

ANGIOTENSIN II INDUCED CEREBRAL MICROVASCULAR INFLAMMATION AND INCREASED BLOOD–BRAIN BARRIER PERMEABILITY VIA OXIDATIVE STRESS

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Abstract—Although hypertension has been implicated in the pathogenesis of vascular disease, its role in inflammatory responses, especially in brain, remains unclear. In this study we found key mechanisms by which angiotensin II (AngII) mediates cerebral microvascular inflammation. C57BL/6 male mice were subjected to slow-pressor dose of AngII infusion using osmotic mini-pumps at a rate of 400 ng/kg/min for 14 days. Vascular inflammation in the brain was evaluated by analysis of leukocyte–endothelial interaction and blood–brain barrier (BBB) permeability. Results from intravital microscopy of pial vessels *in vivo*, revealed a 4.2 fold ($P < 0.05$, compared to vehicle) increase in leukocyte adhesion on day 4 of AngII infusion. This effect persisted through day 14 of AngII infusion, which resulted in a 2.6 fold ($P < 0.01$, compared to vehicle) increase in leukocyte adhesion. Furthermore, evaluation of BBB permeability by Evans Blue extravasation showed that Ang II significantly affected the BBB, inducing 3.8 times ($P < 0.05$, compared to vehicle) higher permeability. Previously we reported that AngII mediated hypertension promotes oxidative stress in the vasculature. Thus, we used the superoxide scavenger; 4-hydroxy-TEMPO (Tempol) to determine whether AngII via oxidative stress could contribute to higher leukocyte adhesion and increased BBB permeability. Tempol was given via drinking water (2 mmol) on day 4th following Ang II infusion, since oxidative stress increases in this model on day 4. Treatment with Tempol significantly attenuated the increased leukocyte/endothelial interactions and protected the BBB integrity on day 14 of AngII infusion. In conclusion, AngII via oxidative stress increases cerebral microvasculature inflammation and leads to greater immune-endothelial interaction and higher BBB permeability. This finding may open new avenues for the management of nervous system pathology involving cerebrovascular inflammation. © 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: angiotensin II, cerebrovascular, inflammation, oxidative stress, blood–brain barrier, hypertension.

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Abbreviations: AngII, angiotensin II; BBB, blood–brain barrier; EB, Evans Blue; EC, endothelial cells; HSP, heat shock proteins; ICAM-1, intercellular cell adhesion molecule-1; i.p., intraperitoneal; i.v., intravenously; NF- κ B, nuclear factor- κ B; PBS, phosphate buffered saline; ROS, reactive oxygen species; SHR, spontaneously hypertensive rat; Tempol, 4-hydroxy-TEMPO; VCAM-1, vascular cell adhesion molecule-1.

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doi:10.1016/j.neuroscience.2010.09.029

Hypertension is one of the major risk factors for vascular disease and endothelial dysfunction. Despite availability of effective antihypertensive drugs, the treatment of hypertension remains suboptimal and the prevalence is increasing. Hypertension continues to be one of the most significant healthcare burdens, causing end-organ damage of brain, heart and kidney (Virdis and Schiffrin, 2003; Di Napoli and Papa, 2005) and is associated with a significant morbidity and mortality (AHA, 2009). With regards to the brain, hypertension impairs brain adaptive responses, causes hypertrophy and remodeling of cerebral vasculature, accelerates atherosclerosis (Dahlof, 2007; Iadecola and Davisson, 2008), and remains the most significant risk factor for stroke, cognitive decline and dementia. It also plays a role in pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (Iadecola and Davisson, 2008).

The major detrimental actions of hypertension on brain are directed towards the dysregulation of cerebral blood flow (CBF) (Iadecola and Davisson, 2008). Indeed, by impairing CBF, hypertension could globally affect the brain function including the cerebral functional mechanisms which are the adaptive responses of brain, encompassing the fast neurovascular coupling, also known as functional hyperemia, neurometabolic and neurobarrier coupling, autoregulation, endothelium dependent vasorelaxation and blood–brain exchange—one of the key functions of blood–brain barrier (BBB). Neurovascular, neurometabolic and neurobarrier coupling maintain an adequate energy flow to the neuronal tissue under the conditions of increased neuronal activity (Cox et al., 1993; Zonta et al., 2003).

There is evidence that pathogenesis of hypertension involves a chronic low-grade inflammatory process, which may contribute to pathophysiology of hypertension, and its complications (Di Napoli and Papa, 2005; Marchesi et al., 2008). A crucial phase in the inflammatory cascade is the enhanced interaction of immune cells with a stimulated endothelium. This complex process involves leukocyte margination (rolling and adhesion) and diapedesis (transmigration through the vascular wall). As a consequence, significant alteration in resident cell function and viability develops (del Zoppo, 2009). In addition, converging lines of evidence have highlighted the involvement of oxidative stress in the pathogenesis of hypertension and endothelial dysfunction (Touyz and Schiffrin, 2008).

The mechanisms through which chronic hypertension may affect the brain, remain to be determined and many unanswered questions remain. A slow-pressor angiotensin II (AngII) model of hypertension has been validated by us

and others in mouse and rat model of hypertension. In our previous studies we have established that chronic infusion of AngII using osmotic mini-pumps in mice at the rate of 400 ng/kg/min is a slow-pressor dose which raises the systolic BP by 20 to 40 mm Hg, and it creates hypertension on day 5 of infusion as oppose to the model of malignant hypertension created by infusion of AngII at the rate of 1000 ng/kg/min causing elevation of BP on day 2. The model of slow-pressor response is pertinent because this model of hypertension mimics the essential hypertension found in humans (Reckelhoff and Romero, 2003).

The goal of this study was to test the hypothesis that slow pressor dose AngII causes cerebrovascular inflammation (CVI), which is mediated via oxidative stress and also affects the permeability of the BBB. Therefore, the objectives of this study were to: (1) investigate the inflammatory response to AngII slow pressor dose in cerebral vessels *in vivo*. (2) Identify the effects of cerebrovascular inflammation on BBB permeability *in vivo*. (3) Examine whether Ang II-induced CVI is mediated by oxidative stress.

EXPERIMENTAL PROCEDURES

Animals

The studies were carried out on 6-week old male C57BL/6 mice from Taconic Farms (Hudson, NY, USA). All experiments were conducted in accordance with the guidelines approved by Institutional Animal Care and Use Committee at Temple University.

For the first series, each group ($n=6$) of mice were anesthetized with an intraperitoneal (i.p.) injection of Ketamine (100 mg/mL, Sigma-Aldrich, St. Louis, MO, USA)–Xylazine (20 mg/kg, Sigma-Aldrich, St. Louis, MO, USA) mixture (1:1) at a dose of 1 ml/kg for insertion of osmotic minipumps (Alzet, Cupertino, CA, USA) to deliver AngII (400 ng/kg/min, Sigma-Aldrich, St. Louis, MO, USA) or vehicle (Veh) s.c. for 2 weeks on the back, slightly posterior to the scapulae. An additional group of mice infused with Ang II (400 ng/kg/min) received a 4-hydroxy-TEMPO (Tempol, Sigma-Aldrich, St. Louis, MO, USA) in drinking water starting on day 4 of infusion of AngII.

4-hydroxy-TEMPO treatment

Tempol was dissolved in sterile PBS and further diluted in the drinking water bag to achieve the concentration of 2 mmol. The drinking water bag was wrapped in aluminum foil to prevent it from light degradation and installed in each animal cage. The drinking water bag containing Tempol was changed every other day.

Cranial window implantation

On the day of cranial window implantation, the animals were anesthetized with an i.p. injection of Ketamine (100 mg/mL)–Xylazine (20 mg/kg) mixture (1:1) at a dose of 1 ml/kg. The head was shaved and positioned in a stereotaxic head holder. A 1 cm area of skin on the dorsal surface of the skull over the right cortical hemisphere was excised and the periosteum was removed. A 4 mm diameter circular craniotomy was performed using a high speed drill (Champ-Air Dental Drill Benco Dental) over the right parietal cortex extending from attachment of the temporal muscle to the midpoint of the sagittal suture in the coronal direction and aligned to the middle of the sagittal suture. Normal saline was dripped over the cranium to avoid thermal injury to the cortex. A 5 mm diameter coverglass was then placed over the exposed brain, and an airtight seal was produced using Nexaband Quick Gel. The

coverglass provided adequate mechanical protection from infection or contamination. A recovery period of 4 days was allowed between implantation and observation of the cranial window to eliminate the inflammation caused by surgery (Ramirez et al., 2010; Zhang et al., 2009).

Intravital microscopy and measurement of leukocyte–endothelial interactions

The animals were anesthetized and immobilized on a plexiglass stage on day 4 and day 14 after osmotic pump implantation. Intravital microscopy was performed with an epi-fluorescence microscope (BX10, Olympus, Japan) which was equipped with a Digital Camera (Cooke 1600, Cooke Corporation, Romulus, MI, USA). Leukocytes were stained *in vivo* by a bolus injection of 0.05 ml of a 0.01% solution of the fluorescent dye Rhodamine 6G (Sigma Inc, St Louis, MO, USA) into the facial vein. The light leaving the lamp housing was filtered to allow light with a peak wavelength of 605 nm to be transmitted to tissue. Excitation of fluorescent dyes in the leukocytes caused a shift in the wavelength of the emitted light. Selective filtering allowed visualization of the fluorescent cells on a dark background. Images from the camera at a video frame rate of 25 frames/s were acquired and recorded using Camware software (Cooke corporation). The interactions between leukocyte and endothelium were investigated offline.

Measurement of leukocyte rolling and adhesion

Three venules (with diameter 20–40 μm) in each animal were assessed. The number of rolling leukocytes was considered to be the total number of leukocytes moving along the endothelial cells at substantially slower velocity compared with the midstream blood cell velocity. They were counted when they passed an arbitrary line perpendicular to the longitudinal axis of the vessel over a period of 30 s. Adhering leukocytes were defined as the total number of the leukocytes firmly attached to the microvascular endothelium that did not change their location during the entire 30 s of observation period. Adhering leukocytes were scored as the number of cells per mm^2 of the vascular surface area, calculated from the diameter and standardized length (100 μm) of the vessel segment under investigation.

Quantitative evaluation of Evans Blue extravasation

Blood–brain barrier disruption was assessed quantitatively by measuring Evans Blue (EB, Sigma-Aldrich, St. Louis, MO, USA) extravasation (Uyama et al., 1988; Belayev et al., 1996). Briefly, on day 14, mice were i.v. injected with 0.1 ml 2% Evans Blue, which was allowed to circulate for 60 min. The animals were perfused transcardially with 0.9% phosphate buffered saline (PBS) to remove intravascular EB dye. The brains were weighed and homogenized in 500 μl 0.1 M PBS and 500 μl of TCA (trichloroacetic acid 100%, Sigma-Aldrich, St. Louis, MO, USA). Samples were incubated at 4 °C for at least 1 h and centrifuged at 10000 g for 30 min. The resulting supernatants were measured for absorbance of EB at 610 nm using a spectrophotometer.

Statistical analysis

Student's *t*-test was used to evaluate the difference of leukocyte rolling and adhesion in vehicle and angiotensin II treated mice at day 4. Bonferroni's test after one way analysis of variance (ANOVA) was used for analyzing differences in leukocyte/endothelial interaction and Evans Blue extravasation among vehicle, AngII and AngII plus 4-hydroxy-TEMPO treated mice on day 14. Data were presented as means \pm SEM. A statistically significant difference was assumed at $P<0.05$.

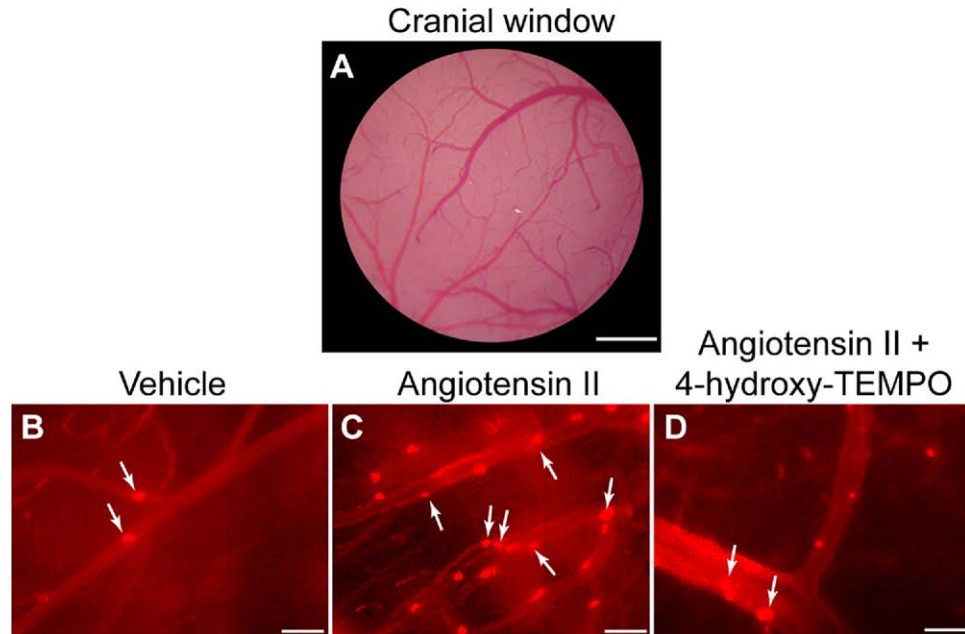


Fig. 1. Typical closed cranial window in a mouse. Scale bar=1000 μm . (A) Representative video images of capturing leukocytes labeled with Rhodamine 6G in the post-capillary venules on day 14. Video images were taken in Veh infused mice (B), AngII infused mice (C) and AngII infused mice treated with 4-hydroxy-TEMPO (Tempol) (D). Arrows indicate Rhodamine 6G-labeled leukocytes flowing through venules. Scale bar=40 μm .

RESULTS

Effects of angiotensin II on leukocyte rolling at post-capillary venules in brain

To evaluate whether a change in the number of rolling and adherent leukocytes can be observed on AngII infused animals, intravital microscopy on pial vessels was performed (Fig. 1A) in Veh (Fig. 1B), AngII (Fig. 1C) and

AngII+Tempol (Fig. 1D) infused mice. There was no change of leukocyte rolling on pial venules on day 4 of AngII infusion (2.17 ± 0.57), when compared to Veh infused mice (2.3 ± 0.37) (Fig. 2A). However, AngII infusion significantly increased leukocyte rolling on pial venules (5.7 ± 0.91) on day 14 compared to Veh infused mice (1.86 ± 0.65). Treatment with Tempol in drinking water, initiated on day 4, significantly decreased leukocyte rolling on venules

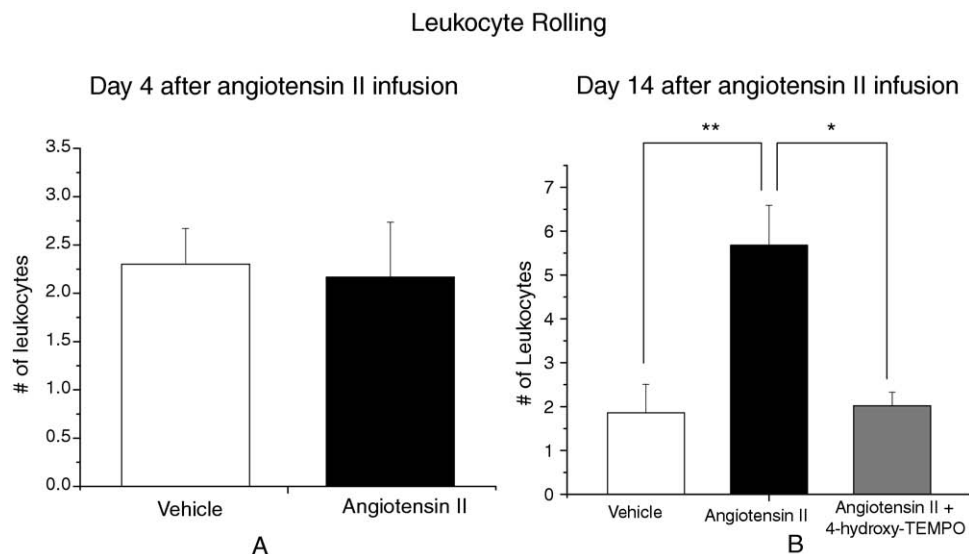


Fig. 2. There was no change of leukocyte rolling on day 4 day of AngII infusion compared to Veh infused mice (A). However, AngII infusion significantly increased leukocyte rolling on pial venules on day 14 compared to Veh infused mice. Treatment with 4-hydroxy-TEMPO (Tempol) in drinking water initiated on day 4 reversed the increased leukocyte rolling on venules induced by AngII infusion (B). (Data were expressed as Mean \pm SEM, $n=6$ in each groups, ** $P<0.01$, * $P<0.05$).

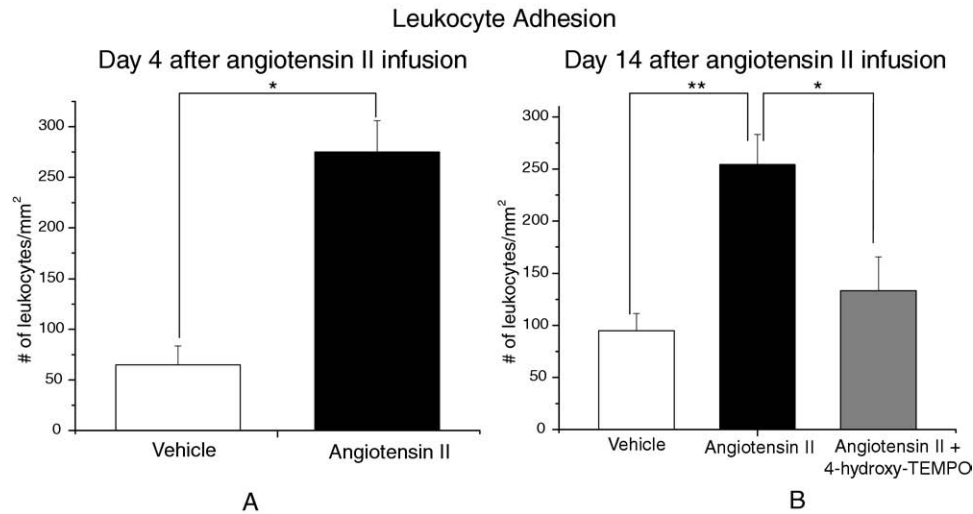


Fig. 3. AngII infusion significantly increased leukocyte adhesion on pial venules both, on day 4 (A) and 14 (B) compared to Veh infused mice. Treatment of AngII infused mice with 4-hydroxy-TEMPO (Tempol) in drinking water initiated on day 4 significantly decreased leukocyte adhesions on venules compared to non-treated AngII infused mice (B). (Data were expressed as Mean \pm SEM, $n=6$ in each groups, ** $P<0.01$, * $P<0.05$).

in Ang II infused mice (2.02 ± 0.30) (Fig. 2B), and there was no significant difference of leukocyte rolling between Veh and AngII+Tempol treated mice ($P>0.05$).

Effects of Ang II on leukocyte adhesion at post-capillary venules in brain

There was significant increase of leukocyte adhesion on pial venules on day 4 of AngII infusion (275 ± 30.96), when compared to vehicle infused mice (65 ± 18.1) (Fig. 3A). On day 14, there was persistent significant increase of leukocyte adhesion on venules in AngII infused mice (254.2 ± 28.8) compared to Veh infused mice (95 ± 16.5). Treatment with Tempol in drinking water, initiated on day 4, significantly decreased leukocyte adhesion on venules (133.4 ± 32.02) in AngII infused mice (Fig. 3B), and there was no significant difference of leukocyte adhesion between Veh and AngII+Tempol treated mice ($P>0.05$).

Effects of angiotensin II on blood–brain barrier disruption

AngII infusion significantly increased Evans Blue extravasation in the brain (1579 ± 376.1 ng/g brain) on day 14 compared to Veh infused mice (414 ± 66.69 ng/g brain). Treatment with Tempol in drinking water, initiated on day 4, significantly decreased Evans Blue extravasation in the brain (579.9 ± 199.4 ng/g brain) in AngII infused mice (Fig. 4). There was no significant difference of Evans Blue extravasation between vehicle and AngII+Tempol treated mice ($P>0.05$).

DISCUSSION

The new findings of this study are the following: 1. Chronic infusion of AngII at the slow-pressor dose causes significant increase of leukocyte adhesion on brain venules on day 4. This effect is maintained on day 14 of AngII infusion,

and also is accompanied with a significant increase of leukocyte rolling; 2. AngII significantly increases the BBB permeability on day 14 of AngII infusion. 3. Treatment with Tempol significantly attenuates the increased leukocyte/endothelial interactions and protects the BBB integrity as demonstrated by reduction of the Evans Blue extravasation on day 14 of AngII infusion.

Our study is the first to demonstrate that an AngII slow-pressor model of chronic hypertension increases leukocyte adhesion and rolling in mice cerebral microvessels, and increases permeability of BBB.

It has been shown that AngII may affect BBB permeability in the tissue culture environment (Guillot and Audus,

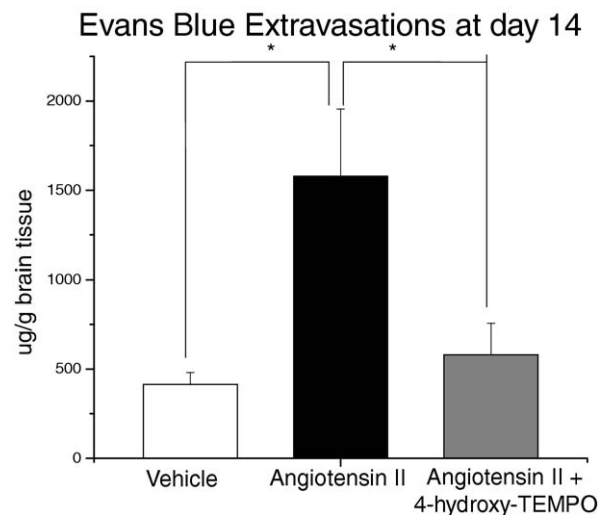


Fig. 4. AngII infusion significantly increased Evans Blue extravasations in brain on day 14 compared to Veh infused control mice. Treatment of AngII infused mice with 4-hydroxy-TEMPO (Tempol) in drinking water initiated on day 4 reversed such increased Evans Blue extravasations compared to AngII infused mice. (Data were expressed as Mean \pm SEM, $n=6$ in each groups, * $P<0.05$).

1991; Fleegal-DeMotta et al., 2009). Studying the effects of slow-pressor dose of AngII is especially important since it results in a gradual increase in blood pressure during chronic infusion of AngII at an initially sub-pressor dose (Dickinson and Yu, 1967; Romero and Reckelhoff, 1999; Granger and Schnackenberg, 2000). This type of response has been shown in different species including mice (Kawada et al., 2002), rats (Lever, 1993), rabbits (Dickinson and Yu, 1967) and humans (Ames et al., 1965), and it is considered as a model of essential hypertension.

These results demonstrate that cerebrovascular inflammation occurs no later than day 4 as it was demonstrated in our experiments by the increased leukocyte adhesion on day 4. Importantly, in our previous studies we observed that blood pressure increases on day 6 in this model (Welch et al., 2006), while, the oxidative stress develops on day 4 (Modlinger et al., 2006) of AngII infusion. We can speculate that perhaps the increased leukocyte adhesion precedes the onset of hypertension, but coincides with the development of oxidative stress.

The fact that leukocyte adhesion at day 4 in AngII infused mice was not accompanied by increased rolling may be explained, perhaps, with possible biphasic effects of AngII, when leukocyte rolling occurs earlier than day 4 and then this effect is lost temporally, however the second phase of increased rolling could be detected on day 14. The initial phase of increased leukocyte rolling is likely followed by increased leukocyte adhesion that could be detected on day 4.

One of the major finding of our study is that, the increased leukocyte adhesion in AngII infused mice is associated with increased BBB permeability as we observed with the Evans Blue extravasation assay. Reversal of cerebrovascular inflammation (leukocyte rolling and adhesion) by Tempol was also associated with restoration of the leaky BBB. Our hypothesis, that oxidative stress mediates cerebrovascular dysfunction in AngII-infused mice, is supported by these effects of Tempol. Our finding that treatment with Tempol corrected both the abnormally increased leukocyte adhesion and rolling, and the increased BBB permeability, could indicate that oxidative stress perhaps contributes to AngII induced inflammation, and damaged BBB in this model. Currently, Tempol is considered rather general redox-cycling agent than just superoxide dismutase mimetic (SOD-mimetic) given its catalase-like activity and its ability interfering in Fenton reaction. It also inhibits the reaction between superoxide anion (O_2^-) and nitric oxide (NO) that yields peroxynitrite ($ONOO^-$), which is a strong oxidant. Tempol improves NO signaling via cGMP dependent pathway, and also prevents activation of multiple crucial signaling pathways, like: AngII mediated mitogen-activated protein kinase, nuclear factor- $\kappa\beta$ (NF- $\kappa\beta$) in rat model of deoxycorticosterone acetate (DOCA)-salt induced hypertension, Rho and Rho kinase in aortic rings where the reactive oxygen species (ROS) are induced by treatment with xanthine plus xanthine oxidase, and protein kinase C in blood vessels from diabetic rats (Wilcox, 2010). It significantly reduces elevated blood pressure in animal models of acute and chronic hyperten-

sion. Recent studies indicate that antihypertensive effects of Tempol is related not only to correction of oxidative stress and increased NO activity (Schnackenberg and Wilcox, 1999), but also the inhibition of the sympathetic nervous system and to the activation of adenosine triphosphate-dependent potassium channels during intravenously (i.v.) administration. In the settings of a prolonged treatment, Tempol also improves kidney oxygen utilization for tubular sodium transport and as a result increases renal tissue oxygen tension, balances salt sensitivity, restores NO signaling between macula densa and afferent arterioles, and reduces renal vascular resistance. All these actions contribute to the reduction of the elevated blood pressure.

There are a number of potential implications of our findings. In previous studies it was observed that oxidative stress can alter brain endothelial tight junctions *in vitro* (Schreibelt et al., 2007) however its impact on the BBB permeability *in vivo* in the context of chronic hypertension had not been demonstrated prior to our study.

The renin–angiotensin system plays an important role in the development of hypertension. AngII is a powerful vasoconstrictor and anti-diuretic peptide, and has been shown to be one of the most significant contributor of vascular remodeling (Touyz et al., 2003). AngII regulates blood pressure and fluid homeostasis via both peripheral (renal and cardiovascular) and central nervous system mechanisms. AngII alters vascular permeability through pressure-related shear stress injury of the endothelium and via nonhaemodynamic mechanisms. It also causes significant endothelial dysfunction.

Cerebrovascular endothelium is the principal controller of leukocyte traffic between the systemic circulation and brain parenchyma. The leukocyte transmigration is mediated through multistep leukocyte–endothelial cell adhesive interactions involving intracellular cell adhesion molecules, selectins and integrins expressed on endothelial cells (EC) and leukocytes. I.p. injection of AngII in rats produces arteriolar leukocyte adhesion (Alvarez et al., 2004) and increases P-selectin, E-selectin, intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expression in arterioles and venules (Alvarez et al., 2004). There is an evidence that the latter is mediated in part by vascular endothelial growth factor (VEGF) and prostaglandins (PGs) (Suzuki et al., 2003). In rat endothelial cells AngII stimulates endothelial VCAM-1 via NF- $\kappa\beta$ signaling which is mediated by oxidative stress (Pueyo et al., 2000). Activation of the AngII type one (AT-1) receptor contributes to vasoconstriction, increased expression of vascular ICAM-1, MAC-1 in polymorphonuclear cells. It was shown that Ang II increases ICAM-1 mRNA and protein expression in cultured brain microvascular endothelial cells, and this effect is abolished when EC are pretreated with AT-1 receptor blocker, Losarten (Liu et al., 2006).

In the genetic model of hypertension, the spontaneously hypertensive rat (SHR), AT-1 receptor blockade normalizes cerebrovascular autoregulation and reduces cerebral ischemia. The increased number of endothelium-ad-

herent and infiltrating macrophages has been shown in SHR model in the context of increased ICAM-1 expression (Ando et al., 2004). The AT-1 receptor blockade with Candesartan reverses these abnormalities and pathological vascular hypertrophy. In a separate study it was shown that SHR has higher expression of many heat shock proteins (HSP) including HSP60, HSP70, HSP90 and heat shock factor-1 along with the enhanced expression of tumor necrosis factor- α , interleukin-1 β , and NF- κ B (Zhou et al., 2005). HSP70 activates the NF- κ B pathway, which is mediated by a rapid calcium influx. As a result, there is a robust increase proinflammatory cytokines (Asea et al., 2000).

The effect of angiotensin on the BBB remains a complex question. Kakinuma and colleagues have demonstrated that astrocyte-dependent angiotensins are crucial in the maintenance of BBB function (Kakinuma et al., 1998). Later, Wosik and collaborators showed that astrocyte derived AngII via AT-1 receptor stabilizes the BBB permeability by threonine-phosphorylation of the tight junction protein—occludin and mobilizing it in the lipid rafts in human with multiple sclerosis (Wosik et al., 2007). Factors contributing to this discrepancy in reports of AngII effects on BBB function could include the source of AngII, with different effects on the luminal and abluminal surfaces of the endothelial cells and concentration dependent effects.

The BBB is the main gateway between the systemic circulation and brain parenchyma. The BBB is defined as the boundary that regulates access of blood components (influx of nutrients and efflux of waste) and immune cells into the brain. The BBB is composed of capillary endothelial cells and forms the neurovascular unit along with glial cells, pericytes and neurons. Compared to other capillary endothelium, brain endothelium has specialized characteristics such as tight junctions, lack of fenestrations, and specialized transport systems. Under normal physiological conditions, the role of the BBB is to protect and maintain the delicate neuronal environment, and disruption of the BBB leaves the CNS vulnerable to neuronal damage. Most common neurological disorders involve some level of BBB dysfunction. In fact, BBB alteration occurs in stroke, head injury, hemorrhage, viral, bacterial and parasitic infections, neurodegeneration (Alzheimer's disease, Multiple Sclerosis, Parkinson's disease), epilepsy and brain tumors. While much is known about how alterations to the BBB contributes to neuronal damage and neuroinflammation; no studies have addressed how chronic hypertension may affect the BBB function and permeability.

As there is a continuous dynamic communication between neurovascular unit components, cerebrovascular inflammation by affecting the BBB permeability would interfere with the ability of the BBB to protect neurons from substances circulating in the systemic circulation that may be harmful to the brain, and also, could affect the mechanism of neurovascular coupling, and also contribute to the disrupted clearance of β -amyloid protein which is important in the pathogenesis of Alzheimer disease. Since the anatomical substrate of BBB is the brain endothelial cell interface, it is not surprising that the barrier properties of

cerebral vasculature depend on the cohesive and electrical resistance properties of the brain EC along with the integrity of the basal lamina.

CONCLUSION

Our data illustrate that, during prolonged s.c. infusion of AngII at a slow pressor-dose, AngII causes cerebral vasculature inflammation and leads to the increased BBB permeability. This increase in permeability was associated with an increase in leukocyte rolling and adhesion. The studies described did not enable us to provide evidence of a cause and effect relationship between these parameters. This effect of AngII is, at least in part, mediated via oxidative stress since Tempol, membrane permeable antioxidant, reverses the leukocyte rolling and adhesion, and prevents the increased BBB permeability. Additional studies are required to further investigate the mechanism of this effect in order to shed light into the role of cerebrovascular inflammation in pathogenesis of neurovascular dysregulation and identify cerebrovascular inflammation as a new target for disrupted neurovascular coupling therapeutics. Obtained knowledge could lead to new avenues for the development of safe and effective therapeutic approaches to inhibit impaired neurovascular unit function.

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