were pooled.

Oxidative stress, namely the increased bioavailability of ROS resulting from an imbalance in which pro-oxidants overwhelm antioxidant capacity, is another major pathophysiological process involved in stroke. ROS are highly reactive to various molecular targets (lipids, proteins, nucleic acids, carbohydrates) and damage vascular walls by inducing lipid peroxidation. In addition, ROS interfere with vasoreactivity even at low concentrations, by acting as signaling molecules but also by reducing NO bioavailability [3, 20, 34, 35]. Furthermore, ROS are potent stimulators of ET-1 synthesis by endothelial and smooth muscle cells [36]. F_2 -isoprostanes are stable circulating end-product of lipid peroxidation and are considered specific and reliable biomarkers of oxidative stress. In addition, their levels in biological fluids correlate with ROS levels and oxidative stress in experimental and human studies, and also with the presence of cardio- and cerebrovascular risk factors [3, 37]. Besides markers, F_2 -isoprostanes can act as mediators of oxidant injury. For example, 8-isoprostane is a potent vasoconstrictor in cerebral and systemic arteries [37, 38]. The serum from all the groups of stroke patients exhibited higher 8-isoprostane levels compared to

those reported in acute stroke patients in the literature [39]. Uric acid has also been hypothesized to be a biomarker and mediator of oxidative stress as well as to act as a scavenger of oxidants, but clarifying evidence is lacking [40]. Nevertheless, in our study uric acid levels at 24 hours were similar and within the normal range in every group.

Although inflammatory and oxidative stress profiles were enhanced in serum from all groups, vascular function was differentially affected. LVD serum caused an increase in myogenic tone compared to control in PCAs. Reduced reactivity to acetylcholine and reduced constriction to L-NAME in the presence of normal reactivity to SNP suggest that serum affected endothelium-dependent relaxation, while smooth muscle sensitivity to NO was preserved. Similar effects were found in MAs perfused with the same serum. A possible explanation for these findings is reduced NO bioavailability due to increased production of superoxide [35]. Raised 8-isoprostane levels could have contributed to the vasoconstricting effect of serum, and also support the hypothesis of an enhanced oxidative stress milieu as the underlying mechanism of endothelial dysfunction. Moderately increased levels of hsCRP, moderate stroke severity and presence of atherosclerosis could imply a pro-inflammatory pattern of circulating factors, possibly further stimulating endothelial production of superoxide [9, 10].

PCAs perfused with serum from CE+HTN patients showed higher myogenic reactivity and tone than the other groups, reduced constriction to L-NAME with reduced reactivity to SNP, suggesting compromised smooth muscle sensitivity to NO. MAs perfused with the same serum exhibited increased tone and tended to have reduced reactivity to SNP, although this was not statistically significant. Thus, in both vessel types, this serum increased tone, but affected smooth muscle prominently in PCAs. The relatively major effect produced by serum components on smooth muscle of PCAs could depend on specific receptor expression of this vascular bed. The increase in tone could also derive from the presence in the serum of a vasoconstrictor. Potent vasoconstrictors such as ET-1 or angiotensin II have been described as raised in stroke patients especially with hypertension [8, 41].

LVD+HTN serum in PCAs produced increased myogenic tone versus control, reduced constriction to L-NAME and reduced reactivity for lower concentrations of SNP, indicating impaired smooth muscle sensitivity to NO. In MAs exposure to this serum did not increase tone and was associated with reduced reactivity to SNP. The relatively lower impairment in smooth muscle measured in PCAs versus MAs could be due to the protective action of the blood-brain barrier. The increase in myogenic tone only in PCAs could be determined by the presence of a vasoconstrictor that, according to different receptor profiles, is effective on PCAs whilst functionally inactive in MAs. Vasoactive factors of this serum could belong to inflammatory and oxidative stress environment, as suggested by the higher levels of hsCRP and 8-isoprostane compared to the other sera. Inflammation and oxidative stress could have been potentiated by the simultaneous presence of atherosclerosis, untreated hypertension and higher stroke severity [1, 2, 5].

The clinical significance of the vasoactive effect of stroke serum in non-ischemic vessels is not clear and may contribute to either clinical improvement or deterioration [14–17]. However, in our set of experiments we found that serum from all groups of patients

produced cerebral vasoconstriction in non-ischemic vessels, which could result in regional CBF reduction. Since ischemic depth, duration and collateral status may be different among stroke subtypes, it is also possible that the impact of serum-induced vasoconstriction may vary [6, 16]. Vasoconstriction of collaterals reduces perfusion of the penumbra and promotes the extension of the infarct (“collateral failure”), especially in cases of already impaired collaterals due to atherosclerosis and hypertension [16]. In cases of early arterial recanalization, as often observed in cardioembolic stroke, vasoconstriction of non-ischemic cerebral vessels can also support the mechanism of neurological worsening known as “hemodynamic steal phenomenon”, characterized by improved perfusion in the ischemic field at the expense of the other cerebral areas [17].

As for mesenteric vessels, the intrinsic vascular tone is the main determinant of peripheral resistance, thus directly responsible for establishment of blood pressure [18]. Independently from previous medical history, transient peri-ictal changes of blood pressure have been described in both directions and represent a *grey area* in terms of best clinical management. High blood pressure occurs in up to 75% of acute strokes while low blood pressure is infrequent, however both are predictive of poor outcome [19]. These alterations are presumably due to multifactorial causes (e.g. undiagnosed preexisting hypertension, treatments, neuroendocrine response) but a role of blood-borne vasoactive factors could be considered among the possible contributors. According to this perspective, the vasoconstricting effect produced by LVD and CE+HTN sera in MAs could support an increase of peripheral resistance and consequently of arterial pressure.

Our study has some limitations that should be acknowledged. First, we measured only one (8-isoprostane) of the vasoconstrictors possibly contained in serum and there may be others that contribute to increased tone or altered reactivity. Second, the results from this in vitro, “single-vessel” model have to be interpreted with caution in terms of hemodynamics. Control of blood flow is determined by changes in vascular resistance that involves more than just one segment of the arterial tree. Third, we determined vascular function after a relatively brief exposure to serum (few hours). It is possible that longer exposure would affect vascular function to a greater extent. Fourth, selecting only patients without other known pro-inflammatory conditions, we had a relatively small number of stroke patients in each group for pooling samples. Lastly, serum concentration in the perfusate was also lower than the physiological in the blood (20 vs. ~55%) for the diseased groups. Nonetheless, due to unavailability of serum from age-matched healthy subjects, our control group was perfused with saline instead of healthy human serum, therefore our results could have been partially affected by this limitation.

In conclusion, our study demonstrates vasoactive properties of acute stroke serum on non-ischemic cerebral and mesenteric vessels, which related to etiological subtypes and were due to circulating factors possibly belonging to the inflammatory and oxidative stress milieu. These effects may potentially contribute to the pathogenesis of early hemodynamic events affecting non-ischemic cerebral and systemic vasculature and, if confirmed by further studies, should be considered in clinical management.

Acknowledgments

The Authors appreciate and gratefully acknowledge the work of the hospital staff of the Department of Neurology / Oregon Stroke Center at Oregon Health and Science University.

Funding

This study was funded by National Institute of Neurological Disorders and Stroke Grants R01 NS-045940 and R01 NS-093289 and National Heart, Lung, and Blood Institute Grant P01 HL095488, and the Totman Medical Research Trust.

References

1. Denes A, Thornton P, Rothwell NJ, Allan SM. Inflammation and brain injury: acute cerebral ischaemia, peripheral and central inflammation. *Brain Behav Immun*. 2010 Jul; 24(5):708–723. [PubMed: 19770034]
2. Domínguez C, Delgado P, Vilches A, Martín-Gallán P, Ribó M, Santamarina E, et al. Oxidative stress after thrombolysis-induced reperfusion in human stroke. *Stroke*. 2010 Apr; 41(4):653–660. [PubMed: 20185784]
3. Ho E, Karimi Galougahi K, Liu CC, Bhindi R, Figtree GA. Biological markers of oxidative stress: Applications to cardiovascular research and practice. *Redox Biol*. 2013 Oct 8.1:483–491. [PubMed: 24251116]
4. Montaner J, Perea-Gainza M, Delgado P, Ribó M, Chacón P, Rosell A, et al. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. *Stroke*. 2008 Aug; 39(8):2280–2287. [PubMed: 18535284]
5. Ormstad H, Aass HC, Lund-Sørensen N, Amthor KF, Sandvik L. Serum levels of cytokines and C-reactive protein in acute ischemic stroke patients, and their relationship to stroke lateralization, type, and infarct volume. *J Neurol*. 2011 Apr; 258(4):677–685. [PubMed: 21424610]
6. Alexander RW. Theodore Cooper Memorial Lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. *Hypertension*. 1995 Feb; 25(2):155–161. [PubMed: 7843763]
7. Alvarez-Perez FJ, Castelo-Branco M, Alvarez-Sabin J. Usefulness of measurement of fibrinogen, D-dimer, D-dimer/fibrinogen ratio, C reactive protein and erythrocyte sedimentation rate to assess the pathophysiology and mechanism of ischaemic stroke. *J Neurol Neurosurg Psychiatry*. 2011 Sep; 82(9):986–992. [PubMed: 21296900]
8. Rodríguez-Pascual F, Busnadiego O, Lagares D, Lamas S. Role of endothelin in the cardiovascular system. *Pharmacol Res*. 2011 Jun; 63(6):463–472. [PubMed: 21296157]
9. Sprague AH, Khalil RA. Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem Pharmacol*. 2009 Sep 15; 78(6):539–552. [PubMed: 19413999]
10. Vila E, Salaices M. Cytokines and vascular reactivity in resistance arteries. *Am J Physiol Heart Circ Physiol*. 2005 Mar; 288(3):H1016–H1021. [PubMed: 15706038]
11. Wityk RJ. Blood pressure augmentation in acute ischemic stroke. *J Neurol Sci*. 2007 Oct 15; 261(1–2):63–73. [PubMed: 17582440]
12. Aries MJ1, Elting JW, De Keyser J, Kremer BP, Vroomen PC. Cerebral autoregulation in stroke: a review of transcranial Doppler studies. *Stroke*. 2010 Nov; 41(11):2697–2704. [PubMed: 20930158]
13. Hakim AM, Evans AC, Berger L, Kuwabara H, Worsley K, Marchal G, et al. The Effect of Nimodipine on the Evolution of Human Cerebral Infarction Studied by PET. *J Cereb Blood Flow Metab*. 1989 Aug; 9(4):523–534. [PubMed: 2661584]
14. Rehme AK, Fink GR, von Cramon DY, Grefkes C. The role of the contralesional motor cortex for motor recovery in the early days after stroke assessed with longitudinal fMRI. *Cereb Cortex*. 2011 Apr; 21(4):756–768. [PubMed: 20801897]
15. Silvestrini M, Cupini LM, Placidi F, Diomedì M, Bernardi G. Bilateral hemispheric activation in the early recovery of motor function after stroke. *Stroke*. 1998 Jul; 29(7):1305–1310. [PubMed: 9660377]

16. Liebeskind DS. Collateral therapeutics for cerebral ischemia. *Expert Rev Neurother.* 2004 Mar; 4(2):255–265. [PubMed: 15853567]
17. Paulson OB. Cerebral apoplexy (stroke): pathogenesis, pathophysiology and therapy as illustrated by regional blood flow measurements in the brain. *Stroke.* 1971 Jul-Aug;2(4):327–360. [PubMed: 4398838]
18. Mellander S. Functional aspects of myogenic vascular control. *J Hypertens Suppl.* 1989 Sep; 7(4):S21–S30. discussion S31. [PubMed: 2553897]
19. Leonardi-Bee J, Bath PM, Phillips SJ, Sandercock PA. IST Collaborative Group. Blood pressure and clinical outcomes in the International Stroke Trial. *Stroke.* 2002 May; 33(5):1315–1320. [PubMed: 11988609]
20. Palomares SM, Gardner-Morse I, Sweet JG, Cipolla MJ. Peroxynitrite decomposition with FeTMPyP improves plasma-induced vascular dysfunction and infarction during mild but not severe hyperglycemic stroke. *J Cereb Blood Flow Metab.* 2012 Jun; 32(6):1035–1045. [PubMed: 22373645]
21. Cipolla MJ, Huang Q, Sweet JG. Inhibition of protein kinase C β reverses increased blood-brain barrier permeability during hyperglycemic stroke and prevents edema formation in vivo. *Stroke.* 2011 Nov; 42(11):3252–3257. [PubMed: 21852606]
22. Pretnar-Oblak J, Sabovic M, Pogacnik T, Sebestjen M, Zaletel M. Flow-mediated dilatation and intima-media thickness in patients with lacunar infarctions. *Acta Neurol Scand.* 2006 Apr; 113(4): 273–277. [PubMed: 16542168]
23. Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE 3rd. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke.* 1993 Jan; 24(1):35–41. [PubMed: 7678184]
24. Brott T, Adams HP Jr, Olinger CP, Marler JR, Barsan WG, Biller J, et al. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke.* 1989 Jul; 20(7):864–870. [PubMed: 2749846]
25. van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke.* 1988; 19:604–607. [PubMed: 3363593]
26. Amburgey OA, Reeves SA, Bernstein IM, Cipolla MJ. Resistance artery adaptation to pregnancy counteracts the vasoconstricting influence of plasma from normal pregnant women. *Reprod Sci.* 2010 Jan; 17(1):29–39. [PubMed: 19767537]
27. Eikelboom JW, Hankey GJ, Baker RI, McQuillan A, Thom J, Staton J, et al. C-reactive protein in ischemic stroke and its etiologic subtypes. *J Stroke Cerebrovasc Dis.* 2003 Mar-Apr;12(2):74–81. [PubMed: 17903908]
28. Ladenvall C, Jood K, Blomstrand C, Nilsson S, Jern C, Ladenvall P. Serum C-reactive protein concentration and genotype in relation to ischemic stroke subtype. *Stroke.* 2006 Aug; 37(8):2018–2023. [PubMed: 16809555]
29. Verma S, Li SH, Badiwala MV, Weisel RD, Fedak PW, Li RK, et al. Endothelin antagonism and interleukin-6 inhibition attenuate the proatherogenic effects of C-reactive protein. *Circulation.* 2002 Apr 23; 105(16):1890–1896. [PubMed: 11997273]
30. Qamirani E, Ren Y, Kuo L, Hein TW. C-reactive protein inhibits endothelium-dependent NO-mediated dilation in coronary arterioles by activating p38 kinase and NAD(P)H oxidase. *Arterioscler Thromb Vasc Biol.* 2005 May; 25(5):995–1001. [PubMed: 15718491]
31. Wang CH, Li SH, Weisel RD, Fedak PW, Dumont AS, Szmítko P, et al. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation.* 2003 Apr 8; 107(13):1783–1790. [PubMed: 12665485]
32. Vongpatanasin W, Thomas GD, Schwartz R, Cassis LA, Osborne-Lawrence S, Hahner L, et al. C-reactive protein causes downregulation of vascular angiotensin subtype 2 receptors and systolic hypertension in mice. *Circulation.* 2007 Feb 27; 115(8):1020–1028. [PubMed: 17283257]
33. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. *Circ Res.* 2001 Oct 26; 89(9):763–771. [PubMed: 11679405]

34. Maneen MJ, Hannah R, Vitullo L, DeLance N, Cipolla MJ. Peroxynitrite diminishes myogenic activity and is associated with decreased vascular smooth muscle F-actin in rat posterior cerebral arteries. *Stroke*. 2006 Mar; 37(3):894–899. [PubMed: 16456123]
35. Chrissobolis S, Faraci FM. The role of oxidative stress and NADPH oxidase in cerebrovascular disease. *Trends Mol Med*. 2008 Nov; 14(11):495–502. [PubMed: 18929509]
36. Kähler J, Ewert A, Weckmüller J, Stobbe S, Mittmann C, Köster R, et al. Oxidative stress increases endothelin-1 synthesis in human coronary artery smooth muscle cells. *J Cardiovasc Pharmacol*. 2001 Jul; 38(1):49–57. [PubMed: 11444502]
37. Comporti M, Signorini C, Arezzini B, Vecchio D, Monaco B, Gardi C. F2-isoprostanes are not just markers of oxidative stress. *Free Radic Biol Med*. 2008 Feb 1; 44(3):247–256. [PubMed: 17997380]
38. Hoffman SW, Moore S, Ellis EF. Isoprostanes: free radical-generated prostaglandins with constrictor effects on cerebral arterioles. *Stroke*. 1997 Apr; 28(4):844–849. [PubMed: 9099206]
39. Seet RC, Lee CY, Chan BP, Sharma VK, Teoh HL, Venketasubramanian N, et al. Oxidative damage in ischemic stroke revealed using multiple biomarkers. *Stroke*. 2011 Aug; 42(8):2326–2329. [PubMed: 21700941]
40. Seet RC, Kasiman K, Gruber J, Tang SY, Wong MC, Chang HM, et al. Is uric acid protective or deleterious in acute ischemic stroke? A prospective cohort study. *Atherosclerosis*. 2010 Mar; 209(1):215–219. [PubMed: 19758590]
41. Vincent JM, Kwan YW, Chan SL, Perrin-Sarrado C, Atkinson J, Chillon JM. Constrictor and dilator effects of angiotensin II on cerebral arterioles. *Stroke*. 2005 Dec; 36(12):2691–2695. [PubMed: 16269635]

Figure 1.

Effect of acute stroke serum on myogenic reactivity in non-ischemic cerebral and mesenteric vessels. **A:** Active diameter changes in response to stepwise increase of intravascular pressure in PCAs. PCAs perfused with CE+HTN serum had significantly smaller diameters than control at the higher pressures. **B:** Active diameter changes in response to stepwise increase of intravascular pressure in MAs. MAs perfused with LVD+HTN serum were characterized by low myogenic reactivity. MAs perfused with LVD and CE+HTN serum had significantly smaller diameters that did not change with increased pressure. * $p < 0.05$ vs. Control; # $p < 0.05$ vs. LVD+HTN serum

Figure 2.

Effect of acute stroke serum on myogenic tone in non-ischemic cerebral and mesenteric vessels at 75 mm Hg. Exposure to LVD and CE+HTN serum produced increased tone in PCAs and MAs, exposure to LVD+HTN serum only in PCAs. * $p < 0.05$ vs. Control; # $p < 0.05$ vs. LVD+HTN serum; [^] $p < 0.01$ vs. PCAs perfused with the same serum

Figure 3.

Effect of acute stroke serum on percent constriction to L-NAME (10^{-3} M) in PCAs and MAs. Serum from all groups of stroke patients reduced constriction to L-NAME in PCAs compared to control, while no differences were measured among MA groups. * $p < 0.05$ vs. Control; ^ $p < 0.01$ vs. PCAs perfused with the same serum

Figure 4.

Effect of acute stroke serum on NO-dependent vasodilation. (A, B) show percent reactivity in PCAs receiving increasing concentrations (10^{-8} M to 10^{-5} M) of acetylcholine and SNP, respectively. The presence of LVD serum significantly reduced reactivity to acetylcholine while did not affect reactivity to SNP. The presence of CE+HTN serum caused reduced reactivity to SNP. (C, D) show the same experimental conditions in MAs as A and B. The presence of serum from all the groups of patients reduced reactivity to acetylcholine at 10^{-8} and 3×10^{-8} M. The presence of LVD+HTN significantly reduced reactivity to SNP. * $p < 0.05$ vs. Control; # $p < 0.05$ vs. LVD+HTN serum; † $p < 0.05$ vs. LVD serum

Table 1

Demographics and clinical features of patients.

	Serum groups			p value * <0.05
	LVD+HTN (n=4 for serum; n=7 for PCAs; n=9 for MAs)	LVD (n=4 for serum; n=9 for PCAs; n=10 for MAs)	CE+HTN (n=4 for serum; n=6 for PCAs; n=8 for MAs)	
Age (mean±SEM)	66.5±6	61.7±5	67±5.6	0.76
Male (%)	75	100	25	0.07
Caucasians (%)	100	100	100	-
NIHSS admission (median [IQR])	21 [16.25–24.25]	13.5 [7.5–19.5]	12 [8.5–17.75]	0.12
Pre-stroke mRS (median [IQR])	0 [0–3.75]	0.5 [0–2.5]	1 [1–1]	0.97
CRP [mg/L] (mean±SEM)	44±1.3	16.6±10	22.2±6.6	0.09
Uric acid [mg/dL] (mean±SEM)	4.6±0.9	4.7±0.3	5±0.4	0.86
<i>Comorbidities (%)</i> :				
• Hypertension	100	-	100	[Selection criterion]
• Diabetes	-	-	-	-
• Dyslipidemia	25	-	25	0.55
• Obesity	50	75	50	0.71
• Smoke	50	25	25	0.69
• Atrial fibrillation	-	-	75	0.01*
• Chronic kidney disease	-	-	25	0.34
• Previous stroke	-	-	50	0.09
• Peripheral Artery Disease	25	-	-	0.34
• Prior Myocardial Infarction / Coronaropathy	50	-	25	0.26
• Alcohol	-	25	50	0.26
<i>Prior medications (%)</i> :				
• Antiplatelets	50	25	100	0.09
• Anticoagulants	-	-	50	0.09
• Statins	25	-	25	0.55
• Antihypertensives	-	-	75	0.01*
<i>Acute treatments (%)</i> :				
• intravenous and/or endovascular thrombolysis	100	75	50	0.26
• none	-	25	50	0.26
Free 8-isoprostane [pg/mL] (mean±SD)	257±12	246±9	227±26	[Measured on pooled samples]